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Progress in Boronic Acid-Based Fluorescent Glucose Sensors

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Effective management of diabetes relies on the frequent monitoring of blood glucose concentrations. The current approach of blood sampling and glucose concentration determination *in vitro* presents many problems. Therefore, there is a drive for the development of non-invasive and continuous monitoring of glucose concentrations. The use of implanted glucose fluorescent sensors represents a promising approach. Due to its strong interaction with diol moieties, the boronic acid group often plays a critical role in the design of such glucose sensors. This paper reviews the progress in this area during the last ten years.

KEY WORDS: Glucose monitoring; boronic acid; fluorescence sensor.

INTRODUCTION

Diabetes afflicts at least 177 million people worldwide (International Diabetes Federation, http://www.idf.org), and this number is increasing due to poor diet and other factors [1,2]. The long-term complications of diabetes are associated with the high blood glucose levels. Currently, there is no cure for diabetes; however, proper control of the glucose concentration can help tremendously to reduce complications and prolong life. A critical step in controlling blood glucose level is appropriate monitoring. Ideally, one would like to have a continuous, real time, and non-invasive monitoring method. However, currently the most commonly used method is a non-continuous and invasive approach that samples blood from a finger followed by *in vitro* glucose concentration determination using a test strip and a meter. Such testing can be quite inconvenient, which creates problem in compliance. Another approach is the "Gluco Watch," which uses iontophoresis [3] as a way to extract biological fluid. This is minimally invasive, but has its disadvantages and is not a continuous monitoring approach.

Potentially, there are several ways to approach continuous, noninvasive glucose monitoring. This includes near-infrared (NIR) spectroscopy [4,5], contact lens type of sensors [6] and implanted sensor devices [7]. Except for the direct spectroscopic method, the others require the use of a sensor as the recognition and transducer unit. Along this line, one can use an enzyme-based sensing device, which is based on reactions involving glucose and is an indirect detection method [8]. A more promising approach is the use of fluorescent chemosensors that have a high affinity for glucose.

Generally, selective sensors consist of three components: a) proper functional groups that afford strong intermolecular interactions, b) a proper 'reporter' event/moiety, and c) the appropriate three-dimensional scaffold as the artificial receptor that provides the appropriated positioning and orientation for the relevant functional groups. 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. This paper presents an overview of the chemistry involved in the binding between boronic acid and saccharides, the fluorescent reporter groups used that signal the binding, and the three-dimensional scaffolds that are appropriate for the selective recognition of glucose in different forms.

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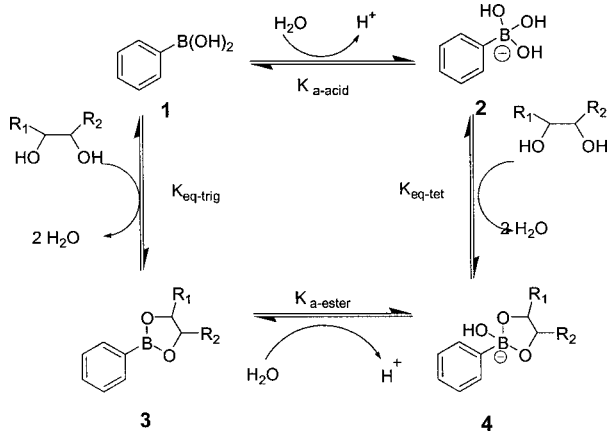
INTERACTIONS BETWEEN BORONIC ACIDS AND DIOLS

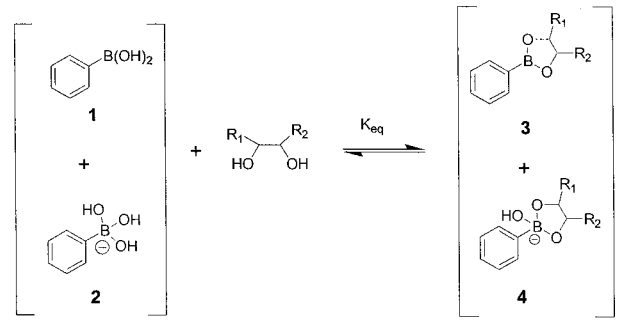
To discuss boronic acid-based sensor designs, we first need to examine how boronic acids interact with a diol and factors that affect the binding. Boronic acids covalently react with 1,2- or 1,3-diols to form five or six-membered cyclic esters in aqueous solution. The adjacent rigid *cis* diols of saccharides normally form stronger cyclic esters than simple acyclic diols such as ethylene glycol and *trans* diols. This complexation is reversible, which makes it an ideal interaction in sensor design. In 1959, Lorand and coworkers published the first quantitative evaluation of the interaction between various diol-containing compounds and phenylboronic acid [9] using the so-called pH-depression method. This method is based on the phenomenon that diol binding to a boronic acid usually lowers the pK_a of the boron containing functional group [9,10]. However, the binding constants determined using the pH-depression method was much higher than those determined later using spectroscopic methods. For example, the binding constant between anthrylboronic acid and fructose was found to be 270 M^{-1} when using a fluorescent method [11] whereas the binding constant of the structurally similar phenylboronic acid with fructose was reported to be 4370 M^{-1} when determined using the pH depression method. There are many similar contradictions in the literature. We considered that gaining an understanding of the reasons for such discrepancy is essential background information for our rational design effort. Therefore we undertook to systematically examine the binding ability of phenylboronic acid to a series of diol-containing compounds [10].

