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Carbohydrate Recognition by Boronolectins, Small Molecules, and Lectins

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Abstract

Carbohydrates are known to mediate a large number of biological and pathological events. Small and macromolecules capable of carbohydrate recognition have great potentials as research tools, diagnostics, vectors for targeted delivery of therapeutic and imaging agents, and therapeutic agents. However, this potential is far from being realized. One key issue is the difficulty in the development of “binders” capable of specific recognition of carbohydrates of biological relevance. This review discusses systematically the general approaches that are available in developing carbohydrate sensors and “binders/receptors,” and their applications. The focus is on discoveries during the last five years.

Keywords

boronic acid; carbohydrate sensing; boronolectin; fluorescent sensor; aptamer; lectin

1. INTRODUCTION

Carbohydrates are known to be involved in a wide range of biological and pathological processes,^{1–8} including cancer metastasis, cell adhesion, cell signaling, embryo development, egg fertilization, protein function regulation, cellular communications, and so on.^{4,7–14} For example, sialyl Lewis X (sLe^X) is known to mediate the metastasis of B16 melanoma cancer;¹⁵ carbohydrate ligand binding to E-selectin mediates the extravasation of cancer cells and organ selectivity of metastasis;¹⁶ sialyl Lewis a (sLe^a) binding to selectin is involved in the extravasation of human colorectal carcinoma cells;¹⁷ endothelial cell adhesion, often mediated by carbohydrate-selectin binding, is correlated with cancer progression;¹⁸ and sLe^a expression on cancer cells is correlated with increased risk of distant hematogenous metastasis,¹⁹ presumably a result of carbohydrate-mediated metastasis. In addition, changes in glycosylation patterns often affect the function of a glycoprotein and are biomarkers for pathological or physiological events. For example, the glycosylation patterns of prostate specific antigen (PSA) from cancer cells in culture²⁰ and prostate cancer patients’ tissue and sera^{21–23} are different from that of normal prostate; human pancreatic RNase 1, a glycoprotein secreted mostly by pancreatic cells, has completely different oligosaccharide chains when produced from pancreatic tumor cells;^{24–27} and deviation from the normal glycosylation pattern on fibrinogen (Fn), a protein critical to blood coagulation, can lead to coagulation disorders.²⁸ In addition, two recent reviews also discuss stem cell surface glycan biomarkers and show that intricate glycan-dependent modulation of signalling molecules such as FGF-2, Wnt, and Notch plays an important role

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in stem cell proliferation and differentiation.^{9,29–32} Table 1 summarizes some additional examples of commonly seen carbohydrate-related biomarkers.

Conceivably, “binders” of carbohydrates of biological importance such as those listed in Table 1 could be used as medicinal agents in the inhibition of pathological events such as metastasis that are mediated by carbohydrate binding, as diagnostic agents for the detection of disease-related glycoproducts, as vectors for targeted delivery of imaging and therapeutic agents, and as research tools in glycomics work. However, up until recently, there has been essentially no effort in the development of carbohydrate “binders” as potential medicinal and imaging agents. Effort in making fluorescent carbohydrate “binders” (fluorescent sensors) has been largely limited to mono- or disaccharides for potential analytical chemistry work including glucose detection^{33–61} with a few exceptions.^{62–65} There had been essentially no activity in developing “binders” for glycoproteins with the ability to differentiate glycoforms until recently when the Wang lab developed a platform approach to select DNA-based aptamers for such applications.⁶⁶ A major issue in this field that is hindering the development of carbohydrate recognition-based therapeutic and diagnostic agents is the difficulty in the design and synthesis of highly specific and tight carbohydrate “binders.” Chemical approaches to this issue will be addressed in detail later in this review. Another key issue that needs to be addressed is the need for improved “communications” between chemists and glycobiologists in addressing carbohydrate recognition problems. The detailed descriptions of the carbohydrate recognition sections below also give a glimpse of the somewhat “silo” approaches in the literature. For example, when one reads the chemical approaches, lectin approaches, and the biological applications discussed in the following sections, they sometimes seem to come from different worlds. A large percentage of publications on carbohydrate sensing and recognition from chemistry labs are mostly focused on the chemistry issues and not directly addressing important glycobiological problems. Of course, such a situation has its historical reasons. For a long time, the chemistry knowledge and platform technologies were not there to apply chemical approaches to address complex glycobiological problems through carbohydrate recognition. However, efforts in recent years have shown great promise for the future development of a large number of carbohydrate binders for various therapeutic, diagnostic, and detection applications. The carbohydrate recognition field is poised to make significant inroads in the biomedical application direction if the significance of such research is widely recognized. In doing so, the chemical and biological fields can and should work together and help each other in addressing some of the most fundamental unanswered questions in carbohydrate recognition and in applying new chemistry tools to solve glycobiology problems. One of the aims of this review is to present carbohydrate recognition studies from different angles, in one place, to facilitate communications among glyco-researchers in different disciplines. The examples will mostly be those published in the last five years. It is hoped that this review will stimulate more research and ideas in taking carbohydrate recognition research to the direction of addressing complex glycobiological and disease problems.

Available carbohydrate “binders” can be classified into the following four categories: (1) antibodies,^{134–135} (2) lectins,^{6,136} (3) aptamers based on nucleic acids,^{137,138} and (4) small molecule lectin mimics.³⁶ Of course, antibodies and derivatives have long been the gold standard in the recognition of a variety of analytes including carbohydrates.^{68–70,75,79,109–115,116,139} However, in research, antibodies have not been widely used in large scale applications such as in arrays. Presumably, cost and stability are factors. Currently, lectins are the major available tools in research for carbohydrate recognition. However, the available lectins often have cross reactivity issues. Therefore, there is a need to develop alternatives for carbohydrate recognition that meet the following criteria: (1) high affinity, (2) high specificity, and (3) suitable for high throughput analysis. The high affinity and specificity points are easy to understand. The need for high throughput stems from the

recent interests in studying carbohydrate/glycan changes at the glycome level.^{67,140–148} To meet these criteria there have been a great deal of recent interest in developing fluorescent sensing methods for carbohydrates for various applications.^{34,149–155} In this regard, there have been very active efforts in developing small molecules and “polymeric” “binders” for carbohydrates. Along this line, there have been work in using the boronic acid moiety as the key recognition unit for carbohydrate sensor development.^{34,149–155} We have termed these boronic acid-based carbohydrate sensors/binders as boronolectins because they mimic the function of lectins and contain the boronic acid unit.³⁶ Therefore, there are small molecule boronolectins (SBL),^{33,155,156} peptidoboronolectins (PBL),^{44,157–159} and nucleic acid-based boronolectins (NBL).^{66,137} There has also been effort in taking advantage of non-covalent interactions such as hydrophobic, hydrogen bond, and ionic interactions,¹⁶⁰ and interactions with metals for the design of carbohydrate receptors.^{34,82,161} Sporadic efforts have also been reported in using aptamers as way to achieve carbohydrate recognition and sensing.^{162,163} Recently, the Wang lab has developed a platform method for the selection of DNA aptamers for glycoproteins with the ability to differentiate glycosylation variations.⁶⁶ This review will discuss progress and future prospects of all three areas along with the current state of using lectins for carbohydrate profiling and analysis. Ways to develop antibodies against carbohydrates, though important, will not be covered in this review for two reasons. First, antibody production is a mature method, which does not face the same kind of challenges and issues as the development of small molecule binders and applications of lectins. Second, though there are many commercially available carbohydrate antibodies, they are not described in the kind of mechanistic details with K_d and selectivity information to allow for careful contrast and analysis.

It should be noted that in this review, we do not strive to be comprehensive. Instead, we focus on describing important concepts and approaches, mostly using examples published in the last 5 years. Almost every week there are new publications in areas covered by this review. In order to finish this review in a timely fashion, we had to have an artificial cutoff point of early 2008. Therefore, we ask the understanding and forgiveness of our colleagues and friends in this field who may feel that insufficient weight might have been given to their publications. At last, we should also note that the labs of Wong,¹⁶⁴ Bertozzi,¹⁶⁵ Kiessling,¹⁶⁶ and others have made very important contributions to the field of using chemical approaches to solving glycobiological problems. However such work is beyond the scope of this review, which is focused primarily on carbohydrate “binders.”

2. BORONIC ACID-BASED SENSORS

Boronic acids have a tendency to react with diols and single hydroxyl groups, and therefore, are the most commonly used moiety for the construction of binders for carbohydrates, which contain many hydroxyl groups. As a prologue to this section, we would like to first note that thus far essentially all boronic acid-based carbohydrate sensing studies were done with arylboronic acids, which sometimes have water solubility and stability problems. α -Amidoboronic acids form an important class of boronic acids with high stability, high water solubility, and known affinity for diols and hydroxyl groups.^{167–171} They could potentially be very useful in carbohydrate sensor design. However, there has never been a systematic effort to study their binding with diols in a quantitative fashion. Aimed at achieving some fundamental understanding of amidoboronic acid-diol interactions, the Wang lab synthesized and studied the binding of a model α -amidoboronic acid (Figure 1) with various carbohydrates and other diol-containing.¹⁷² As expected, this compound showed good water-solubility, comparable affinity for carbohydrates as arylboronic acids, and significant fluorescent property changes upon carbohydrate binding.

2-A. Basic chemistry issues

Boronic acids have been known to form tight and reversible complexes with 1,2- and 1,3-substituted Lewis base donors such as hydroxyl, amino and carboxylate groups. The resulting complexes with diols or alcohols are called boronic esters (neutral trigonal boron) or boronate esters (anionic tetrahedral boron) depending on the ionization state of boron atom.^{3·173·175} Boronic acids are also known to interact with simple Lewis bases such as fluoride^{176·179} and cyanide ions.^{180·182} All these properties have made boronic acid an important functional group for sensor development. Indeed, during the last 15 years, there has been a tremendous amount of effort in using the boronic acid group for sensing and recognition of carbohydrates,^{63·64·183·194} α -hydroxyacids,^{42·195·196} α -amino alcohols,^{175·197·198} cyanide,^{180·182} and fluoride.^{176·179·199} There have been several excellent reviews on sensor development up to about 2005.^{33·35·151·156·188} There have also been a review¹⁵⁶ and several in depth studies on the details of the structures of carbohydrate-boronic acid complexes,^{186·200·203} factors that affect the binding equilibrium,^{173·174} and issues to consider when designing boronic acid-based carbohydrate sensors.¹⁵⁶ There have also been excellent reviews on boronic acid reporters that change fluorescent properties upon binding^{156·188} and a review on using boronic acid in biological applications with an emphasis on inhibition of hydrolytic enzymes.¹⁵¹ In addition, B-N bond formation, when positioned in a relative 1,5 relationship, occupies a special place in the boronic acid-based sensor field because of the work of Wulff^{204·205} and Shinkai,¹⁸⁴ and has been used as a way to modulate boronic acid fluorescence. The Wang lab has studied in detail how the B-N interactions could change its nature depending on carbohydrate complexation.^{206·207} Such results are further studied and supported by structural work from the Anslyn lab.²⁰⁸ All such results are detailed in the relevant reviews and extensive research papers and will not be repeated here. Instead, this review will only briefly describe the salient features of the boronic acid-diol complexation reaction, which is directly relevant to carbohydrate recognition, and then focus on new developments in carbohydrate recognitions during the last 2–3 years.

Boronic acid (**1**) is called an acid because of its boron open shell, which allows for reaction with a protic solvent molecule such as water to form an anionic tetrahedral boronate (**2**) with the release of one proton (Scheme 1). The deprotonation of the boronic acid hydroxyl group has a higher pK_a than the boron open shell reaction with water or alcohol and is actually very hard after anion formation. Therefore, the acidity of a boronic acid is derived from its Lewis acidity *per se*. In this context, a boronic acid can react with a variety of Lewis bases such as hydroxyl, sulfhydryl, and amino groups as well as fluoride and cyanide. After reacting with a diol, boronic acid is converted to boronic ester (**3**), which is also an acid much the same way as a boronic acid—it can react with a water molecule, release a proton, and give the boronate ester species (**4**) (Scheme 1). It is important to note that binding with a diol actually lowers the pK_a of the boron atom in most cases.^{173·174}

Not all boronic acids bind with the diol moiety with the same affinity and not all diols bind to a boronic acid with the same affinity. Understanding the intrinsic preference in binding is very important to the design of boronic acid-based carbohydrate “binders.” Though not always true, boronic acids with low pK_a values tend to have high intrinsic affinities.^{3·156·173·174} For the diol portion, several factors could affect their intrinsic affinities for boronic acids. Low pK_a values, small O-C-C-O dihedral angles, and restricted rotations around the C-C bond of the diol moiety all favor binding.¹⁷⁴ It is commonly believed that high pH favors boronic acid binding to diols. This is actually not always true. Optimal binding depends on the interplay of the pK_a values of the boronic acid and diol, and solution pH.^{156·174} The optimal pH for binding is often between the pK_a values of the boronic acid and diol compounds. With low pK_a diols such as catechols, the optimal binding pH could be below physiological pH.^{156·174} Other factors such as solvent, buffer, and steric hindrance

should also be considered when examining the binding between a diol and a boronic acid.
156·173·174

There are two ways of looking at the binding between a boronic acid and a diol. The first way is to look at individual reactions and binding between either the trigonal boronic acid (**1**, Scheme 1), or the tetrahedral boronate (**2**, Scheme 1) with a diol. In such a representation, K_{trig} represents the binding constant between the trigonal form **1** of boronic acid and the boronic ester **3**; K_{tet} describes the equilibrium between the tetrahedral form **2** of boronic acid and boronate ester **4**. The binding equilibrium between phenylboronic acid and a diol can also be presented in Scheme 2, which describes the overall binding equilibrium between boronic acid/boronate **1**, **2** and boronic and boronate esters **3**, **4**.¹⁵⁶ K_{eq} is used to describe the overall binding constant (Scheme 2), which of course is an apparent binding constant, but is directly relevant to the equilibrium between a sensor and a carbohydrate. In the literature, binding constants commonly refer to the apparent overall binding constant, K_{eq} , which does not take into consideration of the ionization states of either the complexed or the free form of a boronic acid.^{173·174}

There are several methods for determining binding constants between boronic acids and diols including the pH depression method,³ B-NMR method,^{209–211} and spectroscopic methods.^{156·183} The pH depression method is rarely used now because of its requirement for a large amount of sample and the fact it only gives the K_{tet} values, not the overall binding constants.^{156·173} The B-NMR method suffers from similar shortcomings as the pH depression method. Furthermore, isotope effects are often not considered in NMR-based methods, which use deuterated solvents. Therefore, spectroscopic methods are normally the preferred choice whenever possible, especially when dealing with boronic acids that change spectroscopic properties upon diol binding.^{36·155} For those boronic acids that do not change spectroscopic properties upon diol binding and therefore cannot be detected directly by a spectroscopic method, the Wang lab introduced a three-component competition assay by using Alizarin Red S. (ARS) as a fluorescent reporter compound for the sensitive determination of binding constants.^{156·173·212}

From the salient features of the boronic acid-Lewis base (including hydroxyl groups and diols) interactions briefly described above, it is easy to understand why boronic acids are very useful in carbohydrate recognition. However, since monoboronic acids have their intrinsic preference in binding with carbohydrates,^{3·156·173·174} additional functional interactions are needed if the desired selectivity of the boronic acid-based sensors is different from the intrinsic preference of monoboronic acids, which is essentially all the time. The additional interactions can be boronic acid-based in situations of bis-^{63·64·150} or multi-boronic acids²¹³ or the use of other types of interactions such as anionic interactions.^{214·215} Therefore, in developing boronic acid-based carbohydrates sensors, it is important to have the appropriate scaffold and functional group arrangements. Another important issue is the availability of boronic acid fluorescent reporters, which change fluorescent properties upon binding.^{36·155} The following section will discuss recent developments in using boronic acids for carbohydrate detection.

2-B. Boronic acid-based fluorescent reporters

Fluorescence-based detection is among the most sensitive methods. Therefore, in carbohydrate recognition and sensing, using fluorescence as a reporting event has been very popular as well.^{63·64·150·155·178·182·185·206·208·216·240} In designing boronic acid-based fluorescent sensors for carbohydrates, one key requirement is the availability of boronic acids or boronic acid ensembles^{163·192·195·241·244} that change fluorescent properties upon binding to a diol-containing compound. In the following sections, we describe recent developments in this area in detail.

The first class of boronic acid-based fluorescent reporters is the tetrathiafulvaleneanthracenes **5** (Figure 2).¹⁹⁵ In this design, the anthracene unit is the fluorophore. It was said that the fluorescence of the anthracene fluorophore can be quenched by the electron-rich tetrathiafulvalene (TTF) through excited state photoelectron transfer (PET). Addition of a sugar such as fructose could increase the fluorescence intensity of the boronic acid reporter by 5-fold at physiological pH in THF/H₂O (1:1, v/v). The binding constant of **5** with fructose is 115 M⁻¹. The compound has an excitation wavelength of 370 nm and emission wavelength of 419 nm. The mechanism through which this fluorescent intensity change occurs was proposed to involve the competition of PET between the process involving TTF and boronate and the process involving TTF and the anthracene unit. It was reasoned that binding of the boronic acid moiety results in the conversion of the boronic acid unit to the more acidic boronic ester, which is a stronger acid than the starting boronic acid and therefore should be a better electron “sink” to compete for PET. This increased competition for electron transfer from TTF was thought as the reason for the enhanced fluorescence observed. However, one factor that was not considered was that upon conversion of the boronic to its boronic ester form, it would be converted to the tetrahedral anionic boronate ester because of the increased acidity.^{36,173,174} Once it is in the anionic tetrahedral form, the boronate ester can no longer accept electrons in a PET process. More studies are needed to elucidate the mechanism through which the fluorescence intensity changes upon sugar binding. In addition, this compound has poor water solubility and requires lengthy synthesis. Such issues could hinder applications in certain situations.

Also reported from the same group that developed the tetrathiafulvaleneanthracenes were 4-(*N,N*-dimethylamine)benzonitrile (DMABN) derivatives with an appended boronic acid (**6a**) and boronic ester (**6b**).²¹⁸ The DMABN boronic acid (**6a**) showed decreased fluorescent intensity (by over 80%) with the addition of fructose. However, the binding constant is quite high with fructose at 794 M⁻¹.^{36,173,174} One reason could be because the binding constant was determined in a mixed solvent, THF-H₂O (1:1, v/v). Incidentally, the DMABN boronic ester (**6b**) also exhibits absorption and fluorescence spectral changes upon binding with F⁻. These two compounds (**6a**, **6b**) have very limited water solubility with most of the binding studies conducted in THF-H₂O (1:1, v/v). Their excitation (295 nm) and emission wavelengths (393 nm) were also relatively short, which may limit their applications.

Baker and co-workers developed a water-soluble and high quantum yield (ϕ_f 0.453 at pH 7.22) fluorescent carbohydrate reporter (**7**).²⁴⁵ The fluorescent intensity of this 6-morpholinonaphthalene-2-yl boronic acid **7** decreased by 99% with the addition of 100 mM D-fructose (λ_{ex} : 300 nm, λ_{em} : 420 nm, pH = 7.72). The dissociation constants (K_D) for **7** decreased following the order of D-sorbitol (2×10^{-3} M) ≈ D-fructose (3×10^{-3} M) ≫ D-galactose (44×10^{-3} M) > D-glucose (152×10^{-3} M) at pH 7.72.

Lakowicz and co-workers designed, synthesized, and studied spectroscopic properties of boronic acid-based fluorescent sensors (Figure 3), *N*-(o-, m-, p-boronobenzyl)-6-methoxyquinolinium bromide **8** (o-, m-, p-BMQBA) for detection of tear glucose concentrations in contact lens polymers.^{38,246} These compounds showed fluorescent intensity decreases by up to 3 fold upon binding with glucose at 450 nm (λ_{ex} : 345 nm). These probes have the advantage of having good water solubility and high quantum yields (0.5), which is comparable to that of fluorescein. The mechanism through which fluorescent intensity changes was thought to be due to enhanced electrostatic interactions between the quaternary nitrogen and the boron atom upon sugar binding, which convert the boron atom from its neutral trigonal form to the anionic tetrahedral form.

The Wang lab has been working on developing water soluble boronic acid-based fluorescent reporters for a number of years.^{63,151,218,229,243,247,248} Some of them have been

reviewed.³⁶⁻¹⁵⁵ Recent work in the Wang lab includes compounds shown in Figure 4. Among them, six benzo[b]thiophene boronic acid (BTBA) analogs (**9a-f**) (Figure 4) were reported as fluorescent reporters.²⁴⁹ All six compounds showed significant fluorescent property changes upon sugar binding with good water solubility under physiological condition. Among them, 2-BTBA (**9a**), 5-BTBA (**9d**), and 7-BTBA (**9f**) showed fluorescent property changes at 2 wavelengths (λ_{ex} : 274 nm, λ_{em} : 305 nm and 334 nm). On the other hand, 3-BTBA only showed fluorescent intensity changes at a single wavelength (317 nm). 4-BTBA (**9c**) and 6-BTBA (**9e**) were tested as a mixture due to difficulties in separation. This mixture showed a red shift (293 to 303 nm) at one emission wavelength and blue shift (329 to 314 nm) at the other upon sugar binding. Among these reporters, 7-BTBA has the highest binding affinity with sorbitol ($K_a = 4561 \text{ M}^{-1}$) and fructose ($K_a = 1342 \text{ M}^{-1}$). One limitation of this series of reporters is their relatively small fluorescent intensity changes (1 fold) upon sugar binding and the short emission and excitation wavelengths. The fluorescent property changes at two wavelengths on the other hand are an advantage, which allows for ratiometric sensing.

Another type of water-soluble boronic acid fluorescent reporters is dibenzofuran-4-boronic acid (**10**, DBFBA, Figure 4) that changes emission intensities at three wavelengths (301, 318, and 327 nm) upon sugar binding under near physiological conditions.²⁵⁰ The apparent binding constant of this reporter with fructose is 514 M^{-1} . One limitation of this fluorescent reporter is its small fluorescent intensity changes upon sugar binding (less than 1 fold) and short excitation wavelength of 286 nm.

The Wang lab also synthesized and evaluated a series of water-soluble fluorescent naphthalene boronic acid-based carbohydrate-reporters (Figure 4).²²⁹⁻²⁴⁸⁻²⁵¹⁻²⁵² In this series, the substitution pattern seems to have a significant influence on the fluorescent properties of the boronic acid. For example, 5-DMANBA (**11a**) and 5-CMANBA (**11b**) showed ratiometric fluorescence changes upon saccharide binding. For 5-CMANBA (**11b**), the excitation wavelength was at 320 nm and the emission wavelength shifted from 490 nm to 440 nm upon sugar binding. 5-MMANBA (**11c**) and 5-ANBA (**11d**) on the other hand showed dramatic fluorescence increases (by 66–70 folds) at a single wavelength (438 nm) upon binding with fructose. This was accompanied by a quantum yield increase from 0.056 to 0.72 for 5-MMANBA and from 0.041–0.89 for 5-ANBA upon sugar binding. These compounds also showed color changes upon sugar addition.

The naphthalimide scaffold has been of interest in the boronic acid field because of its relatively long excitation and emission wavelengths. The Heagy lab reported the first boronic acid compound using this scaffold with very interesting discoveries.²⁵³⁻²⁵⁴ The Mohr lab also reported a very interesting 4-amino-1, 8-naphthalimide boronic acid **12**, which showed fluorescent property changes upon sugar binding at long wavelength (λ_{ex} : 410 nm, λ_{em} : 530 nm).²⁵⁵ The Wang lab also worked on several long-wavelength boronic acid fluorescence reporter compounds (**13a-c**) based on the 4-amino-1, 8-naphthalimide structure (Figure 4).²⁵⁶ In this series, the *N*-substitution seems to have a significant effect on their fluorescent properties. Among these compounds, **13c** has the best water solubility and showed fluorescent intensity increases by up to 2 fold upon sugar binding with an emission wavelength of 570 nm ($\lambda_{\text{ex}} = 493 \text{ nm}$). However, the *N*-methyl and *N*-benzyl analogs showed poor water solubility that the binding studies had to be conducted in a buffer solution with 50% methanol. Based on the structure of **13c**, the Wang lab also studied substituent effects at the *para*-position of phenylboronic acid moiety. However, surprisingly the different substituents of **13c-f** had little effect on either the binding affinities or fluorescent properties.²⁵⁷

There have also been a number of other fluorescent boronic acid-based carbohydrate reporters, such as **14a–c** (Figure 5) using hemicyanine dyes (termed as fluororeactands by the authors) by Mohr and co-workers.²⁵⁸ In such studies, **14a** showed the most significant fluorescent intensity increase (by 1 fold) upon binding with fructose at long wavelength ($\lambda_{\text{ex}} = 460 \text{ nm}$, $\lambda_{\text{em}} = 600 \text{ nm}$). The binding studies were conducted in phosphate buffer solution at pH 7.13. The binding constant between **14a** and fructose is 280 M^{-1} , which is larger than that of **14b** (40 M^{-1}) and **14c** (200 M^{-1}). These boronic acid fluorescent reporters showed good water solubility and significant fluorescent property changes at long wavelength.

A *p*-dialkylaminobenzanilide-based boronic acid receptors **15** (Figure 5) was developed by Jiang and co-workers.²⁵⁹ Binding studies were conducted in phosphate buffer-methanol (v/v = 1/1) solution at pH 6.7 ($\lambda_{\text{ex}} = 300 \text{ nm}$). Under such conditions, the fluorescent intensities of both **15a** and **15b** at around 380–390 nm decreased by 50% upon binding with D-fructose. The binding constants were in the order of D-fructose > D-galactose > D-glucose. The binding constant of reporter **15a** with fructose is 1550 M^{-1} ($\lambda_{\text{em}} = 392 \text{ nm}$), which is much larger than that of **15b** (389 M^{-1} , $\lambda_{\text{em}} = 382 \text{ nm}$).

ortho-Azo substituted phenylboronic acid-based reporters **17–18** (Figure 5) were reported by Egawa and co-workers.²⁶⁰ The UV absorbance of **17** decreased by 70% at 502 nm upon fructose addition in a methanol/water solution (v/v = 1/1) at pH 10.0 and the binding constant was 36 M^{-1} . Reporter **18** had a better water solubility and higher binding affinity for sugars than **17**. The UV absorbance of **18** decreased by 80% at 521 nm upon fructose addition in the CHES buffer solution at pH 10.0 and the binding constant was 110 M^{-1} . One concern is that these compounds only exhibited reasonable binding under basic condition. For broad application, it is essential that boronic acids show significant binding under near physiological conditions.