Boronic acids are Lewis acid and as such can react with water to go from the neutral trigonal form (**1**) to the anionic tetrahedral form (**2**) (Scheme 1). This is an acid-base reaction. The same is true for the diol-boronic acid complex or the boronate ester (**3**). Because boronic acids and their esters can exist in two different ionization states, there are actually 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. The third binding constant describes the overall binding strength regardless of the ionization state of the boron species, K_{eq} (Scheme 2). Through a careful examination, it was found that the binding constants determined using the pH depression method were the K_{tet}, whereas spectroscopic methods tend to give the K_{eq} [10,12]. Therefore, it is very important that one knows exactly what type of binding constants a particular procedure gives before any comparison can be made with or between literature values. The binding constants mentioned in the subsequent discussions are overall binding constants (K_{eq}, Scheme 2) unless specified otherwise.

Using a three-component competition assay, we have determined the binding constants (K_{eq}) of phenylboronic acid with a series of diol-containing compounds [10]. As it is widely recognized, phenylboronic acid (and other boronic acids) has different affinities for diols depending on their structural features, the most of which is the O—C—C—O dihedral angle. Smaller dihedral angles are often associated with higher binding constants. Another important factor to consider is that, generally speaking, boronic acids with lower pK_a's tend to have higher affinities for diols [13], although the optimal binding also depends on the pK_a's of the boronic acid and diol, and the pH [14]. Next we will discuss the different mechanisms through which the fluorescence of a fluorescent boronic



Scheme 1. Binding process between phenylboronic acid and a diol.



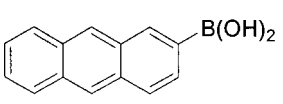
Scheme 2. Overall binding process between phenylboronic acid and a diol.

acid can be modulated upon diol/polyol binding, and how they can be used in the design and synthesis of sensors for glucose.

PHOTOELECTRON TRANSFER (PET) AND FLUORESCENCE RESONANCE ENERGY TRANSFER (FRET) FLUORESCENCE SENSORS

As with any sensor design, there needs to be a signaling event that reports the binding. For obvious reasons, most glucose sensors use fluorescence for the detection. As such, a fluorophore that changes fluorescence upon binding glucose is required. One way to do so is through the modulation of an excited state photoelectron transfer (PET) process that is responsible for the quenching of fluorescence.

The first fluorescence PET sensors for saccharides, the anthrylboronic acids (**5** and **6**), were reported by Yoon and Czarnik. These compounds showed significant fluorescence intensity changes upon binding with saccharide. The intensity change was lower for glucose than fructose [11]. It was thought that the binding triggers a fluorescence intensity change due to the change in the hybridization state of the ester, which has a lower pK_a. Specifically, the boronic acid (pK_a about 8.8) should exist mostly in the neutral trigonl state at physiological pH. In such a state, PET to the open shell of boron can happen in the excited state, which quenches the fluorescence. However, upon ester formation, the boron functionality would exists in the anionic tetrahedral because of a decreased pK_a (about 4.6 and 6.8 for the fructose and glucose esters, respectively) [10]. Such a hybridization change abolishes the excited state PET and therefore removes the fluorescence quenching mechanism, which consequently causes increased fluorescence. Such sensors represent very impressive initial success, although monoboronic acids of any kind would not be expected to have the selectivity needed for specific glucose sensing [10].



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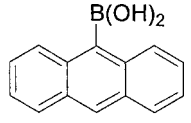

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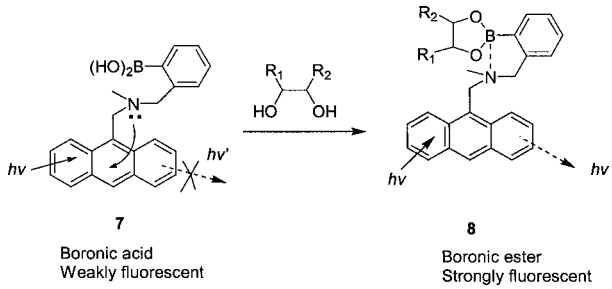
A second system was developed by Shinkai and coworkers [15] (Scheme 3). In this system, an amino group is positioned in a 1,5-relationship with the boronic acid. It had previously been demonstrated by Wulff [16] that such an arrangement promotes dative B—N bond formation. When this happens, it helps to lower the apparent



7

Boronic acid
Weakly fluorescent

8

Boronic ester
Strongly fluorescent

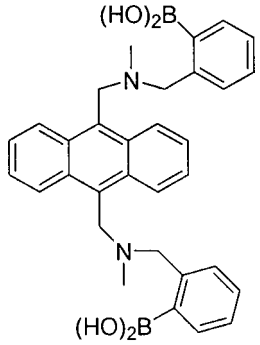
Scheme 3. Illustration of an anthracene-based photoinduced electron transfer system.