These reporters described should be useful as the basic building blocks for the preparation of fluorescent sensors for sugars. One challenge in this field is the design and synthesis of reporters that have excitation and emission wavelengths beyond 500 nm, low molecular weight, and good stability, and are water soluble and easily functionalizable for the construction of bis- or multi-boronic sensors.

2-C. Small molecule boronic acid sensors for saccharides

Of course, the ultimate goal of developing fluorescent reporter compounds is to use them for the design and synthesis of fluorescent sensors for carbohydrates. In this section, we discuss recent developments in using boronic acids as the key recognition moiety for sensing applications. Again, due to the functional similarity of these boronic acid-based sensors to lectins, we have termed them boronolectins.³⁶

In order to develop a general glucose sensing system in aqueous solution, Singaram and co-workers have used anionic fluorescent dyes in combination with boronic acid-based recognition units.^{40,42} The viologen bisboronic acid system was selected as a chromophore and a recognition unit. Viologen is a commonly used dye, which is considered electron deficient and redox sensitive. The addition of a substituted viologen boronic acid into a fluorescent anionic dye solution allows the formation of a non-fluorescent complex due to stacking and quenching presumably through charge transfer.⁴² Upon binding to a sugar, the boronic acid moieties are converted to the corresponding anionic form, which together with the added bulkiness triggers the dissociation of the non-fluorescent complex and causes a significant fluorescent intensity increase upon binding (Scheme 3). The sensitivity can be adjusted by altering the ratio of reporter dye **19** and the bisboronic acid recognition unit **20**.

As shown in Figure 6, the Singaram lab prepared several bisboronic acid-substituted viologens **20a–c** as optimized fluorescence quenchers. Compare to the other two regioisomers (m- and p-BBV), it was assumed that with ortho-substituted boronic acids (o-BBV) there would be stronger electrostatic interactions between the anionic boronate (after sugar binding) and the positively charged quaternary nitrogen. On the other hand, such interactions would be much weaker with the other regioisomers. It was found that the fluorescent intensity of the complexes between anionic dye 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) trisodium salt (pyranine) and three different boronic acid-substituted benzyl viologens (**20a–c**) (λ_{ex} : 460 nm, λ_{em} : 510 nm) increased by 9–17% upon addition of glucose.²⁶¹ The binding constants of these three quenchers with glucose were from 23 M^{-1} to 37 M^{-1} . When tetrakis(4-sulfophenyl)porphine (TSPP, **21**) (λ_{ex} : 414 nm, λ_{em} : 644 nm) was used as the fluorophore, the fluorescent intensity increased by 33% upon sugar addition with the binding constant being 14 M^{-1} with glucose.^{40,261} The long wavelength of the TSPP system is also an added advantage, which helps to reduce background interference for applications in biological fluids.

Since fluorescent quantum dots (QDs) have broad absorption, narrow emission, intense brightness, and good photostability, they were used in place of organic dyes for testing the viologen system.⁶¹ Two sets of core-shell CdSe QDs (**22**) coated with ZnS were used (Figure 6). These QDs were decorated with carboxy and amine groups on the surface.⁶¹ These QD-viologen ensembles showed fairly narrow fluorescence emission centered at 604 nm ($\lambda_{\text{ex}} = 460 \text{ nm}$) and about 1 fold fluorescent intensity increase upon glucose binding. It was said that concentration dependent fluorescent changes were observed in the range of 2.5 to 20 mM of glucose for QDs ($5 \times 10^{-8} \text{ M}$) in their system.

The viologen ensemble systems have the advantage of modular nature with the ability to tune the wavelength by using different fluorophores and being water soluble. It was said that work was on-going in making polymerizable analogs of these dyes for use in glucose sensing. If both components can be immobilized onto hydrogel polymers with similar fluorescent properties as in solution, that would address the issue of multiple components, which could be a limitation for *in vivo* applications.

Using a 3-component method similar to that of the alizarin S assay,^{173,174,212} Zhang and co-workers introduced a nitronyl nitroxide fluorescent quencher **23**. In this case, the quencher is the diol component, which can bind to a fluorescent boronic acid **24** and quench its fluorescence through the formation of complex **25** with an apparent association constant of 2410 M^{-1} (Scheme 4). Addition of a sugar could competitively release the nitronyl nitroxide fluorescent quencher and result in a fluorescent intensity increase.²⁶² In this specific case, an anthracene-based fluorescent boronic acid **24** was used, which has an excitation wavelength of 370 nm and emission wavelength of 419 nm. A maximum of 10 fold fluorescent intensity changes were observed upon sugar addition. In theory, it is possible to use other fluorophores as long as their fluorescence can still be quenched. All studies were conducted in a mixed solvent THF/H₂O (1/1, v:v), which indicate possible water solubility problems.

Anslyn and co-workers have synthesized a cadmium-centered tris-boronic acid receptor **26** (Scheme 5) and determined its binding properties toward various carboxyl and phosphorylate sugars using an indicator displacement assay. In this assay, addition of anionic sugars would change the color of the pyrocatechol violet (PV) indicator in the system.²⁶³ This receptor showed varying degrees of affinities for different anionic sugars in protic media (methanol/water = 3/1, HEPES buffer 50 mM, pH = 7.4). Among all the sugars tested, gluconic acid showed reasonably good binding with an association constant around 107 M^{-1} .

A bisboronic acid fluorescent sorbitol sensor based on the anthracene fluorophore was synthesized and studied by Yoon and co-workers (Figure 7).²⁶⁴ All fluorescent studies were conducted in 50% MeOH/0.1 M aqueous phosphate buffer at pH 7.4 and by using a 6 μ M concentration of compound **27** ($\lambda_{\text{ex}} = 365$ nm, $\lambda_{\text{em}} = 420$ nm). The binding constants of chemosensor **27** with sorbitol, xylitol, fructose, galactose and glucose were 1060, 440, 200, 81 and 12 M^{-1} with fluorescent intensity increases by 2.2, 1.9, 3.9, 2.9 and 2.3 fold, respectively. Such results clearly show that the designed sensor prefers sorbitol, though monoboronic acid also preferentially binds to sorbitol with an over 2-fold selectivity over fructose.^{173,174,212,264}

Based on earlier work,^{63,150} Wang and co-workers synthesized and evaluated additional anthracene-based bisboronic acid sensors for various saccharides.^{231,265} As shown in Figure 8, a series of new anthracene-based bisboronic fluorescent sensors (**28a–d**) and three fluorescent monoboronic as controls (**29b–c**) were synthesized.^{63,64,156,231} These sensors use the anthracene-based fluorophore (**29a**) first developed by Shinkai and co-workers.^{33,266} Compound **28a** was published earlier as a selective glucose sensor.¹⁵⁰ These new compounds (**28b–d**) were designed to examine substituent effect on binding and selectivity. Both cyano- (**28b**) and nitro-substituted (**28c**) sensors had higher apparent binding constants for glucose ($K_a = 2540$ and 1808 M^{-1} , respectively) than the un-substituted sensor (**28a**) (1472 M^{-1}). Although the fluoro-substituted bisfluoroboronic acid (**28d**) had a lower apparent binding constant ($K_a = 630 \text{ M}^{-1}$), it has the best selectivity for glucose under near physiological conditions. Compared to **28a**, the selectivity for glucose over fructose did decrease for all the new sensors (**28b–c**) with the $K_{a\text{-glucose}}/K_{a\text{-fructose}}$ ratio changing from 43 to 3. The binding affinity trend for the bisboronic acids (**28a–c**) parallels that of the monoboronic acid building blocks (**29b–c**). Such results suggest that the affinity of the bisboronic acids (**29b,c**) for glucose was largely correlated with the intrinsic affinity of the boronic acid moiety. Moreover, the introduction of an electron-withdrawing group does not always result in enhanced affinity. Hence, the effect of the electron-withdrawing group on the selectivity and affinity of the bisboronic acid sensors for glucose is hard to predict. In this series, **28b** showed the best binding affinity and the highest fluorescent intensity increase (10 fold) upon binding with glucose. Sensor **28a** showed the best selectivity of glucose. This series of compounds have an excitation wavelength of 370 nm and emission wavelength of 425 to 430 nm. One issue with these anthracene-based bisboronic acid sensors is their lack of water solubility and the need for organic co-solvent in binding studies. In addition, the fluorescence intensity of the anthracene fluorophore seems to be very sensitive to environmental changes and careful controls have to be done under the same conditions in order for results to be comparable. Temperature, oxygen content, and solvents all seem to significantly affect the fluorescence intensity of these sensors.

Stereochemical and regiochemical factors play important roles in the selective detection of saccharides with boronic acid-based sensors.^{44,189,267,268} Several research groups have been working on examining the stereochemical issues in binding. Strongin and co-workers reported several stereoselective and regioselective boronic acid chemosensors (Figure 9 and 10) such as **30**, **31** and **32**.¹⁸⁹ One example is the xanthene dye-based chemosensor **30**, which fluoresces at a long wavelength, 597 nm ($\lambda_{\text{ex}}: 565$ nm), and showed good selectivity for ribose ($K_a = 2400 \text{ M}^{-1}$) comparing to fructose (110 M^{-1}), galactose (310 M^{-1}), and glucose (200 M^{-1}). The fluorescent intensity of **31** increased by 10 fold upon binding with ribose and showed a binding constant of 2400 M^{-1} . Based on stereochemistry studies, the authors proposed that the selectivity of ribose was due to the 2, 3-cis diol structure, and also due to the formation a hydrogen bond between the hydroxyl group of the boronate ester and hydrogen from the amine of **30** (Figure 9, complex **33**). Carbohydrates, which can share the same hydroxyl configurations (2, 3-cis diol) as ribofuranose such as allose, psicose, adenosine and nucleotide, may behave similarly as ribose. This is the first example of a

boronic acid-based chemosensor for ribose with such significant selectivity. In addition, the solution of **30** (0.07 mM) containing **32** (1.9 mM) was observed to generate micromolar level of chromophore **31** *in situ*, which gave spectroscopic property changes. Furthermore, sensor **32** (1×10^{-3} M) changed fluorescent intensity by 1 fold upon binding with lactulose and maltulose, comparing to other di- and trisaccharides (1.85×10^{-3} M), such as lactose, maltose, raffinose, and sucrose, which show no fluorescent intensity changes. Their systems possess good sensitivity and selectivity for the target saccharides. Studies were conducted in 9:1 DMSO/phosphate buffer (0.05 M, pH 7.4), which suggests that water solubility may need further improvement.

The receptor **34** has significant enantioselectivity of sugar alcohols, presumably due to the rigidity of the linker and the crowded binding pockets.²⁶⁸ It also shows very high chemoselectivity for sugar alcohols with six hydroxyl group over those with five or four hydroxyl groups. It can detect monosaccharides at sub-millimolar concentrations. The sensor **34** had enantioselectivity (K_R/K_S) of 1:20000 in binding with D-mannitol (0.05 M NaCl buffer, 52% CH₃OH by weight, pH 8.3). The binding also yielded fluorescence enhancement by 1 fold for the R-sensor (**R-34**) and 7 fold for the S-sensor (**S-34**) ($\lambda_{ex} = 373$ nm, $\lambda_{em} = 421$ nm). Similar to most anthracene-based sensors, water solubility is an issue for biological applications. The same thing is true concerning the chemical and spectroscopic stability of anthracene-based compounds.

During the past decades, the James lab has made very significant contributions towards the boronic acid-based sensing field. They have also been very active in the design and synthesis systems that show stereoselectivity and saccharide selectivity in recognition.^{45,217,269–271} The most recent one uses a chiral fluorescent BINOL boronic acid.²⁷² Compared with previous ones, this sensor shows improved enantioselectivity as well as chemoselectivity toward sugar alcohols, such as D-sorbitol and D-mannitol. The enantioselectivity of this sensor toward D-sorbitol (K_R/K_S) is 1:35 (pH 9.0), and the chemoselectivity for D-sorbitol/D-mannitol is 20:1.

In conclusion, there has been a great deal of interests in making small molecule boronolectins for various applications. With the rapid development of the glycomics field, we expect to see increased activities in this area.

2-D. Sensors for carbohydrates using oligomeric backbones

One of the most challenging tasks in designing carbohydrate sensors is the construction of the 3-dimensional scaffold with the spacing and orientation of the boronic acid units complementary to that of the diols on a carbohydrate. Taking the *de novo* design approach is often very hard because of the difficulties in studying saccharide conformations and in designing scaffolds with precise three-dimensional control. Therefore, there have been interests in using peptides or other oligomers as the basic backbone for making boronolectins. These oligomers not only can serve as structural scaffolds, but also provide certain functional groups for complementary interactions. In addition, using oligomers also gives the opportunities for performing combinatorial work. In the following section, we highlight some of the recent developments in this area.

In the first example, Anslyn and co-workers prepared peptide-based boronic acid receptors (**35**) for pattern-based carbohydrate sensing (Figure 11). In this work, bromopyrogallol red **36** (BPR) was used as an indicator for saccharide binding.¹⁵⁹ It should be noted that this type of indicator-based assay ensemble also originated from the Anslyn lab. The binding response signal was recorded by a CCD camera. Linear discriminant analysis (LDA) was used and discrimination between monosaccharides and disaccharide and within various saccharide groups was achieved. This LDA data set could be used for identifying sucralose

in a real world beverage sample as well. Their chemosensor assay system has the advantage of good water solubility and high sensitivity. This method was one of the first assays where supramolecular pattern-based sensors were used to identify a specific target in a complex beverage.

The Kubik lab synthesized and examined the binding affinities of carbohydrate sensor **37** (Figure 12), which contains two boronic acid moieties on the opposite ends of a cyclic tetrapeptide.⁴⁴ Fluorescent binding studies showed good enantioselectivity of sensor **37** for D-glucose ($K_a = 24800 \pm 1200 \text{ M}^{-1}$) to L-glucose ($K_a = 11900 \pm 1600 \text{ M}^{-1}$) in water/methanol (1:1, pH = 11.7). From mass spectrometric studies using electrospray ionization (ESI), it seems that this sensor forms significantly more stable 1:1 complexes with D- and L-glucose than with D-galactose, D-mannose, and D-allose. The fluorescent intensity of **37** decreased by half with glucose binding ($\lambda_{\text{ex}}: 285 \text{ nm}$, $\lambda_{\text{em}}: 480 \text{ nm}$). Though this sensor can sense glucose with high binding affinity, significant fluorescence intensity changes, and good selectivity and enantioselectivity, it does have the disadvantage of being functional only at high pH and requiring an organic solvent for solubilization.

In this section focused on oligomeric boronic acids, it is important to mention the work from the Hall lab on developing a general solid-phase approach to the synthesis and isolation of functionalized boronic acids, which should be very useful in combinatorial library synthesis for boronic acid-based carbohydrate sensors.²⁷³ As shown in Scheme 6, *N,N*-diethanolaminomethyl polystyrene (DEAM-PS, **38**) can be used for the immobilization of boronic acids. These DEAM-PS-supported arylboronic acids can be used in resin-to-resin transfer reactions (RRTR) or for further reactions. Along a similar line, the Hall lab has established a prototypic bead-supported split-pool library of triamine-derived triboronic acid receptors (Figure 13).²⁷⁴ Well controlled synthesis, bead decoding by HPLC, and preliminary screening of a combinatorial library of 289 triamine-derived triboronic acid receptors for oligosaccharides were accomplished. The peptide-based boronolectins from single beads were decoded from the library and detected by using electrospray mass spectrometry.

Other peptide-based boronolectins have also been reported. The Lavigne lab has recently reported their peptide-based boronolectins for glycomics and cancer diagnostics.¹⁵⁸ In this study, novel peptide boronolectins (PBLs) were synthesized on beads and used to probe glycoproteins and oligosaccharides with fluorescent labels. A “low” diversity 12-mer PBL library was synthesized on aminomethyl PEG PS resin (100 mm) using a biased split-and-pool combinatorial approach. The theoretical diversity of the PBL library is in the order of 10 million distinct peptide sequences containing a statistical average of 4 boronic acid moieties per peptide. Fluorescent microscope imaging of the beads after mixing with various fluorescently labeled glyco-products allowed for the examination of binding. Though no specific PBL was isolated and examined for binding specificity, binding patterns using microplates allowed the establishment that binding was PBL- and saccharide-dependent (meaning that there was selectivity among the different PBLs in the pool). This approach has the potential for application in pattern recognition and development of diagnostic approaches for multiple glycobiomarkers. Future work was proposed to involve small molecule dye competition assay to make this method amenable for biology tests.

Duggan and co-workers also prepared solid-supported peptide boronic acids derived from 4-borono-L-phenylalanine and studied their affinity for alizarin.¹⁵⁷ *N*-Fmoc-4-pinacolatoborono-L-phenylalanine and standard solid phase peptide synthesis methods were used in the preparation of a library of solid-supported pentapeptide-based bisboronic acids including a ‘lysine series’ and an ‘arginine series’. Twelve diboronic acid sequences that have boronic acid moieties at different positions were obtained at 71–90% yield. Six of them

contained two lysine residues for each sequence, while the other six sequences contained two arginine residues for each one. The authors developed a technique for measuring the affinity between a chromophoric diol, alizarin, and the solid-supported peptide-based boronolectins. Binding studies were conducted by measuring absorbance at 507 nm in 50% methanol/50 mM sodium carbonate aqueous solution at pH 10.7. The concentration range of alizarin explored was from 0 to 12 mM. Significant variations in alizarin binding strengths, both within and between arginine and lysine series were observed, with binding constants in the range 200–1100 M⁻¹. The binding affinities of arginine series were 35% stronger than that of the lysine series on average. The binding properties of these peptide-based boronic acids imply their potential application in carbohydrate sensing.

2-E. Other boronic acid-based sugar recognition systems

Though boronic acids are known to bind diol-containing compounds, they have different intrinsic preference for diols of different structures.^{3,173,174} As described earlier, diols with small dihedral angles and low pK_a values tend to have a higher intrinsic affinity for boronic acids. Along this line, it is well-recognized that boronic acids bind to diols on aryl and five-membered rings much more tightly than that on six-membered rings. With bisboronic acids, proper design allows for the construction of sensors that can recognize six-membered ring diols with high affinity.^{208,275} However, with monoboronic acids, the situation is different. Often the binding of a boronic acid with a diol on a six-membered ring is so weak that it is hard to measure a binding constant under near physiological conditions. The only exception is pinanediol,^{167,276} which is often used as a boronic acid protecting group. Even with bisboronic acid, sometimes the binding would start with the recognition of the six-membered ring form and then slowly rearrange to form the complex with a 5-membered ring diol.^{150,186,200} The preference by boronic acids for diols on five-membered rings is especially problematic for the recognition of saccharides released from or as part of a glycoprotein/peptide or glycolipid. This is because these glycans tend to have only linear diols and diols on six-membered rings. Because there have been considerable interests in sensors for many types of glycoproteins,^{62,63,150,277,278} including glycated hemoglobin^{160,279,280} and immunoglobulin G,²⁸¹ there has also been a high level of interest in finding boronic acids that have enhanced ability to recognize diols on a six-membered ring. Along this line, the Hall lab has recently reported an ortho-hydroxymethyl phenylboronic acid (**39a**, Figure 14),¹⁹⁰ which can recognize 1,3-diols on a six-membered ring. The design takes advantage of the known effect of a similarly positioned amino group (**39b**) reported by Wulff,²⁰⁴ which helps to improve the intrinsic binding affinity of the boronic acid unit. In neutral aqueous media, this boronic acid **39c** could complex hexopyranosides primarily using their 4,6-diol, which is presented on most cell-surface glycoconjugates (Scheme 7). The binding constants of **39a** with glycopyranosides were obtained by using the ARS UV assay at neutral pH (7.4) in water.^{173,212} The K_a with methyl α-D-glucopyranoside was 22 M⁻¹, which was slightly lower than the binding constant with glucose. In contrast, the binding constant of phenylboronic acid with glucose is about 5 M⁻¹ at physiological pH.^{173,212} In addition, the Hall compound also has good water solubility and is structurally simple. One can envision a wide variety of applications of this very special boronic acid.

Using the same system (ortho-hydroxymethyl substituted phenylboronic acids) developed by the Hall lab, Hindsgaul and co-workers have developed a method for visual analysis of the terminal glycosylation of glycoproteins.²⁸² The key point of this assay is the application of a boronic acid-based dye reagent, tetramethylrhodamine-boronic acid **40** (TMR-B, λ_{em}: 579 nm), as shown in Figure 15 and Scheme 8. In this method, galactose (or other sugars) can be released from a glycoprotein using an enzyme. The released galactose then can be reacted with an immobilized amine. Subsequent reaction with TMR-B through boronic acid-diol

complexation then allows for labeling and visualization of the beads based on the presence of the immobilized sugar. The bound TMR-B can also be released by adding a solution of glycerol/MeOH/H₂O (1:2:2) to the beads for solution quantitation since the amount of released fluorescent boronate should be proportional to that of immobilized sugar. Using this method, several sugars including galactose (Gal, a hexose), fucose (Fuc, a deoxyhexose), sialic acid (*N*-acetylneurameric acid, Neu5Ac), and *N*-acetylglucosamine (GlcNAc, an aminodeoxyhexose) were analyzed by following the capture/dye-binding/washing sequence for each sugar at 40 μM. The results showed that the relative responses of Gal/Fuc/Neu5Ac/GlcNAc were 1: 0.67: 0.59: 0.36.

There have been many other applications of boronic acid-based sensors for glycoproteins combined with chromatography,²⁸³ polymers,²⁸⁴⁻²⁸⁵ and electrochemistry, which will be discussed in the later sections.^{279-286,287}

2-F. Boronic acid-based polymeric and electrochemical sensors

There have been many studies of polymer-based arylboronic acid sensors for saccharides.⁴⁶ For instance, as shown in Scheme 9, Tao and co-workers invented a new fluorometric glucose detection strategy by utilizing complexation with boronic acid (**41,42**) on polycation (**43**). The formation of a 2:1 complex between compound **41** and glucose (Scheme 9) was the general design for this method. In their studies, boronic acid **42** was used for the fluorescent binding evaluation, and polycation **43** was used to enrich **42** along its polymer chain by electrostatic interaction. The fluorescent boronic acid **42** emits at 376 nm as a monomer. After binding with glucose to form a 2:1 complex, two pyrenyl groups from two molecules of **42** along with **43** would stack and generate an excimer. The polymer chain changes its conformation due to a change of the charge state of boron (from the trigonal form to the tetrahedral form) induced by the 2:1 binding with glucose. This conformational change triggers a change of fluorescent intensity, which can be used to monitoring the binding process. Other sugars such as fructose and galactose did not show such a strong tendency to form a 1:2 complex with a boronic acid as did glucose. Therefore this method could be used for sensing glucose selectively. Complexation results in the generation of excimer emission in the range of 430–600 nm (λ_{ex} : 342 nm).⁴⁶ The fluorescent studies were conducted in aqueous solution containing 10 mM glucose buffered with 10 mM of NH₃ (pH = 10) in the presence of **43** (310 mM). The fluorescent intensity ratio of 482 nm to 376 nm (I₄₈₂/I₃₇₆) was increased by 10 fold with glucose binding. On the other hand, the increase of I₄₈₂/I₃₇₆ with fructose, galactose and ribose was less than 2 fold. The limit of this system is that it functions only under alkaline conditions, which could be improved by lowering the pK_a of the boronic acid through the introduction of electron-withdrawing groups.

Schrader and co-workers developed a fluorescent boronic acid-based polymeric sensor **44** for heparin, a sulfated polysaccharide (Figure 16).²¹⁶ For this sensor, boronic acid and ammonium ion moieties were used for binding, the dansyl group was used as a fluorophore. The ratio of these three moieties in the sensor was 1.0:2.0:0.3. The fluorescent intensity of polymer **44** decreased by 25% at 510 nm upon heparin addition (30–220 nM) in 25 mM HEPES buffer. Sensor **44** was selective for heparin binding (K_a , $3 \times 10^7 \text{ M}^{-1}$) over other glycans and proteins such as dextran (K_a , $3 \times 10^3 \text{ M}^{-1}$), hyaluronic acid (K_a , $2 \times 10^3 \text{ M}^{-1}$), chondroitin (K_a , $4 \times 10^6 \text{ M}^{-1}$), and ovalbumine (K_a , $1 \times 10^6 \text{ M}^{-1}$). This polymeric sensor had good water-solubility, sensitivity, selectivity, and emitted at long wavelength as well. One limitation for this sensor was the small fluorescent property changes upon sugar binding.

Sumerlin and co-workers prepared a block copolymer (**45** or **46**) containing boronic acid and acrylamido fragments via atom transfer radical polymerization (ATRP) (Scheme 10). In their studies, 4-pinacolatoborylstyrene (pBSt) (**47**) was polymerized with 2-

dodecylsulfanylthiocarbonylsulfanyl-2-methylpropionic acid (**48**) as the chain transfer agent (CTA) and 2, 2'-azobisisobutyronitrile (AIBN) as the initiator. Different ratios of monomer/ CTA/initiator were studied. The best ratio was determined to be 250/1/0.4, which gave 99% conversion to poly(4-pinacolatoborylstyrene)- β -poly(N,N-dimethylacrylamide) (PpBSt-b-PDMA, **49**) with a M_n of 60800 g/mol. This method should be very useful for synthesis of water-soluble boronic acid-containing polymers. Their stated future studies were to focus on the responsive properties of the specific functional boronic acid block copolymers, such as spectroscopic property changes upon binding with sugars.²⁸⁸

There have been a number of studies about supramolecular and polymeric structures, which contain the boronic acid moiety. Such examples include, the synthesis of supramolecular boron complexes with different functional groups and their molecular recognition by Hiratani and co-workers,²⁸⁹ a sugar sensing method based on saccharide-induced conformational changes in copolymers containing boronic acid and a fluorophore by Tao and co-workers,²⁹⁰ a new thermo-sensitive fluorescent strategy by Elmas and coworkers for diol sensing using temperature sensitive copolymer with boronic acid incorporation and alizarin red S as an indicator,²⁹¹ fluorescent D-glucose sensors based on supramolecular complexes formed between phenylboronic acid modified beta-cyclodextrin and styrylpyridinium dyes (CISP) by Suzuki and co-workers,²⁹² and using boronate-containing copolymers (BCC) of *N*-acryloyl-m-aminophenylboronic acid (NAAPBA) with *N,N*-dimethylacrylamide (DMAA) or *N*-isopropylacrylamide (NIPAM) for interaction with sugars and yeast cells.²⁹³ In addition, a pH dependent equilibrium mechanism of poly(anilineboronic acid)-sugar complex formation in polymer films was examined by Freund and co-workers.²⁹⁴

Since the structure and charge state of the boronic acid moiety could change upon binding with diols, which may induce potential energy changes for the system, there have been boronic acid-based carbohydrate sensors that rely on electrochemical methods for detection. For instance, Yang and co-workers prepared poly(aminophenylboronic acid) (PABA) on gold electrode surface, which showed changes in dielectric characteristics with sugar concentration variations.²⁴⁷ In their studies, electrochemical impedance spectroscopy was used. For the binding studies, 20 mM PBS buffer (pH 7.0) containing 0.1 M NaF was used as the electrolyte solution. Different kinds of saccharides were examined and good linear relationship and high sensitivity (10^{-9} to 10^{-2} M) were observed. However, the selectivity was not very high for the system.