pK_a of the boronic acid, which helps to increase binding strength [9,10]. Indeed, **7** showed much higher binding affinity to glucose and fructose compared with phenylboronic acid [10,15,17]. This system (**7**) was also shown to exhibit a very significant fluorescence intensity change upon sugar binding. This was proposed to be due to the strengthening of the B—N bond upon ester (**8**) formation [16]. Specifically, the fluorescence of the anthracene system can be quenched by the nitrogen lone pair electrons through PET. However, binding to a diol is known to lower the pK_a of the boron functionality [9,10], which was thought to consequently strengthen the B—N bond. This B—N bond strengthening was thought to "tie up" the nitrogen lone pair electrons and thus lead to a reduced PET fluorescence quenching, as shown in Scheme 3. The latest evidence from our laboratory, however, indicates that this is not the mechanism through which fluorescence intensity changes [18]. Instead, a hydrolysis mechanism is apparently in play. However, the elucidation of a new mechanism does not change the fact that this system (**7**) is and has been a very useful reporter system for the development of fluorescent sensors for sugars.

As discussed previously, the binding of a sugar to a monoboronic acid depends on the intrinsic structural features of the sugar. This is illustrated by the observation that favors fructose over glucose by about 50 fold at physiological pH [10]. In order to increase the binding affinity and selectivity to glucose, it is essential that a more selective recognition element should be built into the system. This can be achieved with the inclusion of an additional binding site, which can be either a) another boronic acid to be recognized by a second pair of diols or b) other recognition moieties.

Shinkai and coworkers with the help of molecular modelling, designed compound **9**, which has two appropriately spaced boronic acid moieties [19]. This compound shows a maximum fluorescence intensity change of about 7-fold upon binding with glucose, and binds with glucose

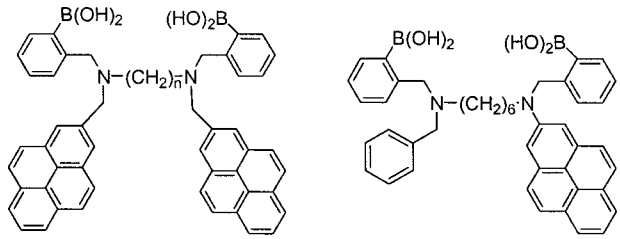
with higher affinity compared to other monosaccharides, indicating that the spacing and orientation of the two bornoic acid moieties are complementary to that of two diol pairs on glucose. For example, the binding constant of **9** with glucose is 3980 M^{-1} in aqueous methanol buffer at pH 7.8, while it shows much weaker binding for fructose ($K_{eq} = 316\ M^{-1}$). Shinkai and co-workers also conducted NMR experiments in methanol to deduce that diboronic acid **9** binds with glucose in the pyranose form. Upon further examination, Norrild and co-workers found that solvents have an important effect on the form in which glucose forms a complex with a diboronic acid [20,21]. It was found that the diboronic acid initially binds with the pyranose form of D-glucose and then slowly converts to the thermodynamically more stable furanose form. Addition of water facilitates this process since the faster mutarotation of glucose occurs in water than in methanol [22].



9

Along a similar line, Shinkai and coworkers also developed a few other potential glucose sensors that use an appropriate spacer to link two fluorescent boronic acid moieties. Among them, compound **10** ($n = 6$) with a hexamethylene spacer between the two phenylboronic acid moieties has high selectivity for D-glucose ($K_{eq} = 1995\ M^{-1}$), whereas it binds to fructose in a 1:2 ratio, indicating that the each boronic acid of **10** behaves as a monoboronic acid toward fructose [23]. In this case, the linker length and the conformational constraints imposed by are the two key elements in determining the specificity for glucose. In another study, Appleton and Gibson further examined the influence of linker length in analogs of **10** and found that C_6 and C_7 linker displayed high specificity to D-glucose, while a longer linker (e.g. C_{12}) behaved like a monoboronic acid showing preference for fructose over glucose [24].

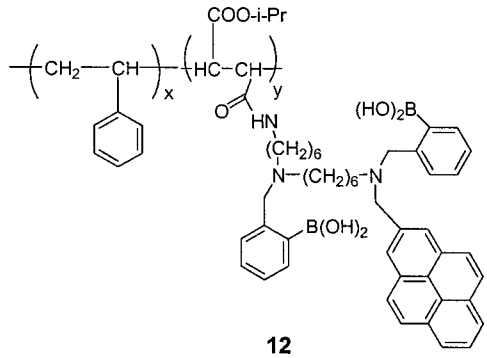
In compound **10**, the two pyrene groups in one molecule caused complication in emission fluorescence spectra due to stacking of two pyrene unit. Therefore, the James group developed the unsymmetrical diboronic acid sensor **11** and the polymeric sensor **12** derived from **10**. Both compounds **11** and **12** showed increased fluorescence intensity with the addition of a saccharide. Compound **11** showed higher selectivity for glucose ($K_{eq} = 962\ M^{-1}$) whereas polymer compound **12** showed higher selectivity for fructose ($K_{eq} = 1124\ M^{-1}$) [25].



10

n= 6, 7, 8, 12

11



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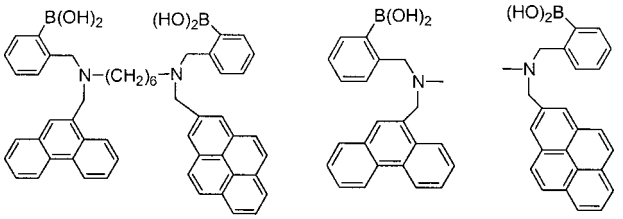
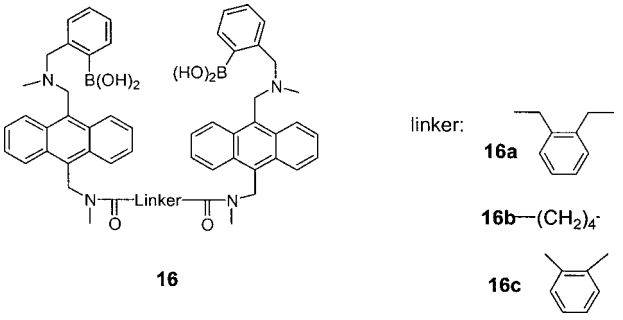
Using polyaryl systems, James and his coworker prepared a fluorescent resonance energy transfer (FRET) saccharide sensor **13** with two different fluorophores, phenanthrene as the donor and pyrene as the acceptor. Compound **13** was designed to be an amalgamation of **14** and **15** because of the overlap in the emission wavelength of phenanthrene and excitation wavelength of pyrene. When excited at 299 nm (phenathrene), the emission intensity at 417 nm from pyrene increased by up to 3.9-fold in the presence of different concentrations of glucose. The results suggested that energy transfer from phenanthrene to pyrene in a rigid 1:1 cyclic D-glucose complex helps to increase fluorescence [26]. The binding constants (K_{eq} between glucose and compounds **13**, **14**, and **15** found to be 142, 30, and 44 respectively, showing again that properly spaced diboronic acid structures help to improve the affinity and selectivity for glucose.



13 **14** **15**

Using Shinkai's anthracene fluorescent reporter system as the basic building block, our group synthesized a series of diboronic acids **16** which have different amide linkers [27]. It was found that **16a**, which has two acetamides attached to phenyl ring in an ortho relationship offers the appropriate diboronic acid orientation and distance for selective binding with glucose. It showed high affinity ($K_{eq} = 1472\ M^{-1}$), and a 43-fold selectivity for glucose over fructose. Neither **16b** nor **16c**, although having the same number of carbons in the linker, showed the kind of selectivity and affinity for the glucose as **16a** did. This again indicates that the rigidity of the linker plays a critical role in determining saccharide selectivity.

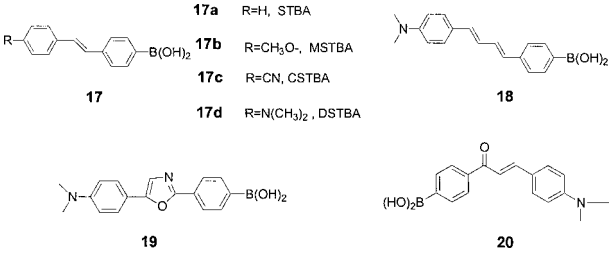


16

NON-PET SENSORS (ICT SYSTEM)

The internal charge transfer (ICT) mechanism has also been employed in a number of fluorescent boronic acid glucose sensing compounds. Usually, an ICT system contains an electron donor group and an electron acceptor group in the same fluorophore. The boronic acid acts as electron acceptor in the neutral form. When the boronic acid group changes to its anionic form (tetrahedral form) at certain pH upon binding with a sugar, it is no longer an electron acceptor. This leads to the spectral changes due to the perturbation of the charge transfer nature of the excited state [28].

The first generations of ICT sensors were developed by the Shinkai group in 1994 and were based on a stillbene core. Such compounds possess a large conjugated π system of electrons, which allows for the possibility of the "terminal" substituent to affect the chromophoric properties of these systems. Four stillbene boronic acid analogs **17a–d** were synthesized and evaluated. Compounds **17b** and **17d** bear an electron-donating group and **17c** bears an electron-withdrawing cyano group. Changing the pH from low to high induced a blue shift in the emission spectrum of **17b** and **17d** and an increase in intensity by about one fold in the presence of sugar. This was thought to be due to the loss of the electron-withdrawing properties of boron functionality when converted to the anionic form of the boronic acid. Similarly, the polyene derivatives **18** [29], diphenyloxazole derivatives **19** [30,31] and chalcone derivatives **20** [32] were prepared and tested for binding with sugars. However, the fluorescence intensity changed only by a maximum of five fold in these ICT systems. Understandably, these monoboronic acids showed preference for fructose over glucose as would have been expected based on the results of phenylboronic acid [9,10]. However, these "reporter" systems could, in principle, be used for the construction of diboronic acid sensors that show selectivity for glucose.