Niwa and co-workers used ferrocenylboronic acid and an enzyme-modified electrode for electrochemically recognition of lipopolysaccharides (LPS).²⁹⁵ The gold electrode was modified by a bovine serum albumin membrane with diaphorase. On this electrode, ferrocenylboronic acid derivatives were oxidized and then regenerated by a diaphorase-catalyzed reaction in the presence of NADH. The cycle of consumption and regeneration of ferrocenylboronic acid derivatives induced a current response, which decreased by 225–425 nA/cm² with the binding of boronic acid and LPS. This current decrease was also amplified by the recycling step. Comparing to LPS, the binding of a monosaccharide such as D-mannose or D-galactose caused no response at the same concentration (1 μ g/mL). Furthermore, this electrode showed a rapid response time for LPS. It is faster than the currently used method. The detection limit of LPS from *E. coli* O127:B38 was as low as 50 ng/mL.

Anzai and co-workers reported a polyphenylboronic acid-modified gold electrode for carbohydrate detection.²⁹⁶ The surface of the gold electrode was coated with a thin film containing poly(allylamine) with the phenylboronic acid (PBA) moiety. The modified gold electrode exhibited a concentration-dependent current decrease with added sugars in the

presence of $[Fe(CN)_6]^{3-}$ ion at neutral pH. The detection range of the electrode was 1–100 mM for glucose and 0.1–10 mM for fructose.

2-G. Other arylboronic acid-based sensors

There have been quite a few other arylboronic acid-based carbohydrate sensors. For example, Koh and co-workers designed and synthesized phenylboronic acid-based self-assembled monolayers (SAMs) on Au surface for monosaccharide sensing (Figure 17).²⁹⁷ They also characterized the surface properties of the SAMs by atomic force microscopy (AFM). Sugar binding was examined by using surface plasmon resonance (SPR) spectroscopy with good sensitivity (SPR angle shift was 0.133°) at very low concentration of saccharide (1.0×10^{-12} M to 1.0×10^{-4} M). For this study, LED was used as a light source to generate light signal at a maximal wavelength of 650 nm. When the alkyl spacer length of the phenylboronic acid derivatives was at $n = 3$, the SPR angle shift derived from binding between phenylboronic acid and monosaccharide was amplified by 10^7 times because of the optimal order of the monolayer structure. However, the selectivity of this system is the same as other monoboronic acids in their binding with various diols. Some other boronic acid-based sensors involving self-assembly have also been reported.^{287,298–303}

Li and co-workers reported a glucose-responsive vesicular sensor based on boronic acid–glucose recognition in co-vesicles (Scheme 11).²³⁷ The amphiphile, 4-(4-dodecyloxybiphenyl-4-yloxy) butyl trimethylammonium bromide **50** (DBBTAB) is a quaternary ammonium salt, which can form vesicles at low concentrations in aqueous media.²³⁷ DBBTAB vesicles have positively charged surface, which attracts negatively charged alizarin red S (ARS) to form co-vesicles. Since ARS has been used as a fluorescent reporter for binding between phenylboronic acid (PBA) and sugar,^{173,212} the authors prepared a vesicular fluorescent sensor based on phenylboronic acid (PBA)–glucose recognition in the ARS/PBA/DBBTAB co-vesicles. Fluorescence studies of the ARS/PBA/DBBTAB vesicles were conducted in an aqueous phosphate buffer at pH 6.8 ([ARS] = 5×10^{-5} M; $\lambda_{ex} = 468$ nm, $\lambda_{em} = 560$ nm). Fluorescent intensity increased by 200 fold with the addition of PBA ($0\text{--}5 \times 10^{-4}$ M), and then the intensity decreased by 10% with the addition of glucose ($0\text{--}43 \times 10^{-3}$ M). Compared with the aqueous PBA/ARS solution, the vesicular sensor system showed increased sensitivity to glucose by 7 to 8 fold. The selectivity of this system was similar to the PBA/ARS system. Although their system worked well in aqueous solution, the small fluorescent intensity change (a 10% decrease) observed with the addition of glucose might become an issue in real life applications.

Duggan and co-workers developed fructose-permeable liquid membranes containing boronic acid carriers.^{304–306} In such a design, the boronic acid contained within the hydrophobic liquid membrane could bind with saccharide at the interface to generate a boronate ester. Then the boronate ester could diffuse through the membrane and hydrolyze back to the boronic acid and release the saccharide to the receiving phase. The transport of fructose and glucose through supported liquid membrane (SLM) promoted by several tetrahedron-shaped lipophilic monoboronic acids and diboronic acids was examined. The selectivity was measured by the ratio of sugar fluxes between fructose and glucose. The diboronic acid gave high fructose/glucose selectivity (7.7:1.0). As shown in Figure 18, their diboronic acid carriers could bind with multiple equivalents of D-fructose to form two types of diesters (**51** and **52**) in the membrane. The method of transporting D-fructose by **51** showed better selectivity for D-fructose over glucose than did **52**. It was proposed that the selectivity was due to the formation of a tridentate 2, 3, 6- β -D-fructofuranose diester **51** and a macrocyclic D-fructopyranose diester **52** within the membrane. This suggestion was supported by computer molecular modeling studies and ^{13}C NMR experiments with labeled ^{13}C -D-fructose.³⁰⁶

Duggan and co-workers also studied the transport of four sialic acid derivatives through lipophilic supported liquid membranes.³⁰⁷ Aliquat 336, a lipophilic quaternary ammonium chloride salt, was added into the membrane to promote the transporting of the sialic acid derivatives. The flux of sialic acid derivatives in the membrane containing Aliquat 336 alone was higher than those in the other two membrane systems (boronic acid alone or boronic acid in combination with Aliquat 336). A mobile-site jumping mechanism was proposed to explain the flux improvement by lipophilic ion pairs. It was proposed that the triol side chain and the amide functional group of sialic acid derivatives form a chloride receptor. The sugar-chloride complex hops from cation to cation in the membrane and then release into the receiving phase. The reason for the impaired transport when a boronic acid is present could be due to the abolishment of the chloride binding ability of the triol side upon boronate formation. It was also interesting to note the very different effect Aliquat 336 has on sialic acid derivatives and D-fructose. For the transport of D-fructose, the flux through ammonium boronate membrane is detectable, while no transport was detected with the membrane containing either boronic acid or ammonium chloride alone. The studies of log P values suggested that D-fructose was more hydrophilic than the four sialic acid derivatives, which explains why only the transport of D-fructose through a lipophilic membrane is favored by the mixture of a lipophilic boronic acid and a lipophilic ammonium salt.

There are other efforts in boronic acid-based saccharide sensing. For examples, Ishihara and co-workers examined the binding mechanism of boronic acid and diols.³⁰⁸ Their studies suggested that boronate ion was not as reactive as boronic acid in an alkaline solution. This was further supported by kinetic evidence such as the estimated upper limit of the rate constants for the reactions of some boronic acids with 2,2'-biphenol and 2,3-dihydroxynaphthalene in a neutral to alkaline (pH 7–14) solution. Tao and co-workers evaluated glucose sensing with boronic acid using enzymatic oxidation.⁴⁷ In their studies, the enzyme glucose oxidase (GOx) catalyzed the conversion of glucose into gluconic acid, which was able to complex with a fluorescent boronic acid through the hydroxycarboxylate moiety in aqueous media. This method could overcome the weak binding issue between glucose and boronic acid because of the relatively strong interactions between gluconic acid and boronic acid. Kondo and co-workers studied diol adsorption on phenylboronic acid modified silica gel.³⁰⁹ Potentiometric titration was used to determine the p K_a of the active site in boronic acid modified gels. Araki and coworkers examined sugar detection by phenylboronic acids in a capillary electrophoresis-chemiluminescence system.³¹⁰ They examined the enhancing effects of several phenylboronic acid compounds for luminol-hydrogen peroxide-horseradish peroxidase reaction in the system. Compared to other boronic acids, only 4-biphenylboronic acid and *p*-iodophenylboronic acid showed an enhancing effect over the range of 0.5–10 μ M in this system. Suslick and co-workers developed a series of metal porphyrin appended boronic acid for sugar sensors.³¹¹ They synthesized two boronic-acid-appended zinc-porphyrins as potential colorimetric sensors for carbohydrates. For the synthesis of these two sensors, boronic acid groups were connected either to a β -pyrrolic position or to the *meso*-position of the porphyrin core. However, no spectroscopic property changes were observed with the addition of sugars.

2-H. Applications of boronic acid in sensing of non-sugar compounds

Since boronic acids can bind with compounds that have two adjacent nucleophilic groups, it can not only be used for recognition of carbohydrates, but also for many other types of compounds such as diamine³¹², fluoride¹⁷⁸·³¹³·³¹⁴, dopamine³¹⁵–³²², L-DOPA,²⁵³ nucleosides,³²³–³²⁵ and amino acids.³²⁶ Boronic acids can also be used in drug delivery,³²⁷ and as building blocks for synthesis.³²⁸ However, such applications are not the focus of this review and will not be discussed in detail.

As a summary to this boronic-acid-based sensor, Table II lists the boronic acid-based sensors described, which are specifically for detection of certain carbohydrates.

3. OTHER CARBOHYDRATE SENSORS

In addition to boronic acid-based approaches, many other methods have been used for the development of “receptors/binders” for carbohydrates. These include (1) aptamer approaches, (2) non-covalent approaches, and (3) metal chelation approaches. In the following sections, we discuss discoveries in those areas in detail. Since discoveries in these three areas have not been reviewed as extensively as the boronic acid field, the literature covered may go back to earlier times than the coverage of the boronic acid field.

3-A. Aptamer-based carbohydrate sensors

The term “aptamer” is often used to refer to short DNA or RNA sequences that have been selected to bind a chosen target.¹⁶²⁻³²⁹⁻³³⁰ With the powerful selection methods first developed in the early 1990s, aptamers to small molecules, proteins and nucleic acid structures can be selected with high affinity and specificity. Naturally, the same methods have also been used for the selection of aptamers that bind carbohydrates. Till now, aptamers to carbohydrates were selected for two purposes: (1) as purification tags for RNA or ribonucleoparticles from RNA mixtures⁷⁴⁻³³¹⁻³³² and (2) to identify and/or block specific sugars on cell surfaces.³³³ Herein we mainly focus on the application of aptamers for cell surface carbohydrate recognition and inhibition.

Yu and co-workers first developed RNA aptamers for sialyl Lewis X (sLe^x , **53**, Figure 19) by using *in vitro* RNA selection.⁷⁴ sLe^x is a tetrasaccharide glycan on cell surface and is a mediator of T-cell actions. It sometimes serves as a cancer biomarker.¹²⁰⁻¹³⁹⁻³³⁴⁻³³⁵ During inflammation, the interactions between sLe^x and selectins on the surface of vascular endothelial cells play a critical role in cell adhesion.¹²⁰⁻³³⁴ In addition, aberrant expression of sLe^x has been implicated in cancer metastasis.¹³⁹⁻³³⁵ Therefore, “receptors” that bind sLe^x could be effective anti-inflammatory and/or anti-metastasis agents. These were the first isolated RNA aptamers for sLe^x with high affinity and high specificity. The K_d values were around 10^{-9} to 10^{-11} M. These aptamers also showed similar or better binding affinities comparing to that of commercially available antibodies. In addition, the selected RNA aptamers also showed inhibition of adhesion of sLe^x -expressing HL60 cells to the E- and P-selectins, which suggests the potential for these aptamers to be used as cell adhesion inhibitors for anti-inflammatory therapy. Although it was said that the isolated RNA aptamers could discriminate minor differences in carbohydrates and the binding affinity to sLe^x is 100 times higher than that of lactose, these aptamers were only able to bind sLe^x with 5–10 fold higher affinity than with other similar Lewis group sugars.

In 2006, Paulino and co-workers developed an aptamer-based assay for the detection of neomycin B (**54**, Figure 20),³³² which is an aminoglycoside antibiotic that selectively targets RNA structural motifs. Although several methods including electrophoresis, derivative fluorimetric and pulsed amperometric detection had been reported,³³⁶⁻³³⁹ neomycin B detection was still a challenging problem because these reported methods were time consuming and neomycin lacked the needed spectroscopic and electrochemical properties. Therefore, a competitive impedimetric assay for the detection of neomycin B was conducted by using a fully 2'-*O*-methylated RNA aptamer sensor, which was developed based on previous RNA motifs.³⁴⁰⁻³⁴¹ The *O*-methylation helped to increase the stability of the aptamer against degrading enzymes. In this approach, neomycin B was first immobilized on a self-assembled monolayer of mercaptopropionic acid (MPA) (Scheme 12). Then aptamer was bound to surface linked neomycin B. Neomycin B in solution can competitively displace the bound aptamer and the dissociation was monitored by using

faradaic impedance spectroscopy (FIS). With this method, a 1:1 neomycin-aptamer complex was observed under near physiological conditions. High specific affinity was also found by comparing with binding of paromomycin, which differs from neomycin B in the substitution of a single NH₂ group by an OH group at C₆. Such results confirmed the previous report that the 100-fold lower binding affinity of paromomycin relative to neomycin341 was not affected by the use of 2'-O-Me bases. Neomycin-enriched whole milk was also analyzed to confirm the compatibility of the device with biological fluids. The study showed a linear range between 25 and 2.5 × 10³ μM in milk.

Sialyllactose (α -Neu5Ac-[2-3]- and [2,6]- β -D-Gal-[1-4]-D-Glc) is a ubiquitous saccharide component of mammalian plasma-cell membrane.^{342,343} It is an important recognition moiety for many animal viruses such as influenza A and C viruses, cardioviruses and Newcastle disease viruses. Thus, blocking the binding of the sialyllactose and animal viruses on cell surface could be a route to the development of anti-viral agent. Sawai and co-workers produced a novel cationic-charged modified DNA aptamer by using modified thymidine residues.³⁴⁴ The idea is to use the cationic protonated amino group at the C₅ position to help enhancing the binding affinity with the anionic carboxyl group of sialyllactose. By using the SELEX method, twenty two clones were selected after 13 rounds of selection. The K_d for the aptamer with the highest affinity was 4.9 μM. The author proposed a three-way junction structure for the aptamer with a stem region containing the modified thymidine. The modified aptamer showed a stronger affinity to sialyllactose than the unmodified counterpart, suggesting that the modified thymidine bearing a positively-charged amino group at the C₅ position is an important contributing factor to the high binding affinity.

Another example is the modified RNA aptamer, which binds moenomycin A (55, Figure 21). Moenomycin A is an amphiphilic phosphoglycolipid antibiotic and kills bacteria by interfering with the transglycosylation process in peptidoglycan biosynthesis.^{345,346} It has two building blocks, a branched pentasaccharide moiety and the non-polar lipid part. Hahn and co-workers described 2'-aminopyrimidine RNA aptamers which can specifically bind to the non-polar lipid part of moenomycin A.^{331,347} The introduction of the 2'-amino group was to increase resistance of the aptamer to enzymatic degradation. All isolated aptamers were shown to be G-rich, indicating a hydrophobic binding site, which might account for the highly specific binding to the non-polar part instead of the saccharide moiety. π-Stacking interactions between the nucleobases and unsaturated side chain of the moenomycin analogs, and “sandwich” formation between hexad (A (GGGG) A) platforms and the lipid moiety were assumed to play a role in stabilizing these complexes. The K_d values of the two aptamers reported in this article were determined to be 350 and 320 nM respectively. Another fact that is remarkable in this study is that the aptamers selectively bind to an unsaturated lipid chain of moenomycin in contrast to the farnesylated Ras aptamer binding with the saturated part chain.³⁴⁸ The authors suggested that the difference might have originated from the different configuration of the isoprenoid moieties.

One challenging area of carbohydrate recognition is the differentiation of glycosylation patterns of a glycoprotein. Conceivably, aptamers can be evolved to recognize glycoproteins. However, conventional aptamer selection approaches do not have the intrinsic ability to do substructure-focused selection, i.e. the selection of aptamers that specifically focus on the recognition of the glycosylation site(s). The Wang lab worked on using boronic acid-modified DNA for the selection of aptamers for the specific recognition of the glycosylation site(s) of a glycoprotein.^{137,349} As discussed in the previous section, boronic acid is known to high intrinsic high affinity for diols and hydroxyl groups. Therefore, it was reasoned that the incorporation of a boronic acid moiety should allow the

aptamer selection process to gravitate toward the recognition of the glycosylation site(s) of a glycoprotein.

The boronic acid moiety was introduced to the 5-position of thymidine.¹³⁷ Therefore, a modified TTP nucleotide containing a boronic acid functional group (BTTP, **56**; Figure 23) was synthesized using copper (I)-catalyzed Huisgen cycloaddition reaction/click chemistry.^{350–360} This boronic acid labeled nucleoside triphosphate can be incorporated into DNA with a similar efficiency as the natural TTP.¹³⁷

DNA libraries with BTTP (**56**, Figure 22) were then used for the selection. Fibrinogen was used as a model glycoprotein because of its known association with the risk of major cardiovascular diseases and nonvascular mortality and the critical role of its carbohydrate moiety in fibrinogen function.^{31,361} The SELEX approach was used for the aptamer selection. Aptamers were selected with K_d in the range of low to mid nM.³⁴⁹ More importantly, removal of the boronic acid, the glycan moiety or both resulted in significantly decreased affinities. Furthermore, sugar and boronic acid can competitively inhibit aptamer binding to fibrinogen. In contrast, aptamers selected without using boronic acid-modified TTP did not bind to the glycosylation sites. All such results suggest that indeed boronic acid modified DNA aptamers can be selected to specifically recognize the glycosylation site(s) of a glycoprotein. The same method can be used for the selection of boronic acid modified DNA based aptamers for other glycoproteins. This represents the very first example that aptamers can be selected to specifically focus on the glycan substructure in a glycoprotein. We refer to these aptamers as nucleic acid-based boronolectins.³⁶

Anslyn and co-workers invented a method of using aptamers to fine-tune the selectivity of synthetic small molecule receptors. Specifically, the Anslyn lab selected an aptamer that could bind to the complex between bis-boronic acid receptor **57a** (Figure 23) and tartrate.¹⁶³ First, organic receptor **57**^{163,362} was immobilized on glyoxal agarose beads through reductive amination. Then the immobilized receptor **58a** (Figure 23) in complexation with tartrate was used for the selection and control compound **58b**, which was incapable of binding tartrate, was used for counter selection. The progress of the selection was monitored by incorporating a radiolabel into the RNA pool. After 13 round of selection, no further improvement in binding was detected. The selected aptamers were then cloned, sequenced, and used for binding studies. Several interesting observations were made. First, aptamer binding appeared to be dependent on methanol and the optimal methanol concentration for binding was around 20%. This was one of the first examples of aptamer selection that has been carried out in high concentrations of an organic solvent. Second, on its own, **57** was only slightly selective for citrate ($1.3, K_d$ for tartrate = 7.1×10^{-6} M, K_d for citrate = 5.5×10^{-6}). However, in the presence of aptamer, the specificity of the receptor was dramatically altered (14 for tartrate, $K_d = 2.1 \times 10^{-4}$ and $K_d < 3 \times 10^{-3}$ for citrate in 20% MeOH). The explanation is that aptamer might form a pocket more precisely accommodate the receptor-tartrate complex, while exclude citrate via steric interactions or charge repulsion. Third, lower affinity of **57** for tartrate in the context of the aptamer was also found. The author explained that at least part of the tartrate-binding energy is used to generate an induced-fit conformation. In summary, by generating an aptamer-receptor complex for the recognition, the author successfully selected an aptamer for a small organic ligand. This work shows a novel route to the development of biosensors for small organic analytes and can serve as a model for future applications to improve the specificity of synthetic receptors.

3-B. Non-covalent sensors

Besides the aptamer approaches, non-covalent sensors is yet another approach for the development of “receptors” for carbohydrates. For the non-covalent sensors, Davis and Wareham published an excellent review in 1999.³⁶³ Therefore, earlier work prior to 1999

will not be discussed. Briefly, the review was divided into two main sections, carbohydrate recognition in organic media and carbohydrate recognition in aqueous solution. In the carbohydrate recognition in organic media part, the author discussed “face-to-face” recognition, encapsulation by steroid-based receptors, the cholaphanes alternative frameworks based on cholic acid valleys, bowls, rings and cages, specific motifs for carbohydrate recognition, 2-aminopyridines and related substructures, recognition by anionic centers, and the phosphate-diol motif recognition without pre-organization. In the carbohydrate recognition in aqueous solution part, the discussion was focused on carbohydrate recognition in nature, the driving force for natural carbohydrate recognition, and recognition in model systems.

Biomimetic carbohydrate recognition has drawn much of interest over the past few years. X-ray crystallographic studies of protein-carbohydrate complexes reveal that the polar groups are bound by hydrogen-bond donor and acceptor groups, occasionally to metal ions, while the hydrophobic regions are complemented by nonpolar surfaces. Furthermore, CH- π stacking interactions and van der Waals interactions both contribute significantly to the recognition of carbohydrates by proteins in nature.³⁶⁴ Such information is very important for the design and synthesis of artificial carbohydrate receptors. In addition, it is also very important to understand the driving force for the replacement of water by carbohydrate-OH in a natural binding site. In this part, we will focus on work developed recently by different groups in using non-covalent forces for the design and synthesis of artificial carbohydrate receptors.

3-B-1. Receptors designed by the Mazik lab—In nature, there are many sugar-binding proteins. Such binding often relies on interactions of planar polar side chains with at least two functional groups engaging in cooperative hydrogen bonds and functional groups capable of π -stacking or hydrophobic interactions. Inspired by this, Mazik and co-workers designed and synthesized a series of pyridine-amide/amino and pyrimidine-amide/amino compounds. Such design is to mimic the hydrogen-bond and π -stacking interactions between sugar and proteins in the nature.^{365–372}

As a starting point for the design of monosaccharide receptors, the author first examined the binding affinities of tripyridine **59** and tripyrimidine **60** (Figure 24). For comparative studies, compounds **61** and **62** were also synthesized (Figure 24).³⁶⁵ Octyl- β -D-glucopyranoside (**63**, Figure 24) and Octyl- α -D-glucopyranoside (**64**, Figure 24) were selected as model sugars for the evaluation of binding affinity in aprotic solvents such as chloroform. The interactions between hosts **59–62** and glucopyranosides were investigated by ¹H NMR and extraction experiments. The NMR studies indicate a 1:1 binding stoichiometry. The binding constants were determined to be $8.7 \times 10^3 \text{ M}^{-1}$ between **63** and receptor **59**, and $4.0 \times 10^3 \text{ M}^{-1}$ between **64** and **59** in CDCl₃ respectively. Crystallographic studies have also been conducted. The results show that binding involves both hydrogen bond CH- π and van der Waals interactions. Receptor **60** showed similar binding affinity for both anomers with a binding constant of $1.4 \times 10^4 \text{ M}^{-1}$ for **63** and $1.3 \times 10^4 \text{ M}^{-1}$ for **64**. In order to test the importance of the presence of pyridine nitrogen in the complex, compound **61** (Figure 24) was further designed and its binding affinity with sugar was studied by extraction experiments. Different from compound **59**, compound **61** showed very poor solubility in chloroform even at high concentrations of glucopyranosides. The results suggest that without the hydrogen bond interactions involving the pyridine nitrogen, glucopyranosides could not pull the receptor into the solution, an indication of lack of binding. Due to the solubility problem of compound **61**, the dimethylphenyl derivative **65** (Figure 24) with better solubility was also synthesized for binding studies. The K_a value (750 M^{-1}) was more than 11-fold lower than that of the complex between **59** and **63** ($K_a = 8.7 \times 10^3 \text{ M}^{-1}$), which further confirmed the importance of hydrogen bonding between

pyridine nitrogen and OH groups of glucopyranosides in complex formation. To examine the need for three pyridine rings for tight binding, the binding constants of receptor **62** were also determined. Compared with **59**, much lower K_a values ($1.1 \times 10^3 \text{ M}^{-1}$ and 460 M^{-1} for **63** and **64** respectively) were obtained. The smaller binding constants for **62** implied the importance of the three pyridine rings for tight binding.

Encouraged by the effectiveness of hydrogen-bonding as a way to improve binding affinity toward glucopyranosides, Mazik and co-workers further modified the structures of receptors **59** and **60**. Several other receptors **66–69** (Figure 25) were designed to explore which hydrogen bonding motifs are essential for recognition of monosaccharides.³⁷¹ Again, octyl- β -D-glucopyranoside (**63**, Figure 24) was selected as a probe for comparison and the interactions of all hosts and **63** were investigated by ^1H NMR studies, which also indicate a 1:1 stoichiometry. The binding constants were determined by titration experiments in chloroform at 25°C . The association constant between compounds **66** and **63** was determined to be $2.7 \times 10^4 \text{ M}^{-1}$. Such results suggest that the three naphthyridine-amide moieties in **66** led to a higher binding constant compared with that of receptor **59** ($K_a = 8.7 \times 10^3 \text{ M}^{-1}$). The author contributed the higher binding affinity to secondary hydrogen bonds (Scheme 13).