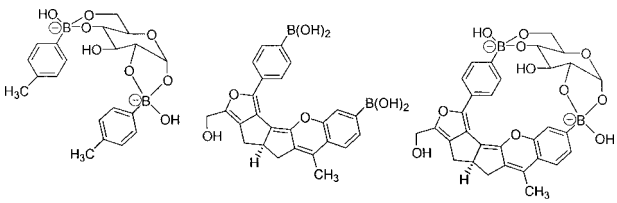


17 **18** **19** **20**

A de novo DESIGN APPROACH TO GLUCOSE SENSORS

Drueckhammer and coworkers reported the first example of a fluorescence sensor for glucose in the glucopyranose form based entirely on *de novo* design [33]. In this approach, two tolylboronic acid moieties were attached to the 1,2 and 4,6 positions of glucopyranose to get the complex structure **21** in the first step. Then methyl-aryl bonds of the two *p*-tolylboronic acid moieties, after quantum mechanical geometry optimization, were considered as two vectors. The CAVEAT program was then used to search for polycyclic scaffolds which possessed bond vectors matching the desired position and orientation of the methyl-aryl bond vectors in the di *p*-tolylboronate. Taking into account of the rigidity, fluorophoric property, ease of synthesis and water solubility, compound **22a** was designed and synthesized as a potential sensor for D-glucopyranose. This

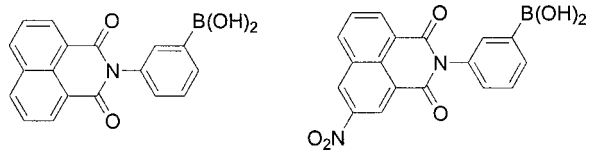
compound consists of two boronic acids attached to a rigid four-fused ring system including a fluorophoric coumarin, a hydrophilic hydroxymethylene group in furan ring, and a chiral center. After geometry optimized with Hartree-Fork 6-31G* method, the lowest energy conformation of the glucose complex **22b** matched very well with the original compound **22a**. The fluorescence intensity of **22a** decreased with the addition of saccharide in a concentration-dependent fashion. As designed, **22a** formed a complex with D-glucose in the pyranose form as identified by ^{1}H-NMR and mass spectrometry studies. Evaluation of the sensor for its affinity and selectivity for D-glucose versus other saccharides including D-galactose, D-mannose, and D-fructose was conducted by monitoring the fluorescence intensity changes. Glucose exhibited at least a 400-fold higher affinity ($K_{eq} = 40000$) than the two other saccharides (galactose $K_{eq} = 100$ and mannose $K_{eq} = 83.3$). The high selectivity for D-glucose suggests that only glucose is able to form a bidentate complex involving both boronic acid moieties of the "receptor."



21 **22a** **22b**

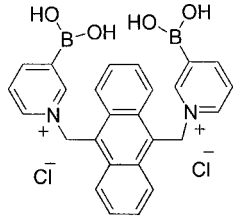
FLUORESCENT SENSORS AND REPORTERS SUITABLE FOR SENSING IN AN AQUEOUS MEDIUM

Despite many initial successes, the application of such sensors for glucose sensing requires the development of new candidates that have photochemical, physicochemical, and chemical properties that make them more "biocompatible." Such factors include water solubility, low toxicity, high chemical and photochemical stability, and long excitation and emission wavelengths. In many fluorescent sensors, the fluorophores are hydrophobic polyarene molecules such as anthracence that limit their water solubility. Therefore, the addition of an organic solvent as the co-solvent is usually required in order to study their binding with saccharides. The application of such hydrophobic sensors in biological systems could be severely hindered by this low water solubility problem, as well as other issues. Aimed at addressing the water solubility issue, Heagy's group developed the naphthalic anhydride sensor **23**, which can be employed in a neutral aqueous environment [34]. This compound is soluble in aqueous solution at least up to micromolar concentrations, which makes it suitable for fluorescent sensor development. The fluorescence intensity of the sensor decreased with the addition of saccharides. It is interesting to note that this sensor showed the greatest fluorescence intensity changes with glucose, while still exhibiting higher binding for fructose over glucose. It is not clear why the binding is not correlated with the intensity of fluorescence intensity changes. When a nitro group was introduced to the naphthalic anhydride ring, the compound (**24**) displayed dual fluorescence and a remarkable pH-dependent sensitivity to glucose over fructose [35].



23 **24**

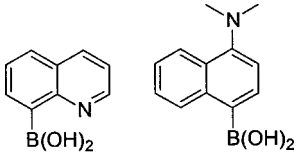
Norrild and coworkers synthesized a water soluble constrained diboronic acid **25** that could bind to D-glucose with a binding constant of 2512 M^{-1} [36]. This boronic acid sensor has a low pK_a of 4.0 due to presence of the cationic pyridinium moieties, which helps to improve the binding with sugar in a neutral aqueous solution. Furthermore, the ionic nature of the two alkylated pyridine moieties helps to increase the water solubility of this compound. Binding studies in aqueous solution at physiological pH indicated that the sensor binds with glucose (1:1) in the furanose form at the 1,2- and 3,5- positions. Although the binding constant ($K_{eq} = 2512$ M^{-1}) and selectivity for glucose over fructose shown by 25 is less than that of Shinkai's PET sensor **9**, its water soluble nature makes it very interesting.



25

A number of water soluble, fluorescent boronic acids recently developed by our group could also be used as building blocks for the design of selective glucose sensors in the future. For example, 8-quinolineboronic acid

(8-QBA, **26**), a novel type of fluorescent probe for saccharides, responds to the binding of a carbohydrate with over 40-fold increases in fluorescence intensity and shows good water solubility, and chemical and photochemical stability [37]. It shows comparable binding affinities for various sugars to those of phenylboronic acid. The mechanism for the fluorescence intensity changes shown by 8-QBA is not yet clear. However, preliminary ^{11}B-NMR results show that the boron atom in both 8-QBA and its carbohydrate esters are in the tetrahedral form at pH 7.4, which indicates that the fluorescence intensity changes are not due to a change in hybridization state of the boron atom.



26 **27**

Compound **27**, a new water-soluble fluorescent reporter, shows large fluorescence intensity increases upon binding diols at physiological pH [38]. In compound **27**, the amino group, an electron donor, and the boron atom, an electron acceptor, are located on the same chromophore. As mentioned earlier, ester formation with sugars lowers the pK_a of the boron functionality by about 2–3 pH units and converts the boron atom from a neutral sp^2 to an anionic sp^3 moiety [10]. The addition of a saccharide consequently brings about changes in chromophoric properties and leads to an increase in fluorescence. Binding studies showed that fructose and sorbitol induce a 40-fold fluorescence intensity increase at 50 mM whereas glucose showed a 17-fold increase at 1 M. The fluorescence intensity of compound **27** increased when the pH was increased from 3 to 10 (Fig. 1). These water soluble fluorescent boronic acids can be used to make bis- or multiboronic acid sensors to detect glucose and other carbohydrates with high selectivity and specificity.

Recently, the Asher group reported a new type of carbohydrate sensing material, which consists of a crystalline colloidal array (CCA) incorporated into a polyacrylamide hydrogel (PCCA) with pendent boronic acid groups [39,40]. The embedded CCA difracts visible light, and the PCCA diffraction wavelength reports on the hydrogel volume, which correlates with the binding between a boronic acid and carbohydrate such as glucose. Thus, this PCCA photonic crystal sensing material responds to glucose in low ionic strength aqueous solutions by

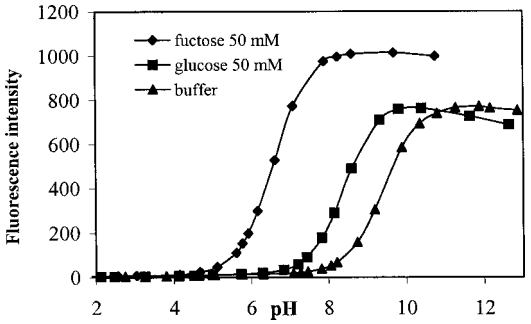
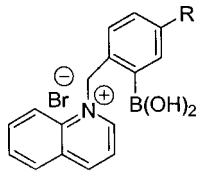


Fig. 1. pH titration of the fluorescence intensity of **27** (1×10^{-5} M) in the absence and presence of sugars in 0.1 M aqueous phosphate buffer, $\lambda_{ex} = 300$ nm, $\lambda_{em} = 445$ nm.

swelling and red shifting its diffraction as the glucose concentration increases. The sensing material responds to glucose and other sugars at <50 μM concentrations. Such materials could potentially be used for the preparation of implantable materials for blood glucose sensing or embedded in a contact lens for tear glucose sensing (See below).

FLUORESCENT BORONIC ACIDS SUITABLE FOR EMBEDMENT IN CONTACT LENS FOR GLUCOSE SENSING

In addition to implanted glucose sensors, the monitoring of tear glucose concentration also holds promise for the development of non-invasive and continuous glucose sensing schemes. In this regard, the Geddes's group reported their preliminary work on testing the feasibility of such an approach [6]. It has been known for many years that tear glucose during hyperglycemia is elevated [41,42]. Therefore, there is the possibility of extrapolating to blood glucose concentration by monitoring tear glucose levels. In monitoring tear glucose concentrations, Geddes's group proposed to use a contact lensbased approach. In such a case, fluorescent sensors would be incorporated into a contact lens, and reading of the fluorescent signals from the contact lens would allow for non-invasive monitoring of glucose concentrations. A series of boronic acids **28** were synthesized for embedment into contact lens. As purpose of the incorporation of a quinolinium moiety was to lower the pK_a of the boronic acid, which as mentioned previously, is known to increase the boronic acid binding affinity with diols. Their initial results indicate that such an approach offers the possibility of continuous monitoring of glucose concentration in the range of 50–1000 μM, which is the normal physiological range in tear [6].