Similar to previous experiments, compound **67** was also designed to demonstrate the significance of the “three-point” recognition model. As expected, lower binding affinity ($K_a = 4.5 \times 10^3 \text{ M}^{-1}$) was obtained, as compared to compound **66**, presumably due to the lack of the third arm for binding. To quantify the influence of the amide NH protons on the formation of binding complex, the binding constant of compound **68** with the replacement of the amide bond by an ester bond was also determined to be 800 M^{-1} , which was more than 10-fold lower than that of the complex between **59** and **63** ($K_a = 8.7 \times 10^3 \text{ M}^{-1}$). Such results are consistent with the hypothesis that the amide N-H is very important for binding. Of course, It is also possible that the ester bonds are much more flexible, which would make the receptor molecule less rigid and result in lower binding affinity. Another interesting experiment done by Mazik and co-workers is the design and binding affinity experiments of compound **69** (Figure 25), which has an added coumarin ring for additional van der Waals interactions with **63**. The 4-fold higher binding affinity ($K_a = 4.3 \times 10^3 \text{ M}^{-1}$) as compared to that of **62** and **63** ($K_a = 1.1 \times 10^3 \text{ M}^{-1}$) indicates that appropriately positioned additional hydrophobic interactions, similar as in protein-sugar complexes, are beneficial to binding.

Furthermore, new effective pyridine-based receptors **70–72** (Figure 26) were designed with replacement of the central phenyl ring by a trimethylphenyl ring to involve more effective CH- π interactions with the CH's of sugar molecules.³⁷⁰ The second methyl group at the 4-position of the pyridine ring in host **71** and an additional amino group in host **72** were incorporated to increase the basicity of the pyridine moiety. The binding constants, again, were determined by ^1H NMR experiments. In this study, different ratios were used between the receptor and sugar. This was to examine whether the binding stoichiometry and whether cooperative binding was involved. The results are shown in Table III.

The binding constants were determined in chloroform at 25°C by titration experiments. The stoichiometry of receptor-sugar complexation was obtained by curve-fitting analysis of the titration data and molar ratio plots. K_{a1} and K_{a2} were defined as binding constant of 1:1 and 1:2 receptor-sugar complexation respectively. The signal in NMR from the amine NH moved downfield and methylene CH_2 moved upfield upon binding. Such results suggested the formation of hydrogen bonds between these groups and the sugar hydroxyls. The relative low solubility of the α -anomer glucopyranoside **74** comparing to the β -anomer **73** in the presence of receptor **71** provided additional evidence for the preference of β -anomer. Specifically, during the extraction experiment, 0.7 equiv of β -anomer **73** was extracted into

the CDCl_3 solution of receptor **71**. Besides, after binding with **73**, the NH and pyridine CH ^1H NMR signals showed significant change in chemical shift, which further indicate complexation. In contrast, α -anomer **74** was not extracted at nearly the same efficiency. It is interesting to note that after addition of a small amount of water (0.07%) to the chloroform solutions, the binding affinity increases by a factor of 2–3. This increase in binding might be due to the formation of water-mediated hydrogen bonds, in line with similar observations in protein-carbohydrate complexes.³⁶⁴

Analogs **75** and **76** were also designed to investigate the influence of steric hindrance at the pyridine nitrogen position on binding affinity (Figure 27).³⁷² The binding constants are shown in Table IV. Again, K_{a1} was defined as 1:1 binding constant of receptor-sugar complex, while K_{a3} was defined as 2:1 binding constant of receptor-sugar complex. The results showed that when the methyl group was in the α -position (**70**), lower binding affinity ($K_{a1} = 8.7 \times 10^3 \text{ M}^{-1}$) was observed as compared to situations when the methyl group is far away from the pyridine nitrogen ($K_{a3} = 2.4 \times 10^4$ for **75–63** and $2.3 \times 10^4 \text{ M}^{-1}$ for **76–63** respectively). For receptor **59**, 1:1 binding was observed. Such results indicate that steric hindrance near the pyridine nitrogen plays an important role in affecting the binding. Decreased steric hindrance at the pyridine nitrogen increased the binding affinity of host ($K_{a3} = 2.4 \times 10^4$ for **75–63** and $2.3 \times 10^4 \text{ M}^{-1}$ for **76–63** respectively). One interesting thing is that, unlike **59**, compounds **75** and **76** showed inversed selectivity, i.e., binding favors the α -anomer (**64**, Figure 24) instead of the β -anomer (**63**, Figure 24). In the case of compound **59**, the axial 1-alkoxy group in α -anomer **64** can form intramolecular hydrogen bonds with the 2-OH group more easily than the equatorial 1-alkoxy substituent in β -anomer **63**, therefore, the 2-OH group in β -anomer **63** is relatively free from intramolecular bonding, thus can bind with receptors more tightly. However, in the case of binding of compounds **75** or **76** with α -anomer **64**, the intermolecular host-guest interactions might play a more important role than the intramolecular H-bonding network, which contributes to the higher binding of **75** or **76** with α -anomer **64**.

Later on, more analogs were further designed (**77–85**, Figure 28).³⁶⁹ The binding constants are shown in Table V.

Based on results from crystal structures, the Mazik lab further designed and synthesized additional receptors, **86** and **87** (Figure 29).³⁶⁶ These receptors used 2-aminopyridine groups as heterocyclic analogues of the asparagine/glutamine side chains, while incorporating a phenyl linker to interconnect these recognition units to provide additional nonpolar contacts to a saccharide by CH- π interaction. Carboxylate groups were also incorporated into the receptors to enhance binding through hydrogen bonds and ion-dipole interactions.

Binding studies were again conducted by NMR method. Again, K_{a1} is used to designate the binding constant of a 1:1 receptor-sugar complex and K_{a2} is used to designate a 1:2 receptor-sugar complex. Incorporation of the carboxylate group improved binding considerably both in organic and aqueous media. Receptor **87** exhibited at least 100-fold higher affinity for β -glucopyranoside ($K_{a1} = 1.2 \times 10^5 \text{ M}^{-1}$ and $K_{a2} = 4.7 \times 10^3 \text{ M}^{-1}$) than the previously described tri-armed pyridine-based receptor **70** ($K_{a1} = 1.1 \times 10^4 \text{ M}^{-1}$ and $K_{a2} = 250 \text{ M}^{-1}$). Besides, compound **87** shows a preference for the β -anomer in the recognition of glucopyranosides ($K_{a1} = 1.2 \times 10^5 \text{ M}^{-1}$ for **63** over $2.2 \times 10^4 \text{ M}^{-1}$ for **64**). Furthermore, compound **86** shows similar binding affinity as compound **87** despite the fact that it does not have the functional groups for ionic interactions. However, both receptors, **86** and **87**, showed dramatically decreased association constants in aqueous solution. For example, compound **87** has K_{a1} of 2 M^{-1} and K_{a2} of 72 M^{-1} with **63** in water. One surprising thing is

that compound **87** showed a preference for the disaccharide D-cellobiose with K_{a1} of 305 M⁻¹ and K_{a2} of 66 M⁻¹ in CDCl₃.

By using the same strategy, the Mazik lab also designed and synthesized compounds **88** and **89** (Figure 30).³⁶⁸ In this case, crown ether was incorporated as another binding site instead of a carboxylate group. The crown ether moiety was designed for hydrogen-bonding interactions with sugar. Again binding constants were determined using ¹H NMR binding titrations and extraction experiments. The data were fitted into a 1:1 (K_{a1}) and a 2:1 (K_{a3}) binding model to give K_{a1} of 5.8×10^5 M⁻¹ and K_{a3} of 1.4×10^4 M⁻¹ for receptor **88** with **63** and K_{a1} of 1.1×10^4 and K_{a3} of 4.8×10^4 M⁻¹ for receptor **89**. The results showed about 10-fold higher affinity for **63** than its precursor **78** ($K_{a1} = 1.3 \times 10^3$ M⁻¹) without the crown ether group. Besides, the two receptors both showed good selectivity for **63** over **64**. For example, receptor **88** has a ratio of 417/1 for binding with **63** over **64**. Although the incorporation of crown ether group could enhance the binding affinity, such results were only obtained in CDCl₃ solution. It would be interesting to see whether the observed selectivity still exists in aqueous solution.

Inspired by the binding affinity of designed receptors with disaccharides, Mazik and co-workers further developed a new biphenyl-based receptor **90** for the recognition of disaccharides (**91a** and **91b**, Figure 31).³⁷³ The hypothesis for this design was that the four heterocyclic recognition groups based on the 2-aminopyridine unit would provide an excellent structural motif for hydrogen bonding. The biphenyl spacer of **90** should provide additional nonpolar contacts to the saccharides. Binding affinity was determined by ¹H NMR with $K_a > 10^6$ M⁻¹ for **91b** in water-containing chloroform solutions (1:10, 1:20) when the data were fitted into a 2:1 (receptor/maltoside) model. Based on spectroscopic changes, it seems that at the initial stage, a 2:1 complex was formed. This was gradually replaced by a 1:1 complex. Compared with the binding of **91b**, **91a** has very poor solubility in chloroform in the presence of receptor **90** indicating weak binding affinity and good β/α selectivity. The relative low binding affinity of **90** for **63** ($K_{a1} = 1.1 \times 10^4$ M⁻¹, $K_{a2} = 300$ M⁻¹) indicates preference for disaccharides.

3-B-2. Receptors designed by the Roelens group—By taking advantage of the well-established hydrogen-bonding property of pyrroles, the Roelens lab described a self-assembled cage receptor containing pyrrole residues for multipoint interactions through hydrogen bonds (**95**, Scheme 14).³⁷⁴ The pyrrolic cage receptor was designed to provide the conformational and functional requirements for carbohydrate recognition.

The designed compound **95** was synthesized by mixing **92** with **93** in methanol in a 2:3 ratio followed by reduction. The structure of **95** was determined by spectroscopic characterization and single crystal X-ray crystallography. Binding experiments were performed by using ¹H NMR in CDCl₃ and a 1:1 stoichiometry with an association constant of 4.8×10^4 M⁻¹ with octyl- β -D-glucopyranoside (Oct β Glc) was obtained. Interestingly, **95** has very high selectivity for the β anomer showing exclusive binding when observed using NMR. The author ascribed the excellent binding to both a precise size fit of the β -glucoside and an excellent complementarity of the amino/pyrrole group compared to the amide group, which is normally used for hydrogen bonding to a sugar moiety. Another interesting observation is that the precursor compound **94**, which has a similar structure as **95**, did not exhibit the same binding ability. Addition of Oct β Glc did not show evidence of complexation. Solubility experiment was further conducted to probe the binding affinity and selectivity. Methyl- β -D-glucopyranoside (Me β Glc) is insoluble in CDCl₃, however, when it was shaken with a 1 mM solution of **95** in CDCl₃, the solid was partially dissolved and the NMR spectra showed that over 40% of the cage was present in the complex form. The bound percentage was increased to 50% in CCl₄ and 75% in C₆D₆. Another competitive experiment to study

selectivity was performed by feeding **95** with an equal molar mixture of Oct β Glc and other selected glycosides. There was less than 10% decrease of binding fraction of **95** with Oct β Glc after addition of various glycosides as compared with binding in the absence of competitors. In summary, the Roelens lab designed and successfully synthesized a self-assembled macrobicyclic cage **95**, which can specifically recognize the β anomer of D-glucose and its alkyl glucosides with high K_a .

The Roelens lab also designed and synthesized a β -mannoside-selective pyrrolic tripodal receptor **96** (Scheme 15) based on the hydrogen bonding property of the receptor.³⁷⁵ Binding properties were determined by NMR, ITC and ESI-MS techniques and the binding affinities were assessed by the BC₅₀ parameter, which was defined as the total concentration of receptor necessary for binding 50% of the ligand. The BC₅₀ for the designed compound with β -mannose is less than 1 μ M in CDCl₃ with selectivity of about 800 over α -mannose or α -galactose, and 680 μ M in CD₃CN solution with selectivity of about 40 over α -galactose.

The Roelen's lab further designed and synthesized a similar tricatecholic receptor for carbohydrate recognition (**97**, Scheme 16).³⁷⁶ The binding affinity of the receptor was investigated with several monosaccharides and disaccharides and the results showed that the receptor binds with octyl glycosides with BC₅₀ of 0.87–5.2 mM and a 6-fold selectivity factor for α -mannoside over α glucoside.

Recently, the Roelen's group designed and synthesized a series of pyrrolic tripodal receptors **98–105** (Figure 32)³⁷⁷ by incorporating the pyrrolic binding groups into a previous receptor featuring a triethylbenzene scaffold bearing three convergent ureidic H-bonding units.³⁷⁸

The pyrrole moiety was conveniently incorporated into the parent triamine by condensation to obtain the Schiff base **100**, which was further reduced to amine **101**. Compound **102** was designed to assess whether the amino group would be more effective than the amido group in binding monosaccharides. The benzylester moiety at the 5 position of pyrrole was incorporated to overcome the solubility problem of **103** and **104**. Compound **105** was designed to ascertain the contribution from the pyrrolic groups to the recognition. Several monosaccharides (Figure 33) were selected as model sugars for binding studies, which were determined by ¹H NMR titrations in CDCl₃ at room temperature. The results are shown in Table VI.

From the results presented in Table VI it can be seen that (1) the incorporation of the pyrrole ring led to improved affinity as demonstrated by the higher binding affinity of **100** and **101** in contrast to that of **99**; (2) compound **100** showed a remarkable binding affinity with β Glc, which is over 400-fold higher than that of **99**, and the highest selectivity for β Glc than all the other compounds studied; (3) The amino receptor **101** generally has a higher affinity than the imino receptor **100** except for α Glc and α Gal, where compound **100** shows higher affinities; and (4) somewhat surprisingly, receptor **102** showed lower binding affinity than **99** and no selectivity. In an effort to understand the structural features of these receptors, the X-ray crystal structures of receptors **100** and **104** were also determined. Alternate arrangement of substituents was observed with the three pyrrole arms on the same side of the aromatic ring forming a cleft. In the center of it is a captured ethanol molecule. The crystal structure conformations were as expected and reaffirmed the original design principles.

3-B-3. Receptors designed by the Inoue group—Inoue and co-workers developed water soluble extended analogs 3poly (**107**, Figure 34)³⁷⁹ of polypyridine structures based on their previous studies including 1poly (**106**, Figure 34).^{380–381} The design was based on the reason that the polymers used are expected to spontaneously form a helical structure by

solvophobic interactions in aqueous solution between one pyridine ring and another at an interval of one pitch (Scheme 17). In the helix form, pyridine rings line up in a *cisoid* conformation and all the pyridine nitrogen pointed to the inside of pore. Since the pore provides a "low-entropy" location compared to the protic solvent molecules outside, which can form hydrogen bonds with each other, the included solvent molecules in the pore thus can be substituted by other hydrogen-bonding substrate such as carbohydrate. The structure of amphiphilic polymer **107** in solution was investigated by UV/vis, ¹H NMR and fluorescence spectra. The results indicated that the major contribution for the highly ordered structure form comes from the intramolecular π -stacking interactions of **107** in polar solvents.

Binding of the water soluble polymer **107** with some common monosaccharides was investigated by using circular dichroism (CD) in MeOH/water (10/1). It seemed that the chirality of the saccharide was transferred to the helical sense of the polymer as evidenced by the appearance of induced CDs (ICDs) in the absorptive region of the polymer. The binding constants were determined to be 14, 5.5 and 4.4 M⁻¹ with D-mannose, D-fructose and D-allose, respectively. However, no significant binding was found with saccharides in 100% of water. The problem of weak binding in aqueous solution can be attributed to two conflicting factors: (1) solvophobic interactions, which cause the helix formation, are favored in water and disfavored in MeOH, and (2) hydrogen bonding, which is the driving force for saccharide recognition, is advantageous in MeOH and disadvantageous in water. More work is needed to improve binding affinity in aqueous environment.

3-B-4. Receptors designed by the A. P. Davis group—In 1998, Davis and co-workers reported a carbohydrate receptor **108** (Figure 35).³⁸² The design of **108** drew lessons from sugar-protein interactions, which again rely on both hydrogen bond and hydrophobic interactions. The target of **108**, the β -D-glucopyranoside unit **109**, possesses axial CH groups and equatorial directed hydroxyl groups (Figure 35). Therefore, for complementary interactions, it is desirable that the binding cavity have parallel aryl rings, appropriately spaced and linked by moieties capable of hydrogen-binding. The tricyclic structure of **108** has two biphenyl rings and eight amide groups that appear to fit these criteria quite well. Molecular modeling on the analogous tetramethyl ester indicated that the cavity defined by the biphenyl and benzene 1, 3-dicarboxamide was able to accept a β -D-glucopyranoside molecule by forming hydrogen bonds and CH- π interactions.

Binding was examined using monosaccharides **109–111** as models (Figure 35) and ¹H NMR in CDCl₃/CD₃OH (92:8). Data were consistent with 1:1 binding with K_a of 980, 20 and 220 M⁻¹ for **109**, **110**, and **111** respectively. Binding affinities in organic solvent CDCl₃ were also determined by using fluorescence spectroscopy. Using a 1:1 binding model, the binding constants were 3.0×10^5 , 1.3×10^4 and 1.1×10^5 for saccharides **109**, **110**, and **111**, respectively. Good selectivity of the β -anomer was also observed. For example, the binding constant for β -glucoside **109** was 980 M⁻¹, which is about 45-fold higher than that of α -glucoside **110** ($K_a = 20$ M⁻¹). Besides, the binding affinity in CDCl₃/CD₃OH system gave a good reason to hope that tricyclic polyamide receptors upon further optimization could be developed as receptors for saccharides in aqueous solution.

Inspired by the remarkable selectivity for the all-equatorial β -glucoside as against α -anomers, Davis and co-workers studied another receptor **112**, an "extended analogue" of **108**, for the recognition of disaccharides through non-covalent interactions (Figure 36).³⁸³ Similar to receptor **108**, receptor **112** possesses extended, parallel non-polar surface linked through spacers containing hydrogen-bond donor and acceptor groups. This geometry is compatible with equatorially substituted carbohydrate derivatives. Based on this, the all-equatorially n-octyl- β -D-cellobioside **113** (Figure 36) was first used for initial binding

studies. Titration ^1H NMR experiments showed a 1:1 binding with a K_a of $7.0 \times 10^3 \text{ M}^{-1}$ in $\text{CDCl}_3/\text{CD}_3\text{OH}$ (92:8), which is higher than that of **108** with β -monosaccharide ($K_a = 980 \text{ M}^{-1}$). No detectable binding was observed when using octyl β -D-lactoside **114**, octyl β -D-maltoside **115**, and dodecyl α -D-maltoside **116** as substrates (Figure 37). Furthermore, binding with **113** was also studied using a fluorescent method. The results also supported a 1:1 binding model with K_a of $2.5 \times 10^3 \text{ M}^{-1}$ with **113** (Figure 37). In contrast, no detectable binding was observed for **114**, **115** and **116**. The fluorescence titration of **112** versus **113** was also repeated in a less competitive solvent system, $\text{CHCl}_3/\text{MeOH}$ (98:2). As expected, the binding constant was much higher under such conditions ($6.4 \times 10^4 \text{ M}^{-1}$). Moreover, weak but unambiguous induced CD was observed when **113** was added to receptor solution in $\text{CHCl}_3/\text{MeOH}$ (98:2), although signals were too noisy for quantitative analysis. Once again, there was no binding observed with **114**, **115** and **116**. All results support high selectivity for **113**.

Since the solubility properties of **108** was low in organic solvent and did not permit carbohydrate extraction studies from aqueous solution, a new variant **117** with a highly lipophilic exterior array of 12 benzylxylo substituents was designed (Figure 37).³⁸⁴ In homogeneous solution, **117** shows similar binding affinity as that of **110** with K_a of 720 M^{-1} in $\text{CDCl}_3/\text{MeOH}$ (92:8) and 980 M^{-1} in $\text{CDCl}_3/\text{MeOH}$ (98:2). In extraction experiments, three hexoses (glucose, galactose and mannose), two pentoses (ribose and xylose) and two methyl glucosides were all extracted into chloroform from 1 M aqueous solutions. Receptor **117** even showed notable affinity and selectivity for glucose by extracting detectable amount even from 0.1 M aqueous solutions. In contrast, there was no extraction observed for other sugars under the same condition (0.1 M).

In 2005, Davis and co-workers reported another variant of **108**, receptor **118** (Figure 38), with dodecacarboxylate side chains to provide the opportunity to study carbohydrate recognition in homogenous aqueous solution.³⁸⁵ It is important to note that this is the first tricyclic polyamide receptor that shows binding affinity in aqueous solution. Using an NMR titration method with freshly dissolved glucose with an anomeric ratio (α/β) of 72:28, a K_a of 4.6 M^{-1} was obtained in D_2O . For glucose equilibrated overnight, which gave an α/β ratio of 40:60, the binding constant was 9.2 M^{-1} . Such results suggest possible selectivity for the β anomer. The binding of **118** with other substrates was also studied (Table VII). The studies with glycosides also show a notable preference for β -glucose units. For example, the binding affinity for methyl- β -D-glucoside is 27.3 M^{-1} . In contrast, the binding affinity for methyl- α -D-glucoside is 6.9 M^{-1} .

The binding affinity of **118** was also determined by means of fluorescence spectroscopy. Similar results were obtained (Table IIX). Although the affinities of receptor **118** are relatively low, the selectivity observed is significant and suggests that further optimization could lead to improve non-boronic acid-based receptors for saccharides that are functional in aqueous solution.

Later on, Davis and co-workers successfully applied their strategy to all-equatorial disaccharides such as cellobiose, designed and synthesized a tetracyclic disaccharide receptor **119** (Figure 39) with good affinity and selectivity.³⁸⁶ Receptor **119** has two building blocks: a meta-tertphenyl structure providing the roof and floor and defining the length of the cavity, and isophthalamide units serving as pillars. Each pillar involves two amide linkages, with potential to form hydrogen bond with the OH groups in cellobiose. Besides, the pillars were also furnished with externally directed tricarboxylate units to promote solubility. Furthermore, five rigid isophthalamides were also incorporated to prevent the cavity from collapsing.

Binding was studied by using an NMR titration method, induced circular dichroism, fluorescence spectroscopy and calorimetry. Ten disaccharides and three monosaccharides were selected as models for selectivity test. Binding constants between receptor **119** and selecting carbohydrates were in the range of 5–910 M⁻¹ (Table IX). The most intriguing result is the excellent selectivity of receptor **119**. Minor changes to disaccharide structures could cause almost complete suppression of complex formation. For example, for the result of gentibiose, which contains an all-equatorial structure that is slightly longer than D-cellulobiose, the change from D-cellulobiose was enough to reduce the binding constant to 12 M⁻¹ from 580 M⁻¹. The NOESY contacts observed for the complex between β-D-cellulobiose and receptor **119** and the computational model of the complex gave a good sandwich-like, 2:1 binding model (Figure 39). It features eight intermolecular hydrogen bonds and about ten CH-π interactions, giving a persuasive explanation for the high binding affinity and selectivity.

3-B-5. Other receptors belonging to different structural classes—The Yu lab reported two relatively simple receptors based on a naphthyridine core: 2,7-di(3'-pyridyl)-1,8-naphthyridine (DPN, **120**, Figure 41) and 2,7-di(3'-quinolyl)-1,8-naphthyridine (DQN, **121**, Figure 41). Both **120** and **121** possess a naphthyridine core moiety, in which two pyridine nitrogen atoms serve as the hydrogen bond acceptors. The binding studies were conducted using fluorescence and UV-vis. Binding constants of about 500–9.0 × 10³ M⁻¹ were obtained for β-D-fructoside, α-D-riboside, α(β)-D-glucoside, α(β)-D-galactoside and α(β)-D-mannoside in CHCl₃. Although these compounds showed good binding affinity, none showed good selectivity. For example, the binding constants for β-D-glucoside and α-D-glucoside are 6.3 × 10³ M⁻¹ and 4.5 × 10³ M⁻¹ respectively. Indeed, the binding constants for all the specific sugars used as model saccharides are similar.

Based on the hypothesis that rigid, folding structures might be used as artificial receptors for molecular recognition or sensing, the Li lab designed two new oligoamides **122** and **123** (Figure 42).³⁸⁷ The oligomers were incorporated with phenyl subunits via amide linkages. The existence of three-centered hydrogen bonds in the oligomers and consequently the folding formation in solution were characterized by ¹H NMR. The binding affinities were investigated using ¹H NMR, fluorescence, and CD spectroscopy. The association constants obtained by fluorescence titration experiments for four selected saccharides were about 1.0 × 10³ – 7.0 × 10³ M⁻¹ with 1:1 binding mode in chloroform. The results support the hypothesis that the new folding structures can efficiently complex saccharide derivatives in chloroform through intermolecular multiple hydrogen bonds.

Lipopolysaccharides (LPS) are the major constituents of the outer cellular membrane of Gram-negative bacteria, which are also known as bacterial endotoxin. They play an important role in the septic shock syndrome.³⁸⁸ The LPS of Gram-negative bacteria normally have a conserved portion, the oligosaccharide core and lipid A part. Lipid A part has an anionic amphiphilic nature, which is able to bind with cationic hydrophobic ligands to neutralize the toxicity of the bacteria. Based on the property of Gram-negative bacteria, Cunsolo and co-workers design and synthesized three calix[4]arene-based ligands as endotoxin receptors, which could be used as potential antimicrobial agents **124**–**126** (Figure 43).³⁸⁹ A tetraproxy-tetraamino-calix[4]arene was chosen as the amphiphilic building block. Tyrosine and tryptophan amino acid residues were used to enhance the hydrophobic and basic properties. Moreover, a 6-amino-hexanoyl arm was designed as a spacer between the calix[4]arene scaffold and the acyl active residue to endow the receptors with high conformational mobility for induced fit recognition of substrates.

Binding of these receptors towards LPS of Gram-negative bacteria was studied by NMR and UV titrations. However, no binding constant was obtained by these two methods. The author

suggested that it might be due to non-specific binding caused by the high stoichiometric ratios. For compound **125**, the molar ratio was 3:1 and 10:1 respectively between the receptor and LPS in the NMR and UV titration experiments.

Another application is the development of highly selective recognition of diols by a self-regulating fine-tunable methylazacalix[4]pyridine (**127**, MACP-4, Figure 44) cavity.³⁹⁰ Gong and co-workers reported the highly selective recognition of diols and benzenediols by MACP-4. The central hypothesis is that the multi-pyridine-containing feature might serve as a strong hydrogen-bond acceptor and form a complex with diols and benzenediols through intermolecular hydrogen bond. Pyridine rings could also serve as other binding units through π - π stacking and CH- π interactions. Besides, the fine-tunable cavity of MACP-4 might be able to recognize marginally different diol and benzene diol derivatives based on the property that it can self-regulate its conformation during binding. For example, the inversion of MACP-4 by resorcinol (1, 3-benzenediol) could be observed by using dynamic ^1H NMR spectroscopic study (Figure 45). Such properties might be attributed to the decreased activation energy of the transition state B in the presence of excess amount of resorcinol.

Molecular recognition of MACP-4 towards various diols was investigated by using ^1H NMR and X-ray diffraction analysis. MACP-4 exhibited excellent selectivity in the recognition of resorcinol (1, 3-benzenediol) with a binding constant of $6.0 \times 10^3 \text{ M}^{-1}$ in CDCl_3 . A very stable 1:1 sandwich complex was formed and the predominant driving force for the formation is the intermolecular hydrogen bonding between the pyridine nitrogen atoms of the host and the hydroxyl groups of the guest.

3-C. Metal chelator-based sensors

Another important approach for carbohydrate recognition is the coordination of a carbohydrate ligand to a metal center. For example, in nature, C-type lectins recognize saccharides in a calcium-dependent manner.³⁹¹ In this part, several carbohydrate sensors based on the idea of bearing both boronic acid for binding diols and metal-chelation for binding of other parts are discussed. Besides, simple water-soluble lanthanum and europium complexes, which are effective at detecting neutral sugars as well as glycolipids and phospholipids, and sugar discriminating binuclear copper complex, will also be mentioned.

Conversion of glucose to glucose-6-phosphate (G-6-P) and the inter-conversion between G-6-P and α -D-glucose-1-phosphate (G-1-P) are essential steps to produce glycogen.³⁹² Thus selective recognition and detection of G-6-P and G-1-P could be used in the study of relevant biological processes. Shinkai and co-workers developed an artificial receptor with high selectivity using boronic-acid-appended zinc (II) porphyrin (**128**, Figure 46).³⁹³ When zinc (II) porphyrin was bound to G-6-P, the δP value in ^{31}P NMR markedly shifts to a higher magnetic field. A strong excitation-coupling band also appeared in CD spectroscopy. However, such big changes are not observable for zinc (II) porphyrin with D-glucose, G-1-P and for boronic-acid-appended porphyrin in the presence of these glucose derivatives. Such result was thought to be due to two reasons: (1) the phosphate in G-6-P can interact with the central metal zinc, (2) the 1,2-diols can bind with the boronic acid.

An artificial receptor for the detection of uronic acids and sialic acids was also developed by the Shinkai lab (**129**, Figure 47).²¹⁵ Uronic acids play an important role in the oxidation process of monosaccharides or in the biosynthetic process of L-ascorbic acid, while sialic acids are the recognition unit by influenza viruses. The central idea for the design of artificial receptor is based on both boronic acid recognition of diols and Zn recognition of the carboxylate unit of the respective analytes.

Specifically, the Zn (II) complex of new receptors **130** and **131** were also developed by the Shinkai lab (Figure 48).²¹⁵ They contain a fluorescent group bearing both an o-aminomethylphenyl boronic acid group for diol binding and the 1, 10-phenanthroline-Zn (II) chelate moiety for carboxylate binding. The association constants (determined in water:MeOH = 1:2 at pH = 8) of **130** were better than that of compound **131**. For example, the log K_a of **130** for D-galacturonic acid is 3.1 in comparison with that of **131** (1.9). The results of **130** also showed some selectivity for uronic acids in aqueous solution, about 2.5:1 in log K_a over D-galactose. The studies were further extended to sialic acids, which also showed similar affinity (log K_a = 2.3 for **130** and selectivity 1.7:1 in log K_a over D-galactose). In conclusion, the described receptor showed a good example for the design concept utilizing cooperative actions of boronic acid and a metal chelate, which can be used for further application.

Inspired by calcium-saccharide interactions found in C-type lectins, the Strongin group successfully developed lanthanum and europium receptors **132** and **133** (Figure 49)⁸² based on the similar properties of lanthanides and calcium.³⁹⁴ The utility of water-soluble salophene-lanthanide complexes addressed three current challenges. (1) the detection of neutral carbohydrate at physiologically relevant pH; (2) the selective detection of gangliosides; (3) the selective detection of lysophosphatidic acid (LPA), which is a biomarker for several pathological conditions including ovarian cancer and is selectively detected by the europium complex even in human plasma samples. The author proposed that the 2-sn-OH moiety of LPA might play a key role in promoting binding to the metal center. Besides, the europium complex showed good selectivity between sialic acid-containing gangliosides and asialoganglioside at physiologically relevant pH value without the interference of other molecules found in common brain ganglioside and phospholipids extracts. The author attributed the selectivity to the cooperative complexation of the oligosaccharide and sialic acid residues to the metal center.

Another example for the metal chelator-based sensor is a sugar discriminating binuclear copper (II) complex **134** developed by Striegler and co-workers (Figure 50).^{34,395} In this work, complex formation between carbohydrates and the Cu complex was investigated by a combined approach of UV/vis and CD spectroscopy. The copper complex showed good discrimination of different carbohydrate such as mannose and glucose due to different binding complexes (**135**, **136**, Figure 50). However, it was only functional at high pH value (>12), which will put a sever limit on potential applications.

As a summary for this section, Table X lists the non-boronic acid-based sensorsdescribed together with their salient binding properties.

4. LECTINS AND CARBOHYDRATE RECOGNITION

In addition to the man-made molecules described in previous sections that can recognize carbohydrates, nature also has its own set of readily available carbohydrate-binding proteins, which can be used for carbohydrate recognition in lab research and potential clinical applications. Currently, several hundred lectins have been identified, and about 60 lectins are commercially readily available from companies such as Sigma-Aldrich and Fisher Scientific. They all have certain specificity based on the overall topology and sugar compositions. For example, there are lectins that recognize poly mannose saccharides and others that can recognize galactose connected to different structures (Table XI). However, almost all lectins have cross reactivity issues. There are many good reviews and books on lectins in general.^{396,397} Therefore, readers are referred to those comprehensive sources for general matters related to lectins. This following section will not try to be comprehensive. Instead, we will focus on ways that lectins can be used for carbohydrate recognition in

research and for potential clinical applications. We will also discuss the major issues to address and potential pitfalls.

Before the discussion of using lectins in biological and analytical analysis, it is important to give a brief overview of the structural basis for lectin binding. The legume lectin family is the most widely researched lectin group and much of what is known about lectin binding mechanism comes from the study of these lectins. The legume family shows a wide range of specificity for carbohydrates as compared to other lectin families. Here only the salient features of lectin-carbohydrate interactions are presented. The structural basis of the binding of lectins of the legume family has been reviewed.³⁹⁸ A few other good reviews have also been written about the mechanism of lectin binding.³⁹⁹⁻⁴⁰⁰ Therefore readers are referred to these reviews for more in depth discussions.

It is interesting to note that in boronlectins, the strong and reversible interactions between the boronic acid group and hydroxyl groups play a key role in binding. In contrast, lectin binding relies mostly on hydrophobic and hydrogen bond interactions. The carbohydrate binding sites of lectins are typically a shallow depression on the lectin surface. There are four amino acids that are generally conserved in all lectins regardless of their binding specificity; these are asparagines, aspartic acid, glycine (arginine in Con A) and an aromatic residue. Site-directed mutagenesis in which aspartic acid and asparagines residue is replaced results in loss of the lectin-carbohydrate binding ability.³⁶ The actual carbohydrate binding site consists of four loops that are typically named A, B, C, and D.³⁹⁹ Aspartic acid is in loop A, glycine in loop B, while asparagines and the aromatic or hydrophobic residue reside in loop C. The amino acids in loop D are highly variable in conformation, length and sequence and have a role to play in the specificity of a lectin. Lectins typically contain a Ca^{2+} and a transition metal ion (usually Mn^{2+}). The two cations are 4 Å apart and are near to the carbohydrate-binding site. These cations are thought to be important in positioning the amino acids residues that interact with the carbohydrate. The correct orientation for the asparagine residue is mediated by a *cisoid*-peptide bond between the asparagine and the preceding amino acid.³⁴³

The binding of the lectin to the carbohydrate involve a number of hydrogen bonds as well as hydrophobic interactions. For example, the X-ray crystal structure of Con A with mannose shows in detail the typical interactions of legume lectins with carbohydrates.⁴⁰¹ The main features of the carbohydrate-lectin interaction of ConA involved key hydrogen bonds with conserved residues. These are an aspartate (Asp 208), that is preceded by the *cisoid* peptide bond and held in place by a water bridge with Ca^{2+} , an asparagine (Asn 14) residue that interacts with the calcium ion and the backbone NH of Arg 228 (in other lectins this is glycine). Hydrophobic interactions mainly involve a number of Van der Waals forces that contribute to the overall binding of the carbohydrate. This is demonstrated in which there is a Van der Waals interaction with aromatic residue Tyr 12 and the sugar ring as well as an interaction between the loop segment (Thr97-Glu102).

It is believed that lectin-carbohydrate interactions typically have moderate affinity. However, there have not been a vast number of methods in the literature used for studying the binding constants of lectins to their corresponding carbohydrates. Some recent examples of lectin binding constant determination using surface plasmon resonance (SPR), fluorescence polarization (FP), fluorescence correlation spectroscopy (FCS), and quartz crystal microbalance (QCM) techniques give a glimpse of the binding strength. In one study, high mannose glycosylasparagines were prepared and labeled with a fluorescence group such as the dansyl (Dns) group, fluorescein (Fl), or tetramethylrhodamine (TMR).⁴⁰² It was found that the association constants of Con A with Dns-Asn(M6)-OH and TMR-Asn(M6)-OH were 2.94×10^3 and $1.79 \times 10^3 \text{ M}^{-1}$, respectively. Similarly, the K_a values of a

mannose/glucose binding lectin, *Castanea crenata* agglutinin (CCA), for mannose, glucose and a-D-Man-(1→3)-D-Man were estimated to be $4.18 \times 10^2 \text{ M}^{-1}$, $1.39 \times 10^2 \text{ M}^{-1}$, and $2.07 \times 10^3 \text{ M}^{-1}$, respectively.⁴⁰³ Using QCM, the binding constants between ConA and maltose and Jacalin and fetuin were determined as $4.5 \times 10^2 \text{ M}^{-1}$ and $6.4 \times 10^4 \text{ M}^{-1}$, respectively.⁴⁰⁴ All these are modest binding affinities.

Lectin-monosaccharide binding is relatively weak. This is primarily due to the shallow binding site. However, lectins still show higher affinity for oligosaccharides. Therefore, multivalent interactions between lectin and carbohydrates are usually involved in a recognition event leading to the higher affinity. For example, there are 13 mannose residues in the carboxypeptidase Y (CaY). The association constant between Con A and CaY in saturation binding experiments was $2.7 \times 10^6 \text{ M}^{-1}$, which was much higher than binding with a monomer.⁴⁰⁵ There had been previous studies indicating the association equilibrium constants of Con A with oligosaccharides containing mannose in the range of 2×10^6 to $30 \times 10^6 \text{ M}^{-1}$, depending on the type of ligands studies studied.⁴⁰²⁻⁴⁰⁶⁻⁴⁰⁸ Overall, lectin-carbohydrate binding constants are commonly seen in the range of 10^2 – 10^6 M^{-1} range with monosaccharide binding in the lower range and polysaccharide/multivalent binding in the higher range.

Since glycosylation is an important and abundant post-translational and co-translational protein modification⁴⁰⁹ and carbohydrates have been shown to be important in tumor formation and metastasis, cell-cell adhesion, infection, autoimmunity and inflammation, there is a need for methods that allow for the examination of glycans individually as well as in an array format to allow for global glycan profiling. Developing methods to evaluate glycosylation can be very challenging due to the diversity and complexity of glycans.¹⁶⁵ There is currently limited technology to identify carbohydrates (glycans). Current methods for analyzing glycans such as western blotting, LCMS and MS requires advanced expertise, is time consuming and requires the glycans to be removed from proteins. Such methods only allow for the analysis of glycans individually. To examine a large number of glycans simultaneously, one will need to have arrays of carbohydrate “binders” with certain specificity and affinity. One such approach is the use of carbohydrate-binding lectins.

In recent years, there have been a number of examples of the use of lectin microarrays for recognition of glycosylation patterns. General information about the makeup of the glycans can be determined such as whether it is *N*-glycosylated, O-glycosylated, high-mannose, core-fucosylated and fully or partially sialylated. These microarrays can also monitor changes in glycosylation patterns associated with changes in cell-adhesion and tumor cell states.⁴¹⁰⁻⁴¹¹ The advantage of microarrays is that it allows for a profile or fingerprint to be taken of the carbohydrate landscape.

In one such example from the Mahal lab, nine common lectins were arrayed on aldehyde or epoxide derivatized glass slides¹⁴⁴ and the glycoproteins to be examined were labeled by conjugation to lysines carrying a fluorescent dye, Cy3. The array was then used to analyze the protein ovalbumine that is known to have high mannose and/or hybrid *N*-linked glycans.⁴¹² The glycopattern observed showed positive signals for the lectins WGA, ConA and GS-II. This was consistent with the known glycosylation pattern for ovalbumine since these three lectins are known to bind to *N*-linked glycans and ConA to mannose. This pattern is visible to the detection limit of 10 µg/mL. In order to test the ability of the microarray to differentiate between different glycans, the glycopatterns for ovalbumine was compared to that of two other glycoproteins, bovine submaxillary mucin (BSM) and porcine gastric mucin (PGM). The observed pattern for ovalbumine is different from that of BSM or PGM. The observed glycosylation patterns using this nine-lectin array demonstrate its ability to distinguish minimal differences in glycosylation of proteins.

This group utilized this same microarray method to monitor cell-surface glycosylation of *Escherichia coli*.⁴¹³ The glycosylation of bacterial cell surfaces has been shown to be important in symbiosis, immunity and cell-cell interaction.¹⁴⁴⁻¹⁴⁴ However, the limitations of currently available techniques hinder a more detailed examination of the role of glycans in these processes. The method presented by the Mahal group uses a lectin microarray approach to analyze bacterial cell-surface carbohydrates in an efficient manner. The lectin microarray consisted of 21 lectins. Each of the glass slides were printed with 14 isolated subarrays containing five spots per lectin using a SpotBot Arrayer (95 μM spot size). They examined the binding of fluorescently labeled bacteria (stained by nucleic-acid dye SYTO 85) to the microarray. Significant differences were not observed for the uptake of the dye by different bacterial cell lines; however these strains showed unique binding patterns to the lectin arrays. This observation was especially significant for closely related *E. coli* strains JM101 and HB101 that could not be distinguished using traditional hemagglutination assays. The two showed reproducible differences in their lectin fingerprints. Both strains showed strong binding to lectins GSII, HPA and WGA, however only JM101 showed moderate binding to MAA and weak binding to BPA, DBA and ECA. They also examined two pathogenic bacteria *E. coli* RS218 (neonatal meningitis pathogen) and *Salmonella enterica* serotype *tryphimurium* LT2. Both of these bacteria showed binding to α -2, 3-IMAA and α -2-6-(SNA)-sialic acid residues as well as for α -1,2-fucose binding as indicated by lectin UEA I. The presence of sialic acids and fucose has been implicated in immune evasion of pathogens.⁴¹⁵ RS218 also showed binding to BPA, DBA, ECA and GSI. To further probe the specificity of these interactions, binding of certain lectins was inhibited by a known inhibitor; lactose.

The same microarray technology was also used to examine real-time glycosylation changes in bacterial surfaces. It is known that alterations in bacterial carbohydrates occur in response to environmental stimuli and play a role in pathogenesis of bacteria such as *E. coli* and *Salmonella*.⁴¹⁶ Therefore, glycosylation of RS218 was monitored as a function of growth, where OD₆₀₀ of less than 0.4 represented the lag phase, OD₆₀₀ between 0.4 and 2.0 were considered exponential growth phase and OD₆₀₀ above 2.0 was the stationary phase. A decrease in the binding of all positive lectins was observed associated with bacterial growth. These results suggest a possible role of glycosylation in growth dependent invasion of RS218.

Although a very useful microarray technology, several limitations were indicated. Firstly, only accessible carbohydrate motifs rather than the entire glycome were observed using the microarrays, though the most relevant glycans are typically on the accessible surface. Secondly, there is a lack of availability of lectins that recognize unique bacterial sugars. We hope that as more lectins that bacteria recognize are discovered, so will the detection capabilities of this array technology. Despite the limitations, the microarray technology presented can provide a powerful tool to quickly determine changes in surface glycans of bacteria in response to environmental stimuli.

Conventional microarray methods already discussed can analyze microgram quantities while the use of evanescent-field fluorescence detection microarray technology⁴¹⁷⁻⁴¹⁸ offers a more sensitive method of glycoprofiling using lectin microarray technology with the ability of analyzing picogram quantities of glycoprotein samples.¹⁴⁰ Essentially, an electromagnetic wave called an evanescent-field is propagated within a wavelength distance from the sensor surface (~100–200 nm) in the lower refractive index sample medium. The evanescent-field technology is able to analyze relatively weak interactions (such as those between lectins and glycoproteins with K_d values in the 10⁻⁴ – 10⁻⁷ M range) because it can detect ongoing interactions under equilibrium conditions *in situ* without the need to wash after a probing reaction. The evanescent field lectin microarray has been applied to profile

glycans of Lec mutants.⁴¹⁹ In this application, cell glycan analysis was performed for Chinese hamster ovary (CHO) cells and their glycan profile was compared to those of their glycosylation-defective Lec mutants. Lec1 (defect in GlcNAc-T1), Lec2 (CMP-sialic acid transporter) and Lec8 (UDP-Gal transporter) were chosen since their biosynthetic features are well characterized. The Lec mutant and CHO cells were grown harvested and washed with PBS to remove any glycoprotein containing ingredient. The cell-surfaces were then labeled with Cy3-succimidyl ester, and a 20 µL aliquot of each sample was subjected to lectin microarray. The result of the lectin microarray of the CHO cells and the Lec mutants each showed different profiles. Lec1 showed a decrease in signals for a number of lectins such as MAL, ECA, RCA 120, PHS (L), PHA (E), WGA, DSA and LEL and an increase in signals for GNA and HHL. The similarity among the profiles were evaluated using correlation coefficients, that is, a high similarity is indicated by a correlation coefficient higher than 0.9 and a low similarity if the coefficient is lower than 0.5. Keeping these coefficient limits in mind, the resemblance of CHO and Lec1 cells is relatively low with a correlation coefficient of 0.57 whereas Lec2 is relatively similar with a coefficient of 0.81. Lec8 showed the biggest difference among the Lec mutants with a coefficient of 0.46. Lec 8 showed significant decreases in signal for MAL, ECA, RCA120, ACL and PHA (E) which recognize non-reducing terminal sialic acid or galactose. In addition, the Lec 8 mutant showed increased binding with WFA, ABA, EEL, NPA and SBA lectins. These results were compared to results from flow cytometric analysis and glycosidase digestion with neuraminidase. For the latter technique the cells were washed extensively after treatment with neuraminidase and before Cy3 labeling, resulting in a substantial change in glycan profile for CHO cells. On the other hand, no difference were observed for any of the three Lec mutants which can be explained by the fact that Lec mutants do not have sialic acid and are not affected by neuraminidase treatment.

For flow cytometry, 11 lectins were used as well as anti-Tn (α GAI^NAc) antibody. Both lectin and flow cytometry gave similar results; however this lectin microarray has an advantage over flow cytometry because a number of lectin-biding experiments can be carried out at the same time with increased sensitivity. Their results determined that evanescent field microarray technology can be applied to glycan profiling of crude samples.

To address the need for better interpretation of the resulting glycan fingerprints created by the lectin microarray, one group developed a convenient and rapid lectin-microarray method, Qproteome™ GlycoArray kit. In this method, glycoanalysis is performed on intact glycoproteins that require about 4– 6 hours in most cases. The major advantage of this kit over other methods is that it uses a set of proprietary algorithms that can provide analysis of the resulting histogram with the click of a mouse.⁴²⁰ The technology consists of arrays of 24 plant lectins with overlapping specificities. Binding of a glycoprotein to the array results in a characteristic fingerprint of glycan composition of the protein. The use of such a large number of lectins ensures a high sensitivity to changes in glycosylation pattern. The lectins were spotted in duplicate arrays on nitrocellulose membrane-coated glass slides with 6 replicate spots each and the array was then incubated with intact glycoprotein. The arrays are scanned and the resulting images are analyzed using propriety image analysis software that converts the images into a fingerprint, that is, a histogram of the signal obtained for each lectin. The robust average of the signals is obtained from the 6 replicates and the fingerprint is interpreted by proprietary knowledge-based algorithms. The algorithm creates a list of epitopes with their relative abundance. To develop the algorithm, the specificity of lectins was determined by studying their binding to a large collection of carefully characterized glycoproteins. The glycoproteins in which literature information was not available were fully characterized by mass spectrometric and chromatographic methods. From this information, thousands of fingerprints were created of several hundred glycoform mixtures and resulted in detailed specificity definitions for many lectins. The performance of

this microarray analysis kit was demonstrated by the analysis of a series of glycoproteins and their glycovariants. For example, the glycoprofile of a well-studied protein bovine pancreatic ribonuclease B (RNase B) was examined that contains a single *N*-linked glycosylation site at Asn34 and also contains high-mannose-type-*N*-linked glycans. Glycoanalysis of RNase B showed the presence of high levels of high-mannose type *N*-linked glycans only, which is in agreement with literature data. Strong signals were observed for Glc/Man-and Man-recognizing lectins and essentially no signals for complex-type and antenna-termini-recognizing lectins. The glyco-array kits method also stayed consistent to literature data in the analysis of prostate-specific antigen, porcine thyroglobulin, Tamm Horsfall glycoprotein and recombinant human erythropoietin. This method has been provided as a useful tool for glycobiologists for more detailed analyses and characterization of their proteins of interest.

The use of lectin microarray technology has far reaching implication in the understanding the role of glycans in cell processes and diseases. This technology can be especially useful in understanding the changes in glycosylation that accompany some diseases. Advances in interpreting glycan profile fingerprints with algorithms have improved the gathering of structural information that will enhance this field.

Because of the ability for lectins to specifically recognize certain glycan biomarkers, the potential of using them for targeting has been recognized.⁴²¹⁻⁴²³ Glycotargeting using lectins have applications in antiviral-therapy⁴²⁴ and anti-tumor therapy.⁴²⁵⁻⁴²⁷ Lectins have been explored for application in selective drug targeting systems. Since carbohydrates are the most abundant modification in cells and lectins have the ability to bind to and recognize terminal sugar residues, they provide a means to introduce small drug molecules into cells. A comprehensive review of lectins in drug delivery has been presented by Woodley and co-workers.⁴²⁸ Glycotargeting with lectins is exemplified by the potential use of lectins as anti-HIV therapies.

The increased risk and fatalities associated with viral diseases such as hepatitis C and HIV-1 demands the development of novel anti-viral treatments, especially since current therapies have only limited success. Carbohydrates on viral envelopes play an important role in viral entry into the cells. Therefore, if an agent can bind to these glycans it may interrupt the function of the virus. Many such avenues have been explored in which lectins are used to develop anti-HIV therapy. The infection of cells by HIV-1 requires the fusion of the viral membrane with cellular membranes. This fusion is mediated by viral envelope proteins such as gp120 and gp41 along with cell surface receptors (CD4 and chemokine receptor) on the target cells⁴²⁹. Therefore, the viral envelope proteins are attractive targets for anti-HIV-1 therapy. Agents that interact with the viral envelope may interfere with the entry into target cells. The binding of lectins to the glycans on the viral envelope may also force the virus to delete a portion of its glycans shield, making the virus more susceptible to attack.¹⁰²

Several lectins had been isolated, identified and studied for anti-HIV activity. Cyanovirin (CV-N) is a 11kDa protein with 101 amino acids. It is isolated from the cyano bacterium *Nostoc ellisporosum*⁴³⁰ and has affinity for high-mannose glycans especially α -(1,2)-linked mannose oligomers.^{431,432} CV-N inactivates T-lymphocyte-tropic, laboratory strains of HIV type 1 and HIV type 2, as well as T-tropic, M-tropic and dual tropic primary clinical isolates of HIV-1. The anti-HIV activity of CV-N is related, in some part, to its binding to envelope protein gp120. As a result, it prevents in vitro fusion and transmission of HIV-1 between infected and uninfected cells. Continuous treatment of uninfected CEM-SS cells in the presence of high concentrations (9000 nM) of CV-N did attenuate the lethal effect of the virus. CV-N had an anti-HIV activity in CEM-SS cells with an EC₅₀ of 0.1 nM. SVN (scytovirm) is a 9.7kDa peptide with 95 amino acids and has been shown to have affinity for

$\alpha(1,2)$ - $\alpha(1,6)$ -mannose trisaccharide units. SVN inhibit HIV infection in T-tropic laboratory strain HIV-1 in CEM-SS cells with an EC₅₀ of 0.3 nM.⁴³³ According to ELISA studies performed, the anti HIV activity is related to its binding to the glycosylated viral core proteins gp120, gp160 and to a lesser extent gp41. However, SVN did not bind to the CD4 receptor. Additional experiments also showed that SVN had to be present in the first eight hours of virus infection for it to be effective. Also pretreatment and removal of SVN led to normal HIV infection in uninfected CEM-SS cells. Altogether these studies suggest that SVN interferes with binding/fusion mechanism of the virus.

The lectin actinohivin has been shown to recognize mannose-type glycans¹⁰³ and can inhibit T-cell and macrophage infection by HIV-1 in cell culture. Actinohivin inhibits T-tropic and M-tropic syncytium formation in HeLa/T-env/Tat and HeLa/CD4/Lac-Z cells with an IC₅₀ of 60 nM and in HeLa/M-env/Tat and HOS/CD4/CCr-5/Lac-Z cells with an IC₅₀ of 700 nM.

There are many other similar examples. For example Gerardia Savaglia (GSA) is a D-mannose – calcium dependent specific lectin dimer that has shown complete suppression of HIV-1 infection in the H9 cell line at a concentration of 0.2 μ M¹⁰³ and plant lectins from *Galanthus nivalis* (GNA) and *Urtica dioica* (UDA) have activities against HIV-1 in CEM cell cultures with an EC₅₀ of 0.01 and 0.1 μ M respectively.^{101,424,434,435} A lectin derived from an invertebrate *Chaetopterus variopedatus* (CVL) is specific to β -galactose and has an anti HIV activity in the range of 0.004–0.06 μ M.⁴³⁶

All the examples above demonstrate that indeed binding of glycans important in pathological processes is a feasible strategy for developing therapeutics. If small molecule glycan “binders” can be developed as described in the previous sections, they will also have the potential as therapeutic agents.

Overall, lectins are also very useful tool and potential therapeutics due to their ability to recognize carbohydrates with certain specificity. One potential problem is the size and protein nature of lectins, which may present delivery problems and may limit their application *in vivo* and under harsh conditions where stability might be an issue.