R = OCH_3 or CH_3

28

CONCLUSIONS

A great deal of progress has been made in making boronic acid-based glucose sensors that can potentially be used for non-invasive and continuous glucose sensing. This may include implanted sensing devices or contact lens type sensors. The development of water soluble and chemically and photochemically stable fluorescent boronic acid reporter compounds will undoubtedly aid in the design of clinically useful sensors. However, the short excitation and emission wavelengths for most of these fluorescent boronic acids leave much to be desired. More work is needed to address these practical problems in order to develop a final product.

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REFERENCES

1. G. Y. Daniloff (1999). Continous glucose monitoring: Long term implantable sensor approach. *Diabetes Technol. Ther.* **1**, 261–266.
2. E. Renard (2002). Implantable closed-loop glucose-sensing and insulin delivery: the future for insulin pump therapy. *Curr. Opin. Pharmacol.* **2**, 708–716.
3. R. T. Kurnik, B. Berner, J. Tamada, and R. O. Potts (1998). Design and simulation of a reverse iontophoretic glucose monitoring device. *J. Electrochem. Soc.* **145**, 4119–4125.
4. H. M. Heise, R. Marbach, T. H. Koschinsky, and F. A. Gries (1994). Noninvasive blood glucose sensors based on near-infrared spectroscopy. *Ann. Occup. Hyp.* **18**, 439–447.
5. J. J. Burmeister, H. Chung, and M. A. Arnold (1998). Phantoms for noninvasive blood glucose sensing with near infrared transmission spectroscopy. *Photochem. Photobiol.* **67**, 50–55.
6. R. Badugu, J.R. Lakowicz, and C.D. Geddes (2003). A glucose sensing contact lens: A non-invasive technique for continuous physiological glucose monitoring. *J. Fluorescence* **13**, 371–374.
7. D. Gough and J. C. Armour (1995). Development of the implantable glucose sensor: What are the prospects and why is it taking so long. *Diabetes.* **44**, 1005–1009.
8. E. R. Kenneth and K. J. Ernest (1999). Issues and implications in the selection of blood glucose monitoring techniques. *Diabetes. Technol. Ther.* **1**, 3–11.
9. J. P. Lorand and J. O. Edwards (1959). Polyol complexes and structures of the benzeneboronate ion. *J. Org. Chem.* **24**, 769–774.
10. G. Springsteen and B. Wang (2002). A detailed examination of boronic acid–diol complexation. *Tetrahedron.* **58**, 5291–5300.
11. J. Yoon and A. W. Czarnik (1992). Fluorescent chemosensors of carbohydrates. A means of chemically communicating the binding of polyols in water based on chelation-enhanced quenching. *J. Am. Chem. Soc.* **114**, 5874–5875.
12. G. Springsteen and B. Wang (2001). Alizarin red S. as a general optical reporter for studying the binding of boronic acids with carbohydrates. *Chem. Commun.* 1608–1609.
13. T. D. James and S. Shinkai (2002). Artificial receptors as chemosensors for carbohydrates. *Top. Curr. Chem.* **218**, 159–200.
14. J. Yan, G. Springsteen, S. Deeter, and B. Wang (in press). The relationship among pKa, pH, and binding constants in the interactions between boronic acids and diols—It is not as simple as it appears. *Tetrahedron.*
15. T. D. James, K. R. A. S. Sandanayake, and S. Shinkai (1994). Novel photoinduced electron-transfer sensor for saccharides based on the interaction of boronic acid and amine. *Chem. Commun.* 477–478.
16. G. Wulff (1982). Selective binding to polymers via covalent bonds—the construction of chiral cavities as specific receptor-sites. *Pure. Appl. Chem.* **54**, 2093–2102.
17. W. Yang, J. Yan, H. Fang, and B. Wang (2003). The first fluorescent sensor for D-glucarate based on the cooperative action of boronic acid and guanidinium groups. *Chem. Commun.* 792–793.
18. W. Ni, G. Kaur, G. Springsteen, B. Wang, and S. Franzen (in press). Regulating the fluorescence intensity of an anthracene boronic acid system: A B—N bond or a hydrolysis mechanism? *Bioorgan. Chem.*
19. T. D. James, K. R. A. Sandanayake, R. Iguchi, and S. Shinkai (1995). Novel saccharide-photoinduced electron transfer sensors based on the interaction of boronic acid and amine. *J. Am. Chem. Soc.* **117**, 8982–8987.
20. J. C. Norrid and H. Eggert (1995). Evidence for mono- and bisdentate boronate complexes of glucose in the furanose form. application of ^{1}J C–C coupling constants as structural probe. *J. Am. Chem. Soc.* **117**, 1479–1484.
21. M. Bielecki, H. Eggert, and J. C. Norrid (1999). A fluorescent glucose sensor binding covalently to all five hydroxy groups of D-glucofurnaose. A reinvestigation. *J. Chem. Soc., Perkin Trans. 2*, 449–455.
22. F. P. Worley and J. C. Andrews (1927). Mutarotation. I: velocity of mutarotation of alpha glucose in methyl alcohol and water. *J. Phys. Chem.* **31**, 742–746.
23. K. R. A. Samankumara, T. D. James, and S. Shinkai (1995). Two dimensional photoinduced electron transfer (PET) fluorescence sensor for saccharides. *Chem. Lett.* 503–504.
24. B. Appleton and T. D. Gibson (2000). Detection of total sugar concentration using photoinduced electron transfer materials: Development of operationally stable, reusable optical sensors. *Sens. Actuator. B Chem.* **65**, 302–304.
25. S. Arimori, M. L. Bell, C. S. Oh, S. Chan, K. A. Frimat, and T. D. James (2001). Modular fluorescence sensors for saccharides. *Chem. Commun.* 1836–1837.
26. S. Arimori, M. L. Bell, C. S. Oh, and T. D. James (2002). A modular fluorescence intramolecular energy transfer saccharide sensor. *Org. Lett.* **4**, 4249–4251.
27. V. Karnati, X. Gao, S. Gao, W. Yang, S. Sabapathy, W. Ni, and B. Wang (2002). A selective fluorescent sensor for glucose. *Bioorg. Med. Chem. Lett.* **12**, 3373–3377.
28. N. DiCesare and J. R. Lackowicz (2002). Charge transfer fluorenscent probes using boronic acid for monosaccharide signaling. *J. Biomed. Opt.* **7**, 538–545.
29. N. DiCesare and J. R. Lackowicz (2002). Spectral properties of fluorophores combining the boronic acid group with electron donor

or withdrawing groups. Implication in the development of fluorescence probes for saccharides. *J. Phys. Chem. A* **105**, 6834–6840.
30. N. DiCesare and J. R. Lakowicz (2001). Wavelength-ratiometric probes for saccharides based on donor-acceptor diphenylpolyenes. *J. Photochem. Photobiol. A* **143**, 39–47.
31. N. DiCesare and J. R. Lakowicz (2001). A new highly fluorescent probe for monosaccharides based on a donor-acceptor diphenyloxazole. *Chem. Commun.* 2022–2023.
32. N. DiCesare and J. R. Lakowicz (2002). Chalocone-analogue fluorescent probes for saccharides signaling using the boronic acid group. *Tetrahedron Lett.* **43**, 2615–2618.
33. W. Yang, H. He and D. G. Drueckhammer (2001). Computer-guided design in molecular recognition: Design and synthesis of a glucopyranose receptor. *Angew. Chem. Int. Ed.* **40**, 1714–1718.
34. D. P. Adhikiri and M. D. Heagy (1999). Fluorescent chemosensor for carbohydrates which shows large change in chelation-enhanced quenching. *Tetrahedron Lett.* **40**, 7893–7896.
35. H. Cao, D. I. Diaz, D. DiCesare, J. R. Lakowicz, and M. D. Heagy (2002). Monoboronic acid sensor that displays anomalous fluorescence sensitivity to glucose. *Org. Lett.* **4**, 1503–1505.
36. H. Eggert, J. Frederiksen, C. Morin, and J.C. Norrid (1999). A new glucose-selective fluorescence bisboronic acid. First report of strong a-furanose complexation in aqueous solution at physiological pH. *J. Org. Chem.* **64**, 3846–3852.
37. W. Yang, J. Yan, G. Springsteen, S. Deeter, and B. Wang. (2003). A novel type of fluorescent boronic acid that shows large fluorescence intensity changes upon binding with a carbohydrate in aqueous solution at physiological pH. *Bioorg. Med. Chem. Lett.* **13**, 1019–1022.
38. X. Gao, Y. Zhang, and B. Wang (2003). New boronic acid fluorescent reporter compounds II. A Naphthalene-based sensor functional at physiological pH. *Org. Lett.* **5**, 4615–4618.
39. V. L. Alexeev, A. C. Sharma, A. V. Goponenko, S. Das, I. K. Lednev, C. S. Wilcox, D. N. Finegold, and S. A. Asher (2003). High ionic strength glucose-sensing photonic crystal. *Anal. Chem.* **75**, 2316–2323.
40. S. A. Asher, V. L. Alexeev, A. V. Goponenko, A. C. Sharma, I. K. Lednev, C. S. Wilcox, and D. N. Finegold (2003). Photonic crystal carbohydrate sensors: Low ionic strength sugar sensing. *J. Am. Chem. Soc.* **125**, 3322–3329.
41. D. Michail, P. Vancea, and N. Zolog (1937). Sur l'elimination lacrymale du glucose chez les diabetiques. *C. R. Soc. Biol., Paris* **125**, 1095.
42. D. Michail and N. Zolog (1937). Sur l'elimination lacrymale du glucose au cours dr l'hyperglycemie alimentaire. *C. R. Soc. Biol. Paris* **126**, 1042.