5. CONCLUSIONS

Carbohydrates are known to play important roles in a large number of biological and pathological processes. Therefore, high affinity and specificity “binders” for biologically important carbohydrates are potential medicinal and diagnostic agents. They can also be important research tools. The use of lectins in various applications has shown some initial promise. Progress in recent years in boronic acid-based carbohydrate binders (boronolectins), non-covalent binders, and metal-based binders have laid an excellent foundation for the future development of medicinal and diagnostic agents based on carbohydrate recognition. Especially important and new are ways of making peptide-based boronolectins and nucleic acid-based boronolectins, which give tremendous modularity and structural diversity for a wide range of application. The use of boronic acid-modified DNA aptamers for glycoproteins with the ability to differentiate glycoforms represents a very significant progress in glycoprotein detections. However, it is also true that peptide- and nucleic acid-based boronolectins may only be useful for *in vitro* applications and for targeting of cell surface biomarkers because of their potential membrane permeability problems. In this regard, small molecule lectin mimics including boronolectins continue to hold an advantage for intracellular applications. Because of the need for carbohydrate “binders” for a wide range of applications including concentration determination, cell-surface labeling, as diagnostic agents, delivery of imaging agents to cell surface, binding to

cell surface carbohydrates involved in pathological events (such as metastasis) for therapeutic applications, and intracellular recognition and labeling, all the different methods of developing carbohydrate “binders” discussed will be very useful. We hope with this review, we can pursue more people in the carbohydrate sensing field to work on problems directly related to therapeutic and diagnostic applications.

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Biographies

Binghe Wang was born in 1962 in Beijing, China. He obtained his B.S. degree in medicinal chemistry from Beijing Medical College (Now Beijing University Health Sciences Center) in 1982, and his Ph.D. degree in medicinal chemistry from the University of Kansas, School of Pharmacy, in 1991. Subsequently, he did postdoctoral work with Professor Victor Hruby of the University of Arizona and Professor Ronald T. Borchardt of the University of Kansas. He started his independent career in 1994 as an Assistant Professor of Medicinal Chemistry at the University of Oklahoma, College of Pharmacy. In 1996, he moved to the Department of Chemistry, North Carolina State University, and was promoted to Associate Professor with tenure in 2000. In 2003, he moved to his current institution, Georgia State University, as Professor of Chemistry, Georgia Research Alliance Eminent Scholar in Drug Discovery, and Georgia Cancer Coalition Distinguished Scientist. He is Editor-in-Chief of Medicinal Research Reviews, published by John Wiley and Sons, and the chief editor of a book series entitled "Wiley Series in Drug Discovery and Development." His research expertise includes drug delivery, drug design and synthesis, bioorganic chemistry and molecular recognition, fluorescent sensor design and synthesis, and new diagnostics. He has co-edited two books together with his colleagues: "Pharmaceutical Profiling in Drug Discovery for Lead Selection" by AAPS and "Drug Delivery: Principles and Applications" by John Wiley and Sons.

Shan Jin was born in Beijing, China. He received a bachelor's degree in Chemistry from Peking University in Beijing, China in 2004. In the same year, he joined Professor Binghe Wang's group at Georgia State University as a graduate student. He earned his M.S. degree from Georgia State University in 2007 and continued to pursue a PhD degree in the same group. His research interests focus on boronic acid-based carbohydrate sensors.

Yunfeng Cheng received his B.S. degree in chemistry from Zhejiang University of Technology in 2001 and M.S. degree in medicinal chemistry from Zhejiang University in 2004. He is presently a Ph.D. graduate student at Georgia State University. His current research directions are to study aptamer-based carbohydrate sensors and the antagonists of AI-2-mediated bacterial quorum sensing.

Suzette Reid received her B.S. degree in chemistry from Morgan State University in Baltimore, Maryland and her M.S. degree in Organic Chemistry from Georgia Institute of Technology. She is presently a Ph.D. candidate at Georgia State University. Her research involves the design and synthesis of novel small molecule inhibitors of the Hypoxia Inducible Factor (HIF) pathway.

Dr. Minyong Li is an adjunct assistant professor in the Department of Chemistry, Georgia State University. He received his BS and PhD degrees from China Pharmaceutical University in 1999 and in 2005, respectively. He began his research career in 2005 with Dr. Binghe Wang at Department of Chemistry, Georgia State University as postdoctoral research associate. In 2007, he was appointed adjunct assistant professor. He is a member of Sigma Xi-the Scientific Research Society (2006), America Chemical Society (1999) and QSAR and Modeling Society (2006). His research interests are in the general areas of medicinal chemistry and chemical biology. He has published about 30 peer-reviewed papers.

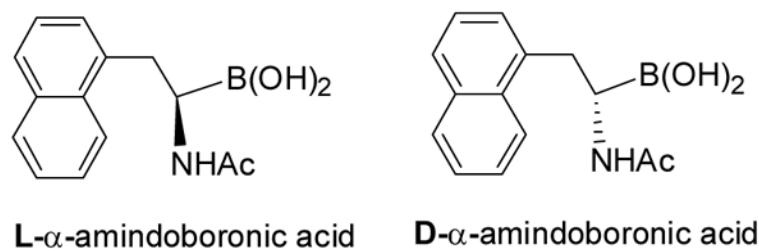


Figure 1.
Structures of an enantiomeric pair of α -amindoboronic acids studied for their binding with diols

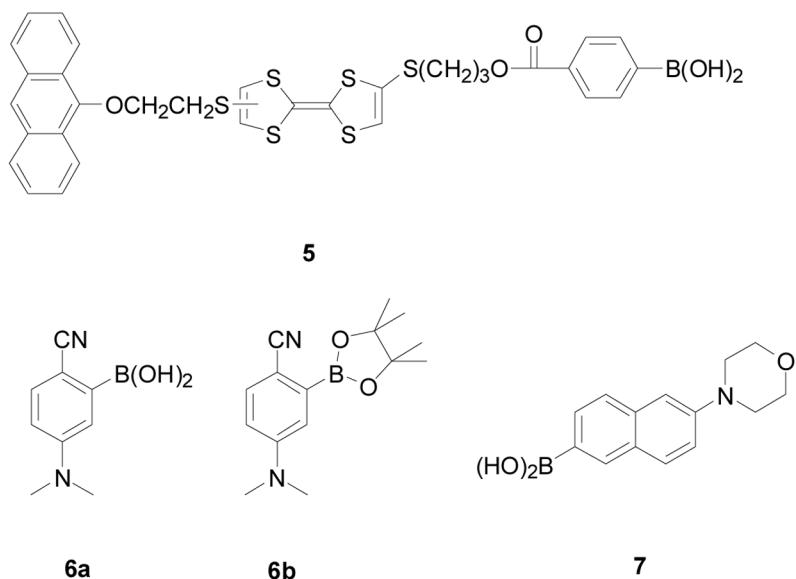


Figure 2.
Structures of boronic acid-based fluorescent reporter compounds **5–7**.

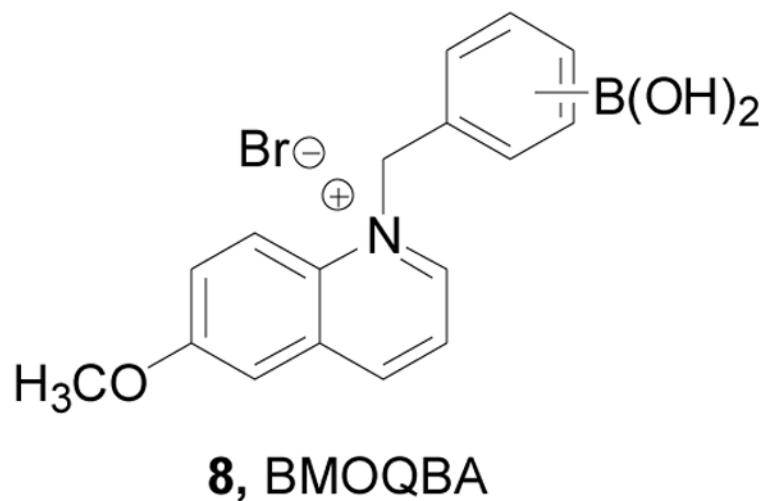


Figure 3.
Structure of compound **8**

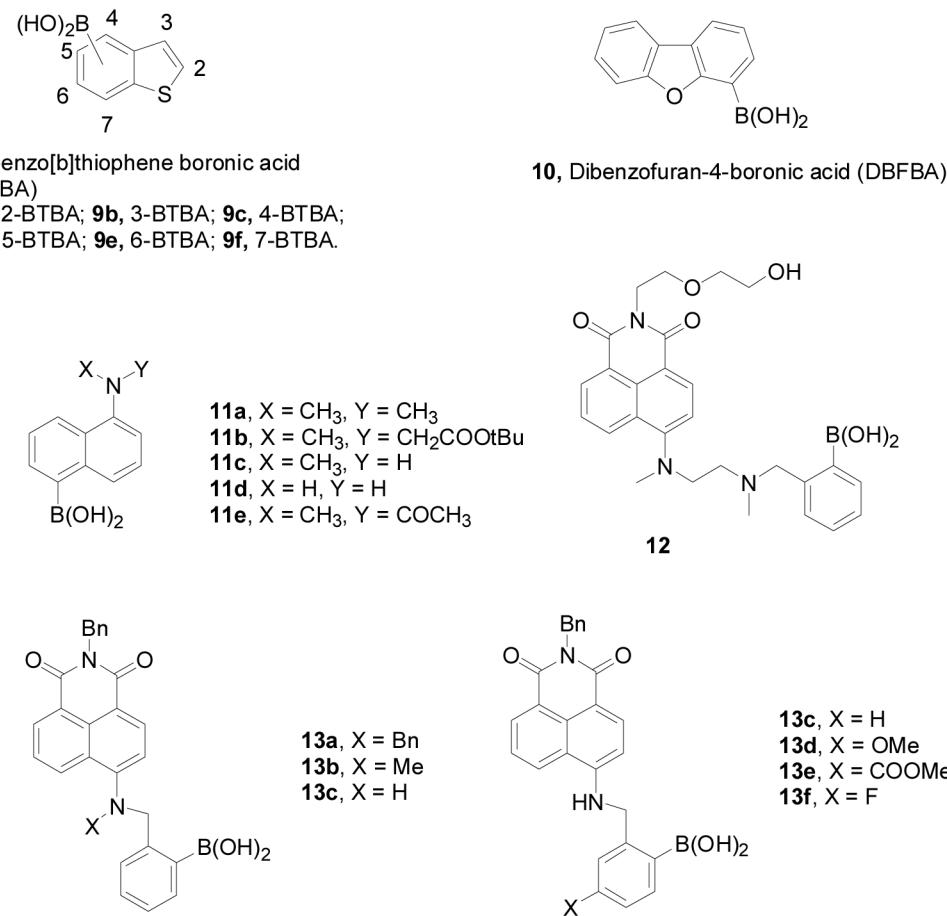


Figure 4.
 Structures of boronic acid-based fluorescent reporters **9–13**

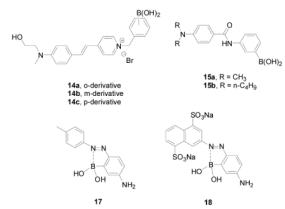


Figure 5.
Structures of boronic acid-based reporters **14–18**

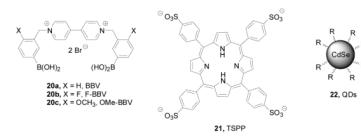


Figure 6.

Structures of compounds **20–22** in the Singaram glucose sensing system

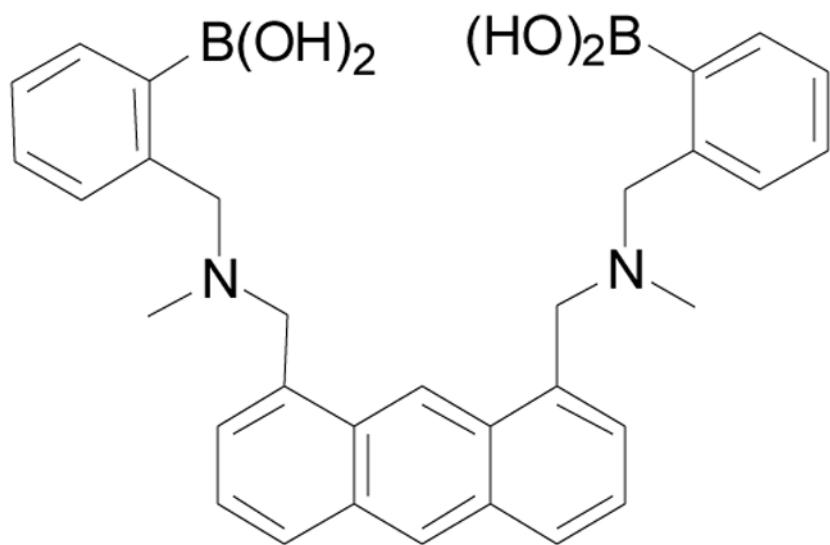


Figure 7.
Structure of compound 27

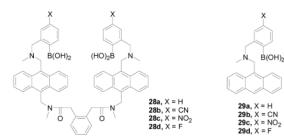


Figure 8.
Structures of boronic acid-based sensors **28–29**

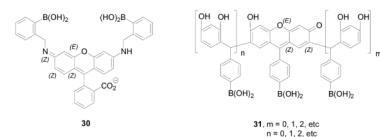


Figure 9.
Structures of boronic acid-based sensors **30** and **31**

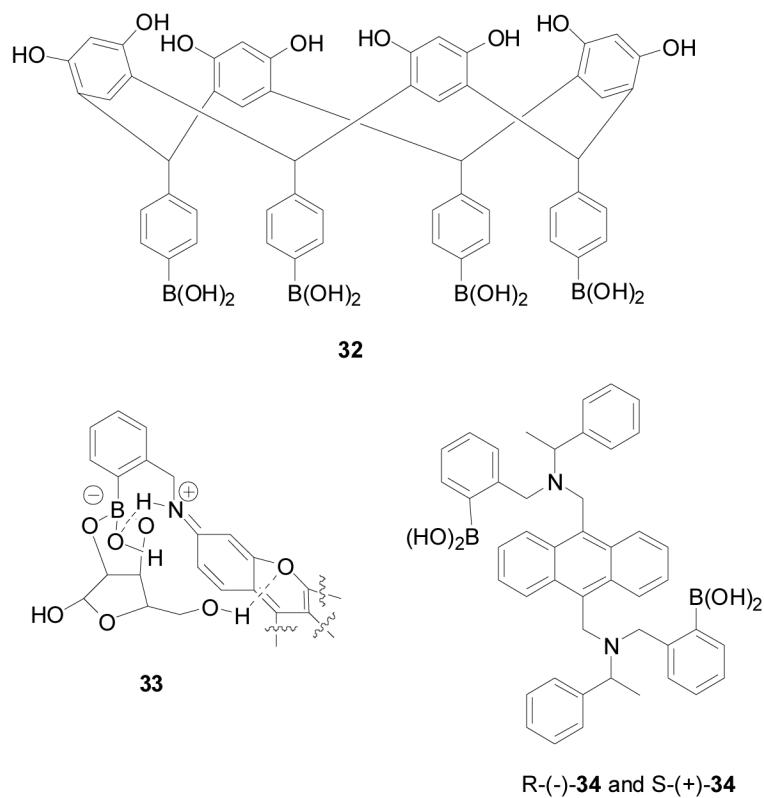


Figure 10.

Structures of boronic acid-based sensor **32**, complex **33**, and sensor **34**

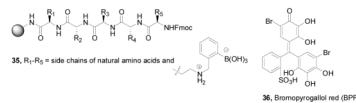


Figure 11.
Structures of boronic acid-based sensors **35** and BPR indicator **36**

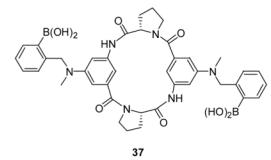


Figure 12.
Structure of boronic acid-based sensor **37**

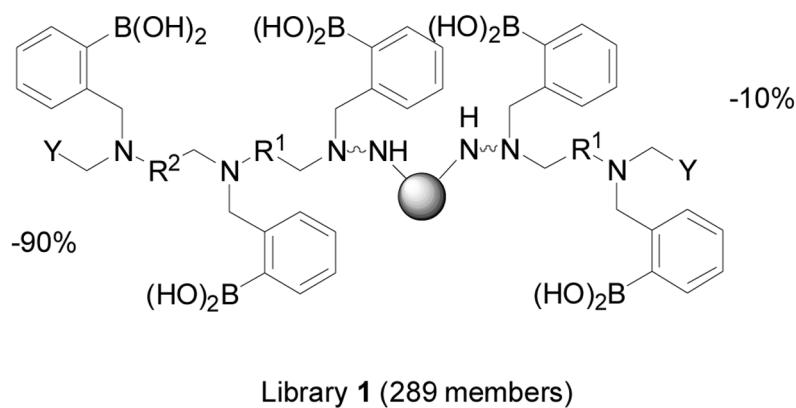


Figure 13.

Structure frame of a peptide library **1** with 289 members

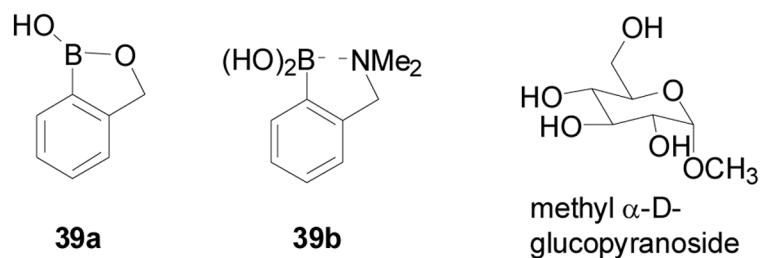


Figure 14.

Structures of ortho-hydroxymethyl phenylboronic acid **39a** and its dialkylamino (Wulff type) analogue **39b** as well as methyl α -D-glucopyranoside

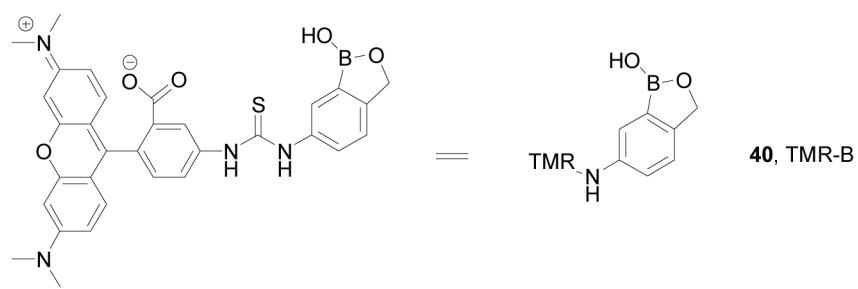


Figure 15.
Structure of tetramethylrhodamine-boronic acid (**40**, TMR-B)

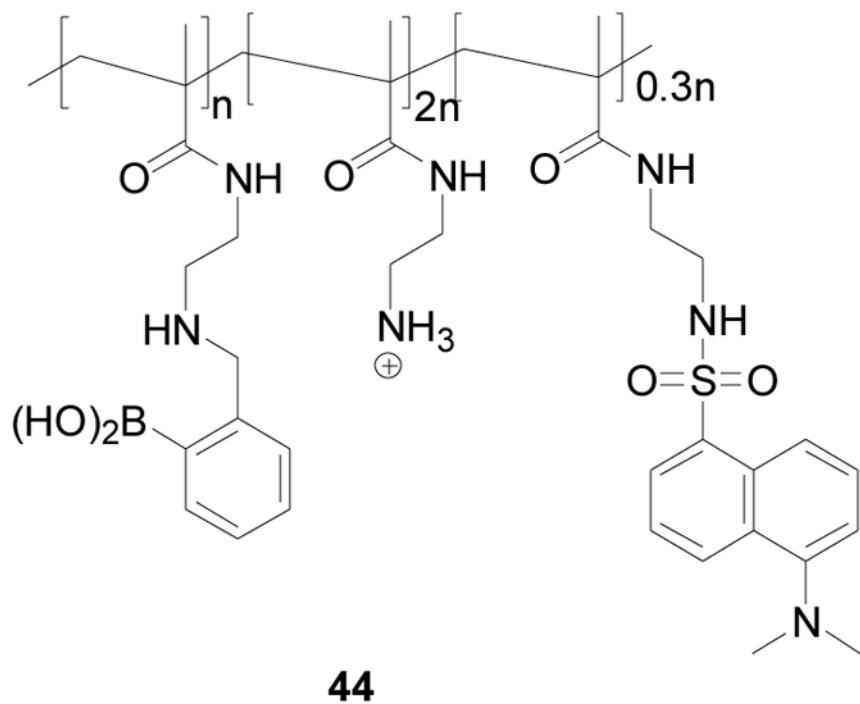


Figure 16.
Structure of polymer **44**

Phenylboronic Acid Monolayer

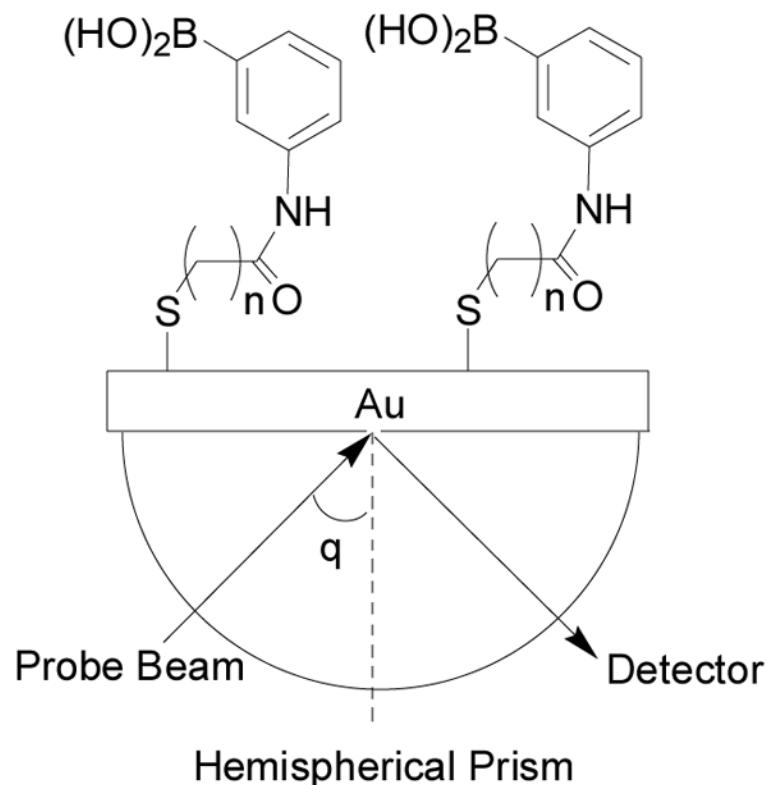


Figure 17.
Structures of boronic acid-based sensors on SAMs

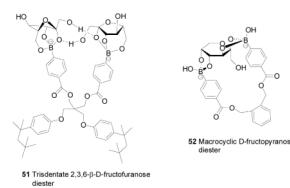


Figure 18.

Structures of a tridentate β -D-fructofuranose ester **51** and a macrocyclic β -D-fructopyranose diester **52**

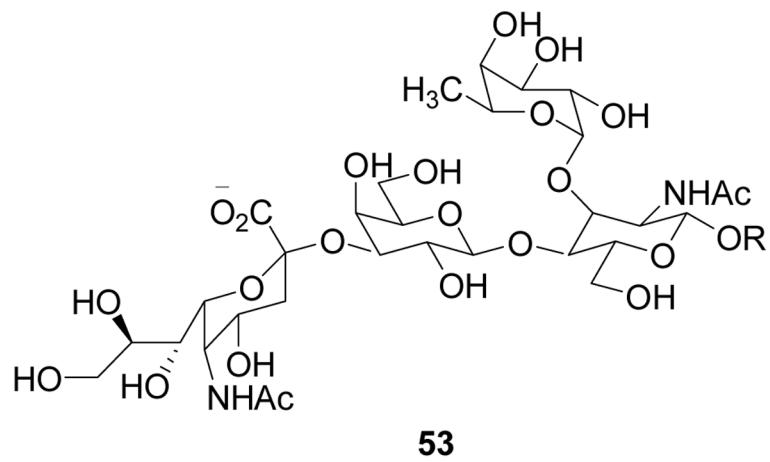


Figure 19.
sLe^x structure

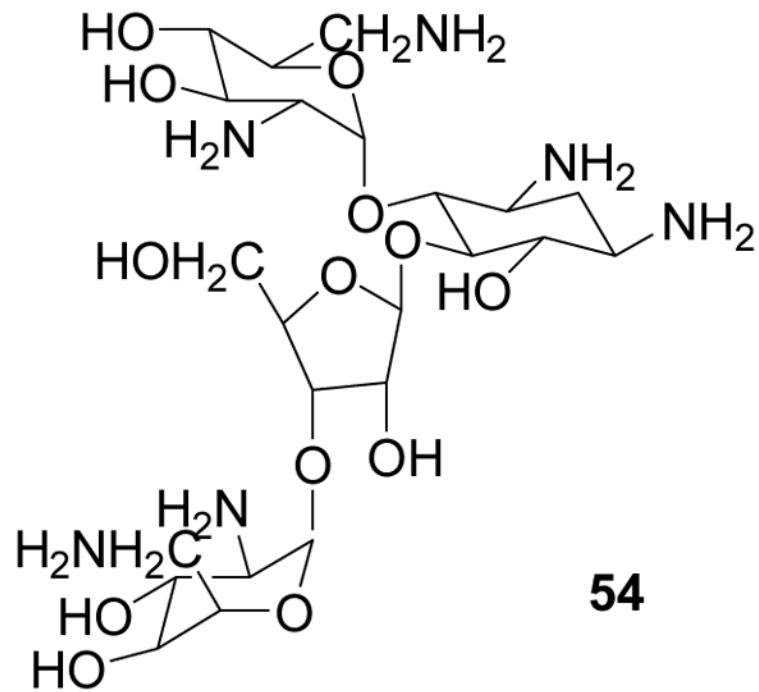


Figure 20.
Structure of neomycin B

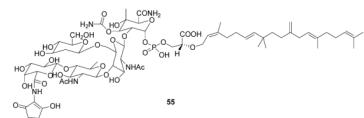


Figure 21.
Structure of moenomycin A

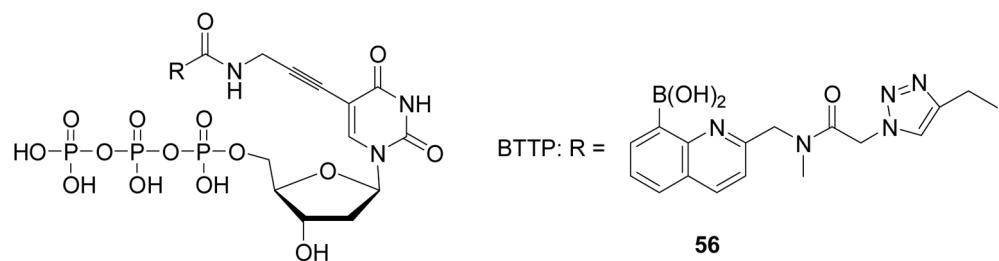


Figure 22.
The chemical structures of BTTP and MTTP

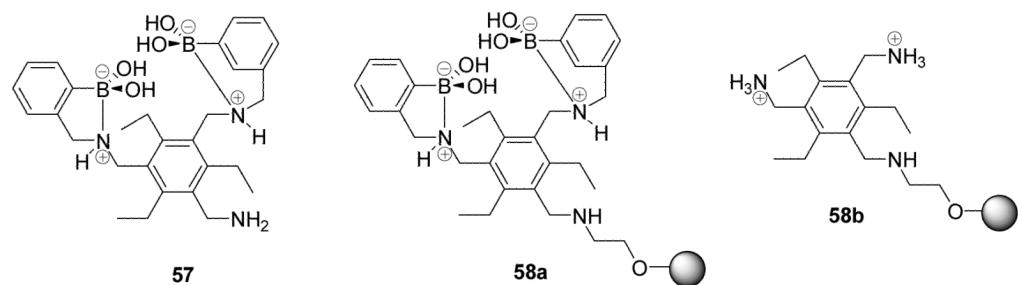


Figure 23.
Structures of compounds **57**, **58a**, and **58b**

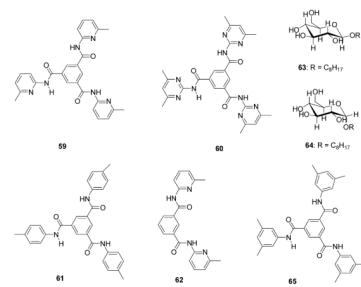


Figure 24.
Structures of compounds 59–65

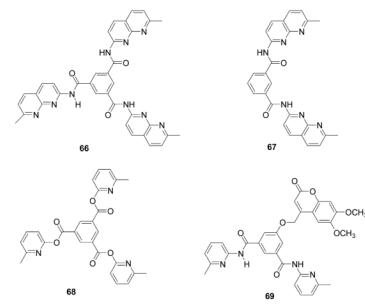


Figure 25.
Structures of compounds 66–69

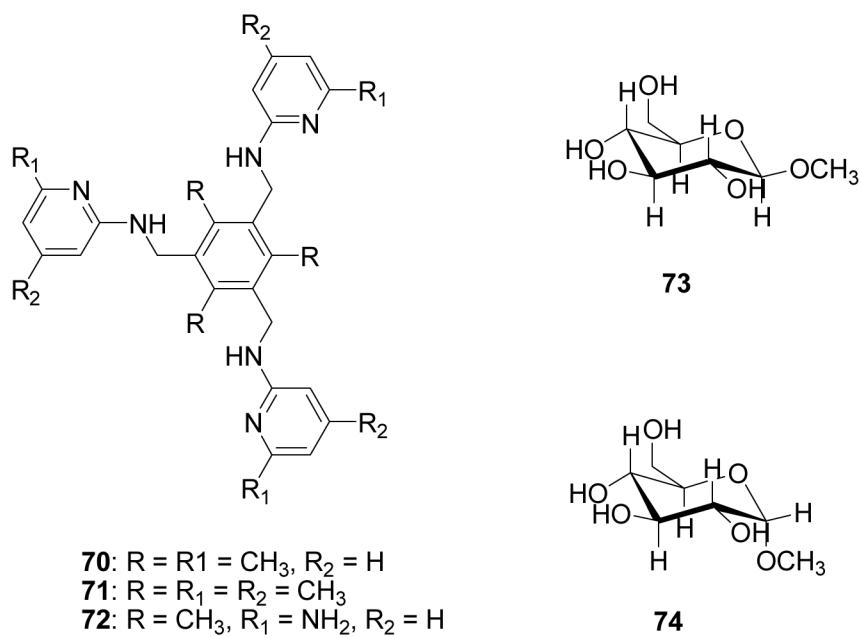


Figure 26.
Structures of compounds 70–74

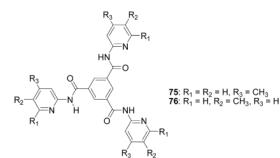


Figure 27.
Structures of compounds **75** and **76**

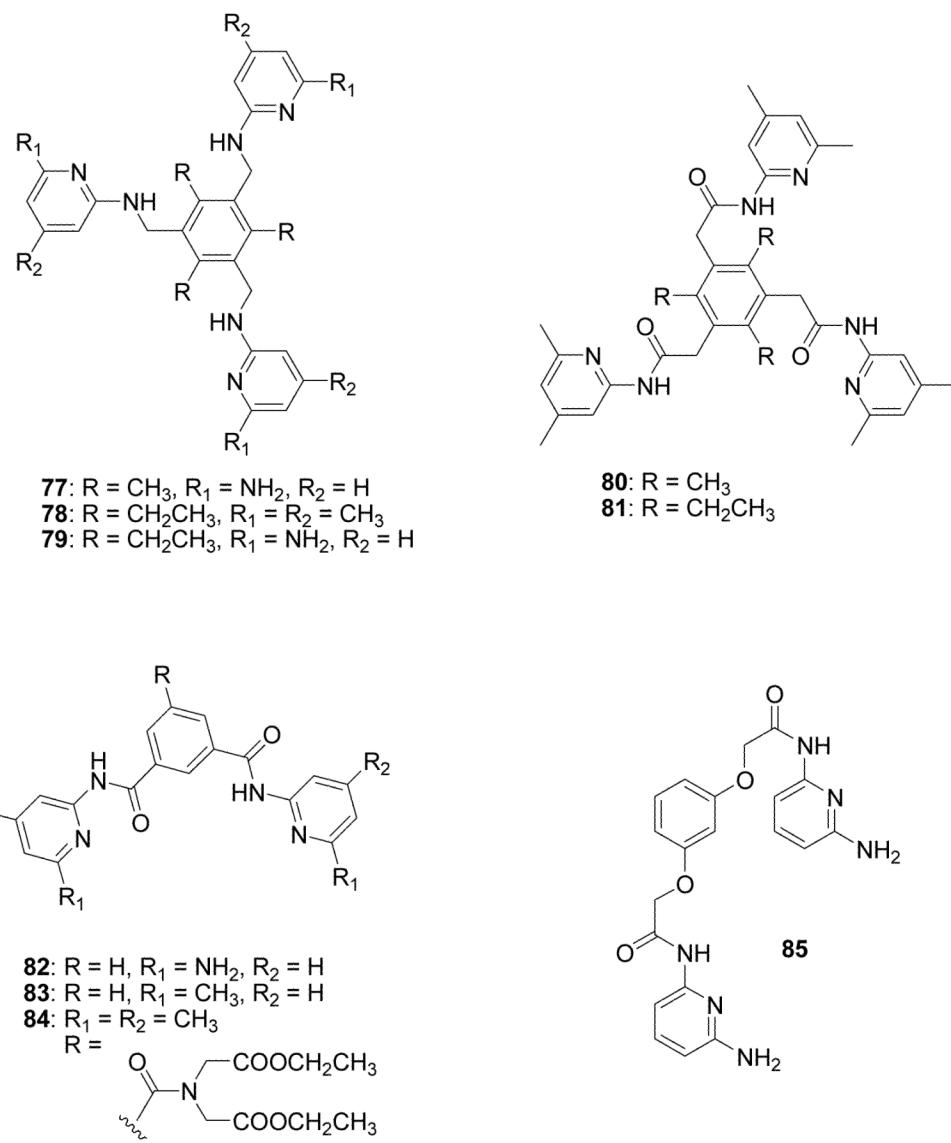


Figure 28.
Structures of compounds 77–85

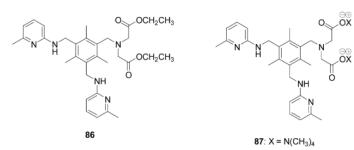


Figure 29.
Structures of receptors **86** and **87**

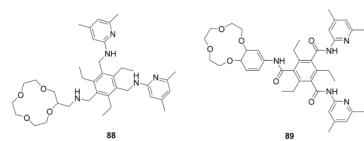


Figure 30.
Structure of crown ether receptors **88** and **89**

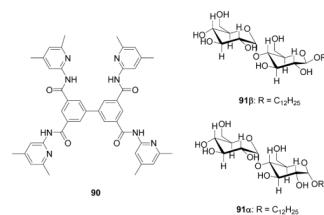


Figure 31.
Structures of compounds **90**, **91 α** and **91 β**

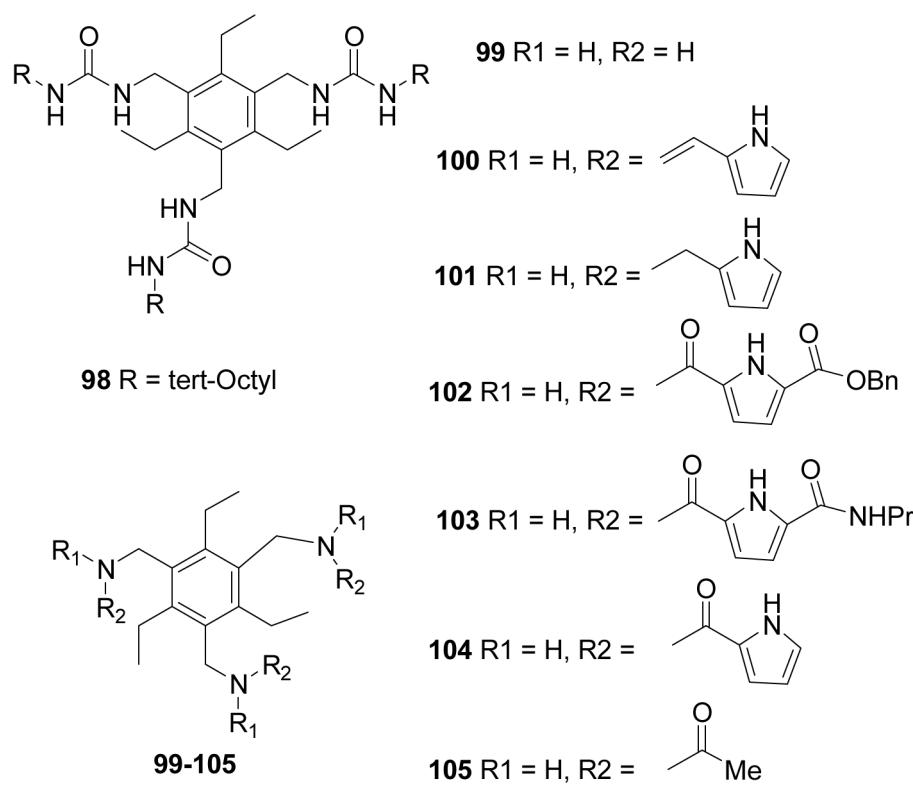


Figure 32.
Structures of pyrrolic tripodal receptors **98–105**

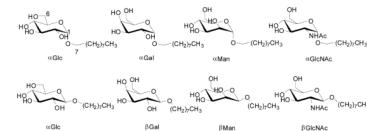


Figure 33.
Structures of model sugars for binding studies

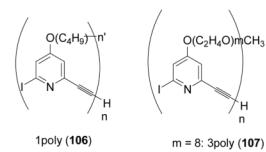


Figure 34.
Structures of **106** and **107**

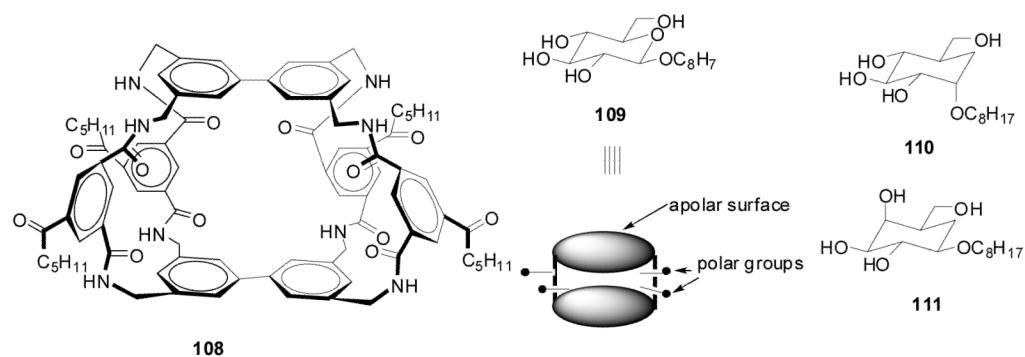


Figure 35.
Structures of compounds **108** and monosaccharides **109–111**

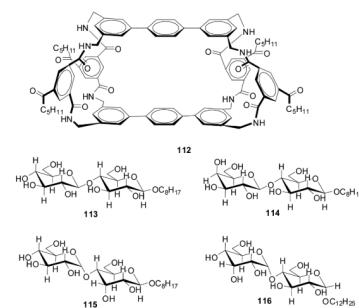


Figure 36.
Structures of compounds 112–116

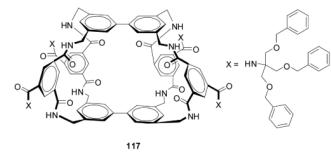


Figure 37.
Structure of 117

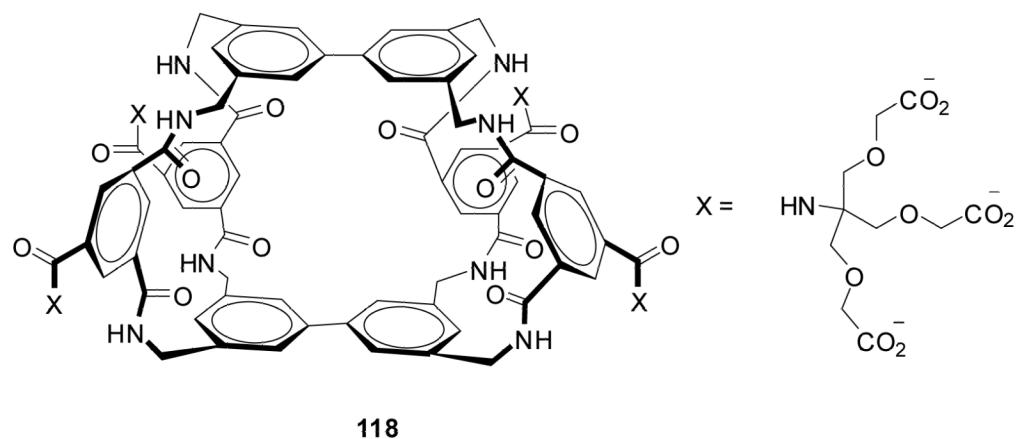


Figure 38.
Structure of compound **118**

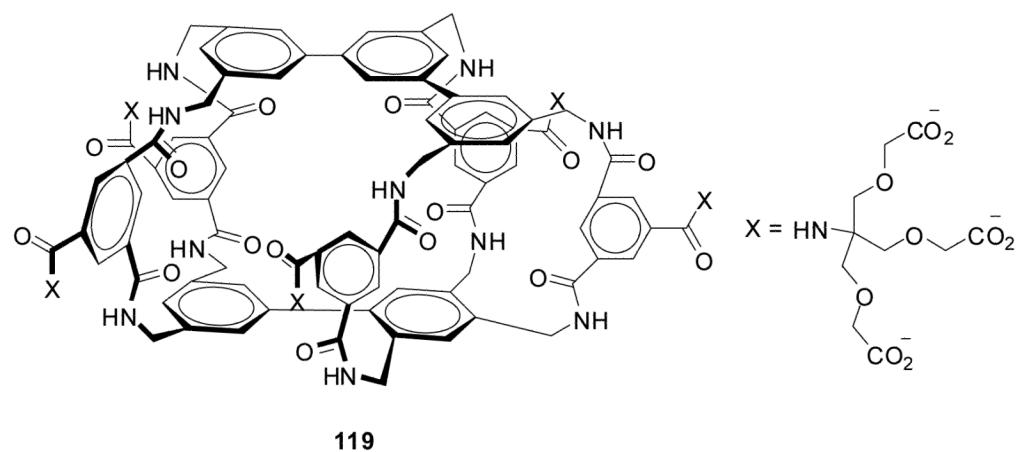


Figure 39.
Structure of compound **119**

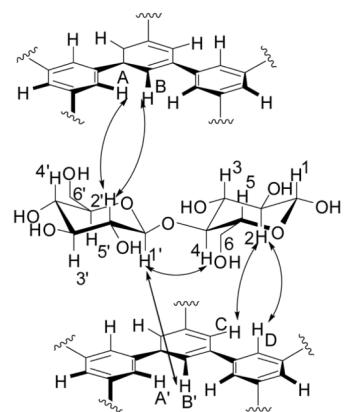


Figure 40.

Figure 1:
NOESY contacts observed for the complex between β -cellobiose and receptor **119**

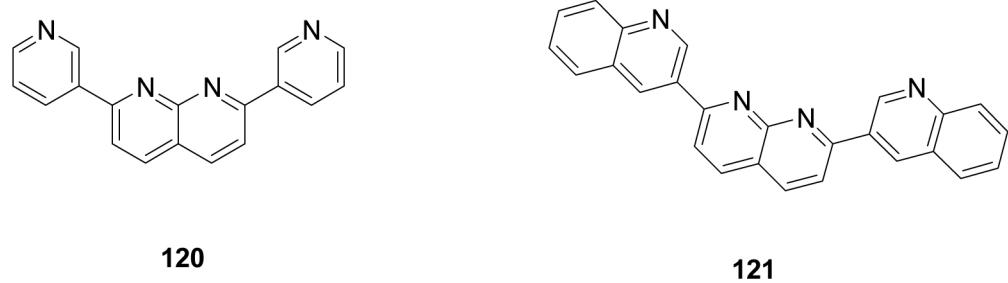


Figure 41.
Structures of compounds **120** and **121**

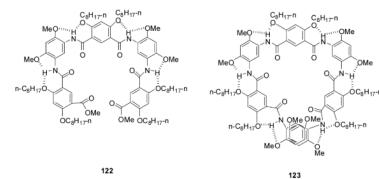


Figure 42.
Structures of hydrogen bonding-driven foldamers **122** and **123**

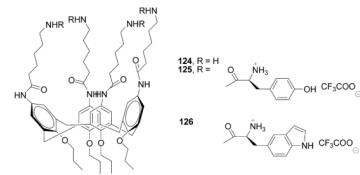


Figure 43.
Calix[4]arene-based ligands as endotoxin receptors **124–126**

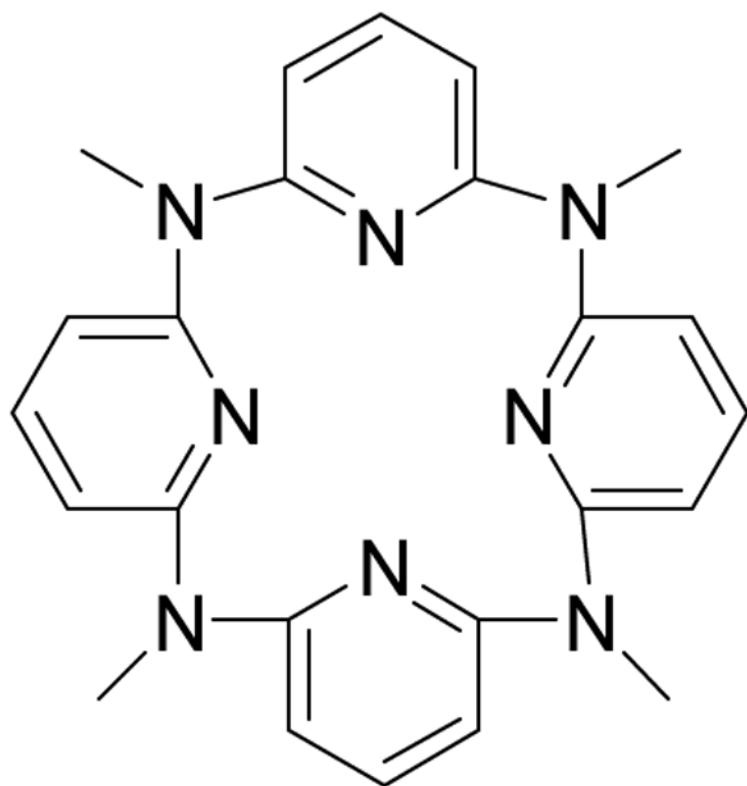


Figure 44.
Structure of MACP-4

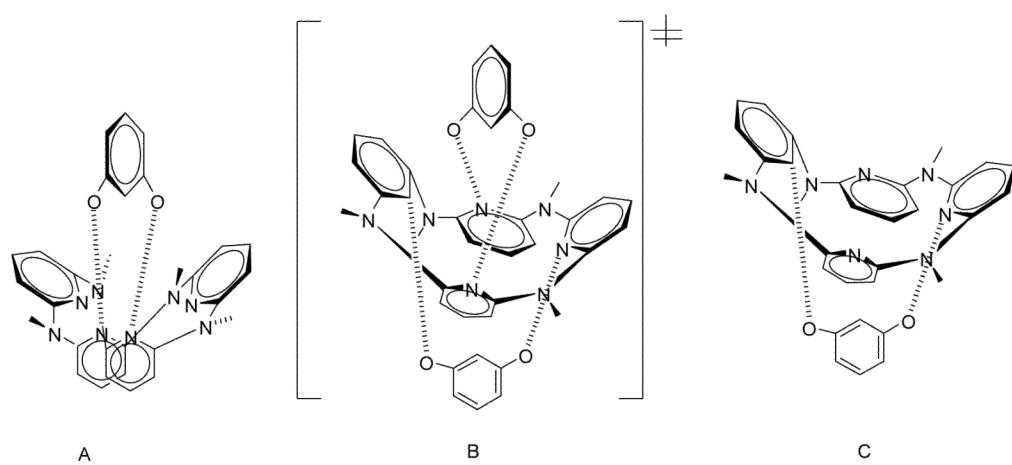


Figure 45.
Transition states of MACP-4-resorcinol complex

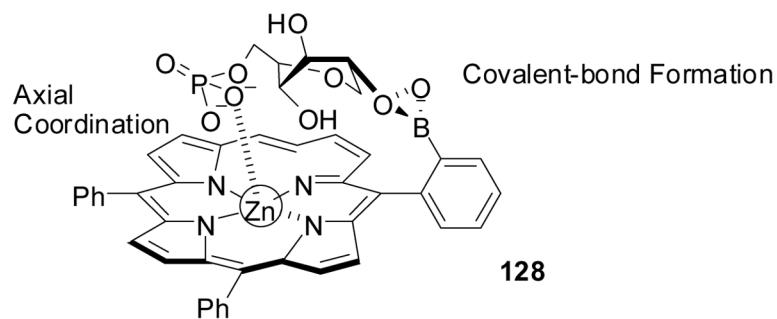
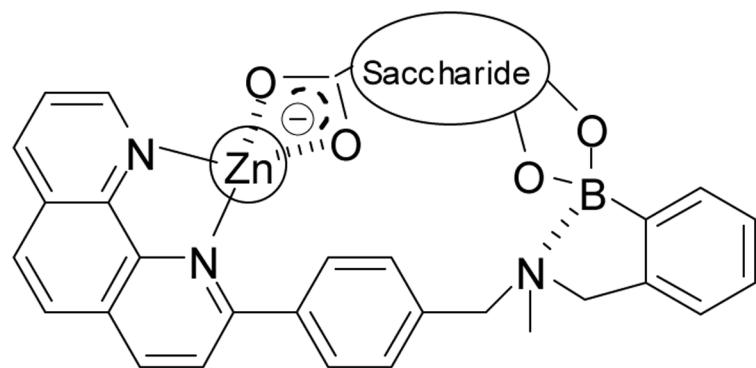


Figure 46.

Complex Structure proposed for boronic-acid-appended Zinc (II) porphyrin plus G-6-P



129

Figure 47.

Complexation mode proposed for the two-points binding of uronic acids to the Zn(II) complex

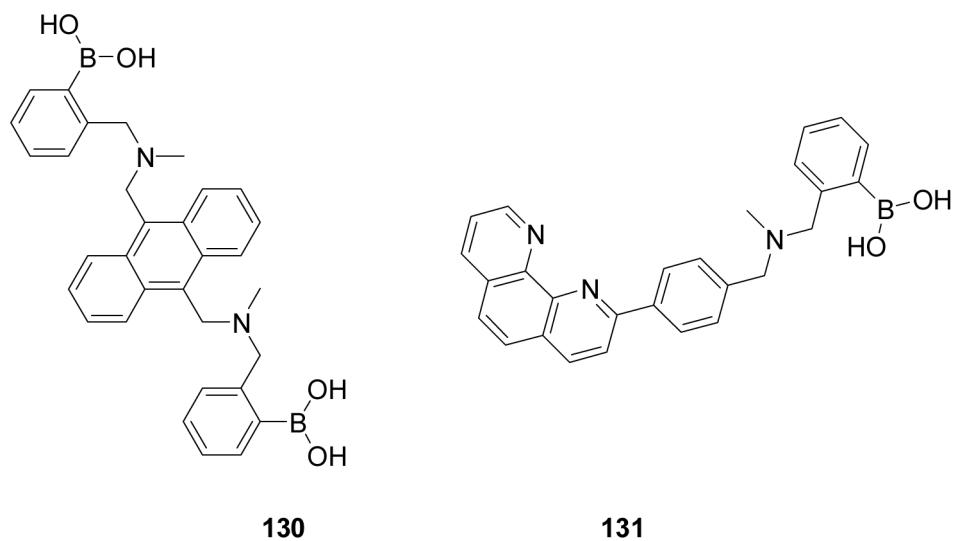


Figure 48.
Structure of **130** and **131**

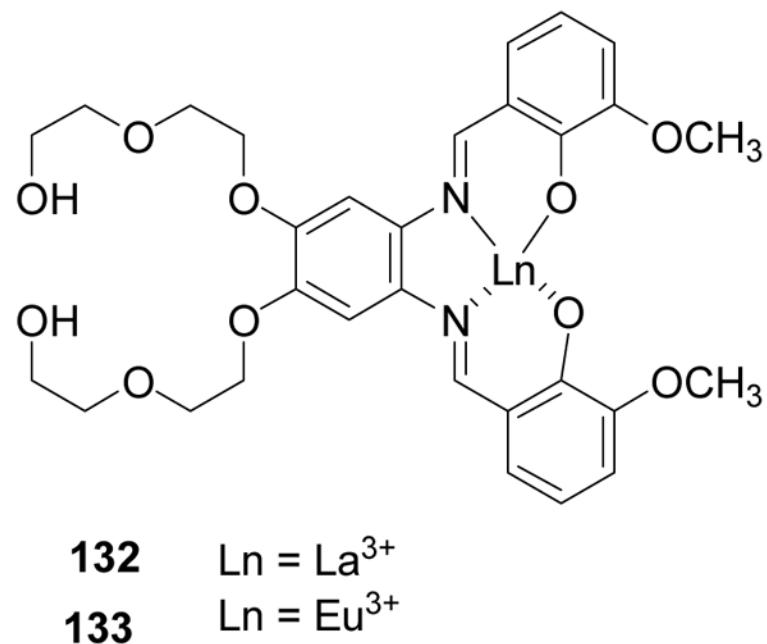


Figure 49.

Structures of salophene-lanthanide (europium) complexes

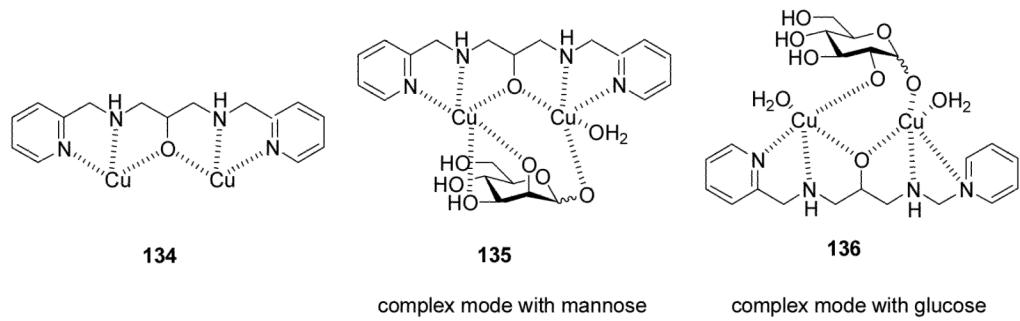
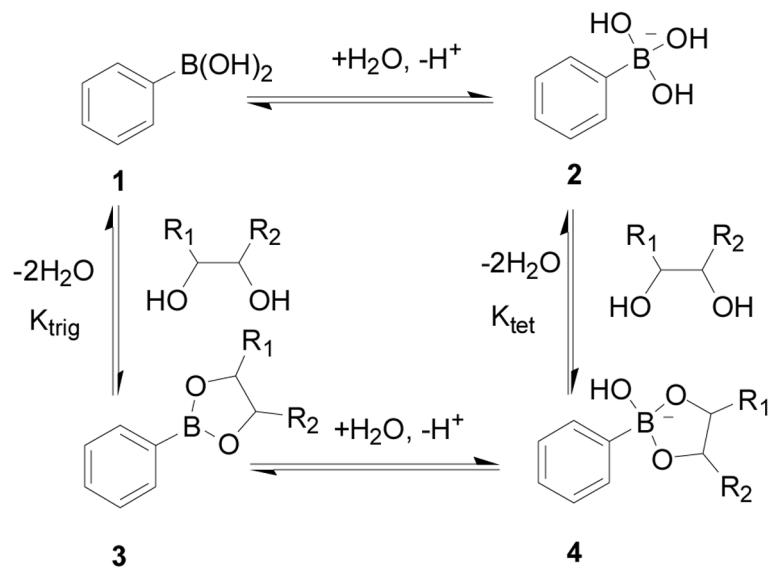
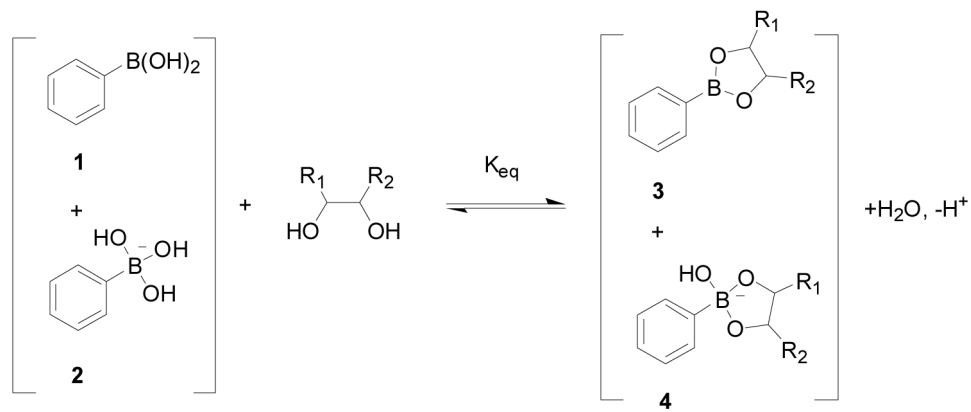


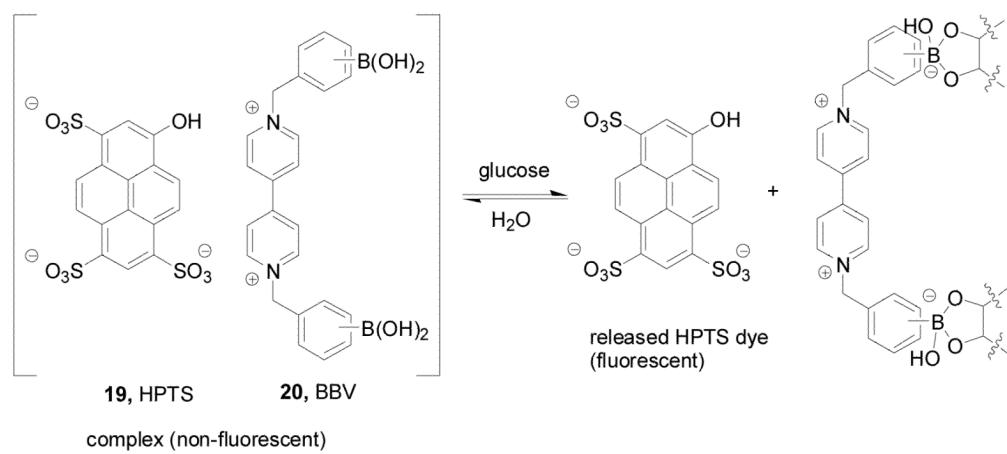
Figure 50.
Complexes modes with mannosidase glucose



Scheme 1.
Binding of phenylboronic acid with a diol

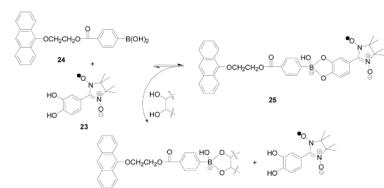


Scheme 2.
Overall binding of phenylboronic acid with a diol



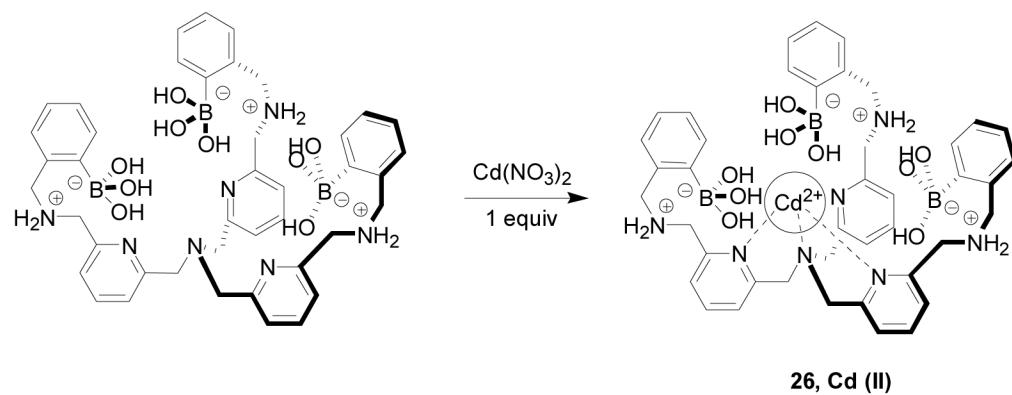
Scheme 3.

Mechanism of sugar sensing by the viologen-boronic acid system



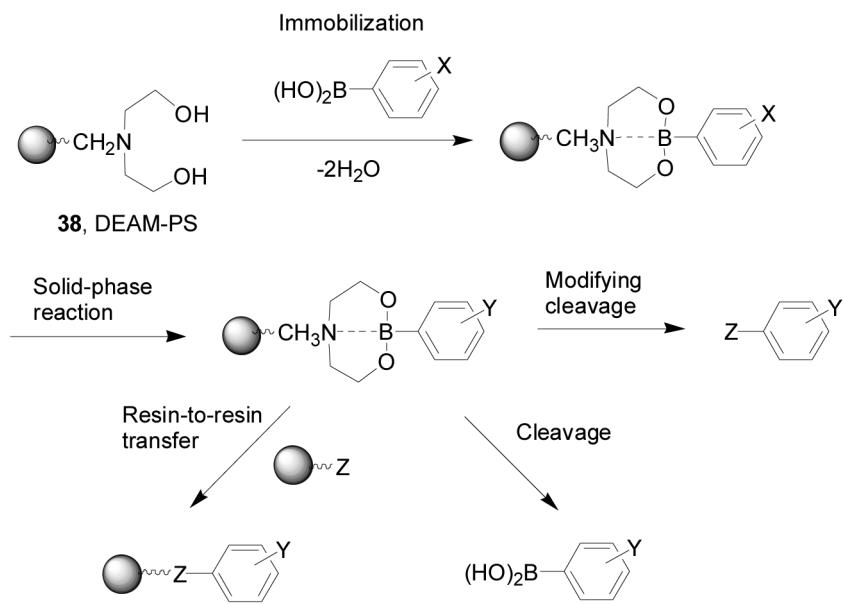
Scheme 4.

Carbohydrate sensing using a 3-component assay with a nitronyl nitroxide quencher

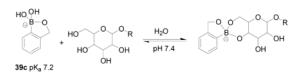


Scheme 5.

Synthesis of a cadmium-centered tris-boronic acid receptor **26**

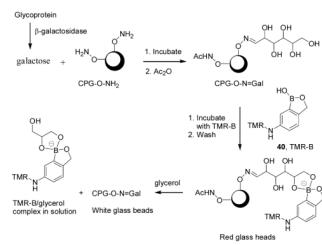
**Scheme 6.**

Immobilization and derivatization of boronic acids using *N,N*-diethanolaminomethyl polystyrene (DEAM-PS **38**) for combinatorial library synthesis

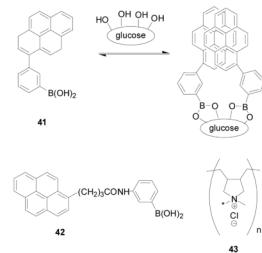


Scheme 7.

Binding between ortho-hydroxymethyl phenylboronic acid **39c** and glycoconjugates

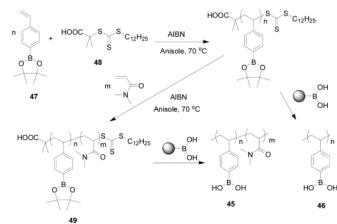
**Scheme 8.**

A strategy for the visual detection of the terminal glycosylation state of a glycoprotein

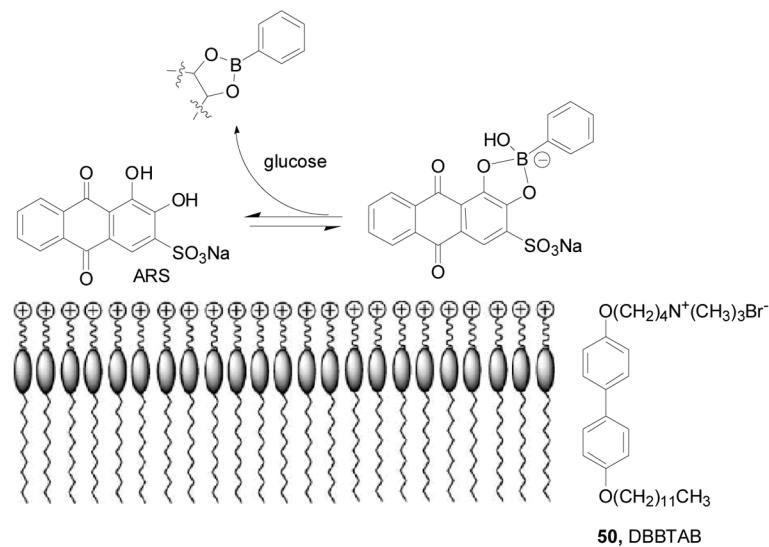


Scheme 9.

Arylboronic acid (**41**, **42**)-polymer (**43**) based sensors for saccharides

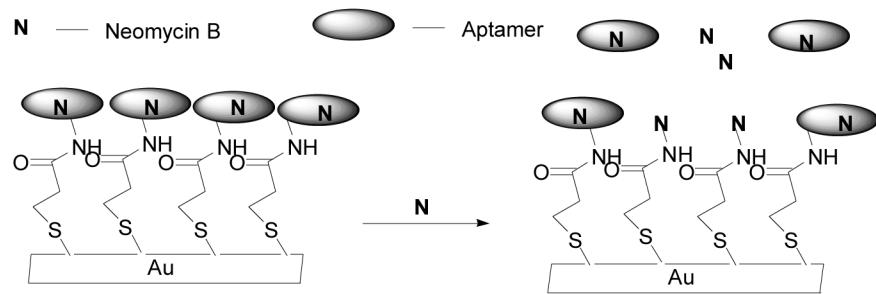
**Scheme 10.**

Formation of a block copolymer (**45**, **46** and **49**) containing boronic acid and acrylamido fragments via atom transfer radical (ATRP) polymerization



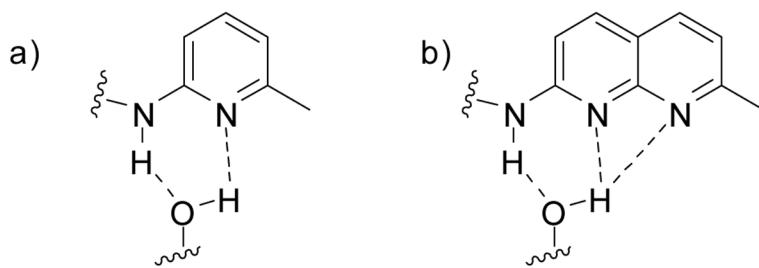
Scheme 11.

A fluorescent glucose sensing system based on ARS/PBA/DBBTAB co-vesicles



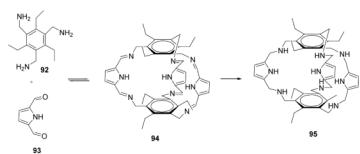
Scheme 12.

Schematic illustration of the modified electrode and competitive assay

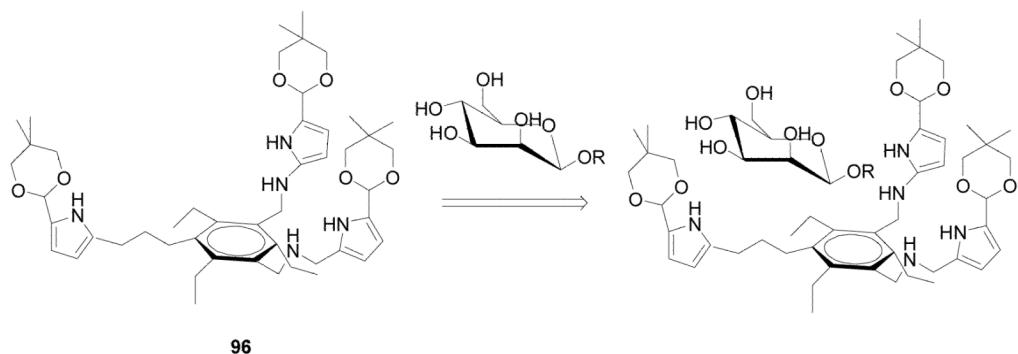


Scheme 13.

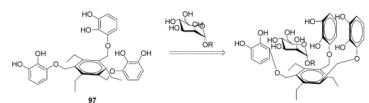
(a) A cooperative hydrogen-bond pattern in carbohydrate complexation with **59**. (b) A cooperative and secondary hydrogen-bond pattern in carbohydrate complexation with **66**



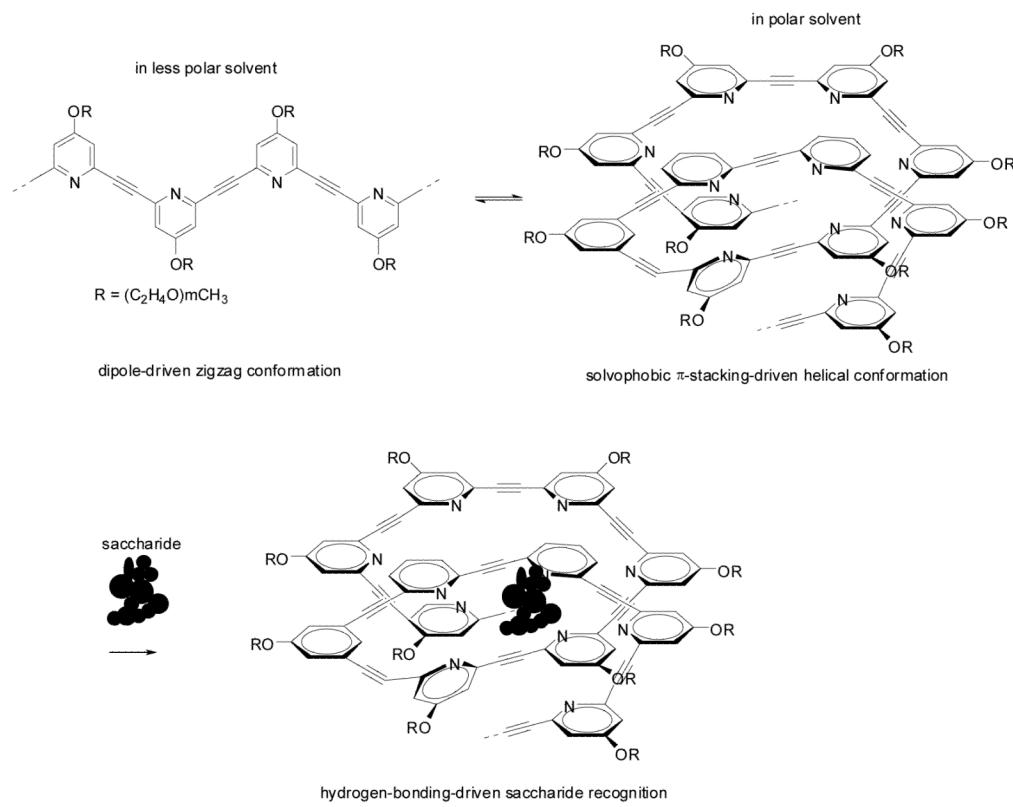
Scheme 14.
Synthesis of the macrobicyclic cage **95**



Scheme 15.
β-Mannoside-selective pyrrolic tripodal receptor



Scheme 16.
Tricatecholic receptor for carbohydrate recognition



Scheme 17.
Hydrogen-bond-driven saccharide recognition

Table I

Carbohydrate-based Biomarkers

Carbohydrates	Characteristics
Tn antigen67-73	An oligosaccharide (GalNAc-O-Ser/Thr) that is a tumor associated antigen and a precursor of the T antigen. It has been used for the development of carbohydrate-based cancer vaccine.
sLe ^x 15-63-64-74	A tetrasaccharide that is usually attached to O-glycans on cell-surface. It is a ligand of the E-selectin family, and is implicated in mediating cancer metastasis.
SLX75-78	Sialylated SSEA-1 (stage-specific embryonic antigen-1) has been identified as a biomarker for pancreatic and lung cancer.
SPAN-179	A sialylated carbohydrate antigen, which is a biomarker for pancreatic cancer.
DUPAN280	Sialyl Lewis C, a sialylated carbohydrate antigen for some cancer. It is commonly used as a marker of pancreatic cancer.
α-Gal-α-Gal81	The causative antigen of acute tissue rejection for transplantation from animal into human. It has been identified as a cell and matrix surface carbohydrate antigen called the alpha-galactosyl epitope (α-Gal).
LPA82-84	The level of LPA (lysophosphatidic acid) has been suggested as a possible test for ovarian cancer.
ST-43985	A sialylated carbohydrate antigen that is used as a clinical marker for a variety of cancers.
CA 12586-87	Cancer antigen 125, a tumor-associated mucinous glycoprotein, commonly seen in tumors of the ovary.
CA 19-9, CA 15-3 CA 27-29, CA 242, CA 50, CA 72-4, CA 195, CA 549, M26, M2928-87-89	These carbohydrate cancer antigens are similar to CA 125. For examples, CA 19-9 is used for the diagnosis of pancreatic, colorectal, gastric, or biliary cancer and for monitoring the clinical response to therapy. CA 15-3 and CA 27-29 are used for the diagnosis of breast cancer.
TAG-12, TAG-72, TAG-72.390-92	Tumor associated glycoproteins made by some cancer. TAG-12 is used as a marker for breast cancer; TAG-72 is used for gastric cancer; and TAG-72.3 is used for lung cancer.
CEA86-89-90-93-98	Carcinoembryonic antigen, a glycoprotein produced by gastrointestinal neoplastic epithelium of glandular origin. CEA is a tumor-associated mucin antigen.
GP120 (polymannose)99-104	An envelope glycoprotein essential for HIV-1 infection. It is a common target for HIV vaccine research.
ABO blood group antigens68-73-97-105-106	The glycoprotein antigens which determine blood types: O, A, and B. The only difference is the composition of carbohydrates.
A2-PAG107-108	Pregnancy-associated alpha-2 glycoprotein made by some forms of cancer.
MCA68-69-72-109	Mucin-like carcinoma-associated antigen that appears at elevated levels in certain breast cancer.
BCM110-112	Breast cancer mucin and tumor associated glycoproteins that appear at elevated levels in certain forms of breast cancer.
CAM17-1, CAM26, CAM29113-114	Tumor associated antigens. CAM17-1 has been suggested as marker for pancreatic cancer and CAM26 and CAM29 are suggested as breast cancer markers.
PMA68	Prostate carcinoma mucin-like antigen, a high M.W. human tumor-associated mucin antigen and biomarker of prostate cancer.
MUC169-109-115-119	Mucin 1 is a member of the mucin family and a glycosylated phosphoprotein, which is over expressed in carcinomas. It is a tumor-associated antigen and a marker of breast and colon cancer.
PEM120-121	Tumor-associated polymorphic epithelial mucin and a tumor-associated mucin antigen. It is produced by gastrointestinal neoplastic epithelium of glandular origin.
M34468	A high molecular weight, mucin-like antigen over expressed on superficial bladder tumor. It is a tumor-associated mucin antigen and a biomarker of bladder cancer.
Galectin-1122-124	A β-galactoside binding animal lectin and biomarker for colon cancer.
Galectin-369-125-127	An endogenous lectin. It is considered a tool for monitoring cell differentiation in head and neck carcinomas and biomarker for colon cancer.

Carbohydrates	Characteristics
Homodimeric Galectin-7128 ⁻ 130	A β -galactoside binding animal lectin on the cell surface, that is capable of inhibiting cancer cell proliferation.
Galectin-9131 ⁻ 133	A β -galactoside binding animal lectin. It is a functional predictive factor for metastasis of breast cancer.

Table II

A summary of boronic acid-based sensors

Compound No.	Sensor Target	Binding Constants (K_a)	Study conditions
19, 20a–c	Glucose	23 M ⁻¹ to 37 M ⁻¹	λ_{ex} : 460 nm, λ_{em} : 510 nm, in phosphate buffer, pH = 7.4
21, 20a–c	Glucose	14 M ⁻¹	λ_{ex} : 414 nm, λ_{em} : 644 nm, in phosphate buffer, pH = 7.4
22, 20a–c	Glucose	around 100 M ⁻¹	λ_{ex} : 460 nm, λ_{em} : 604 nm, in phosphate buffer, pH = 7.4
26	gluconic acid	107 M ⁻¹	UV studies in 75% methanol/HEPES buffer, pH = 7.4
27	sorbitol	1060 M ⁻¹ (5 fold over fructose)	λ_{ex} = 365 nm, λ_{em} = 420 nm, in 50% MeOH/aqueous phosphate buffer, pH = 7.4
28a	glucose	1472 M ⁻¹ (43 fold over fructose)	λ_{ex} = 370 nm, λ_{em} = 430 nm, in aqueous phosphate buffer, pH = 7.4
30	ribose	2400 M ⁻¹ (24 fold over fructose, 8 fold over galactose, and 12 fold over glucose)	λ_{ex} = 565 nm, λ_{em} = 597 nm, in 90% DMSO/phosphate buffer, pH = 7.4
34	D-mannitol	enantioselectivity (K_R/K_S) of 1:20000 in binding with D-mannitol	λ_{ex} = 373 nm, λ_{em} = 421 nm, in 52% CH ₃ OH/aqueous buffer, pH = 8.3.
37	D-glucose	D-glucose (24800 ± 1200 M ⁻¹) to L-glucose (11900 ± 1600 M ⁻¹)	λ_{ex} = 285 nm, λ_{em} = 480 nm, in 50% CH ₃ OH/water, pH = 11.7
44	heparin	Heparin (3 × 10 ⁷ M ⁻¹) over dextran (3 × 10 ³ M ⁻¹), hyaluronic acid (2 × 10 ³ M ⁻¹), chondroitin (4 × 10 ⁶ M ⁻¹), and ovalbumine (1 × 10 ⁶ M ⁻¹).	λ_{em} = 510 nm, in HEPES buffer

Association constants (K_a) for receptors 70–72 with glucopyranosides 63 and 64

Table III

Host-guest Complex	70-63	70-64	71-3	71-4	72-63	72-64
$K_{a1} \text{ (M}^{-1}\text{)}$	1.1×10^4	1.1×10^4	690	2.1×10^4	9.5×10^3	620
$K_{a2} \text{ (M}^{-1}\text{)}$	250	NA	790	NA	4.8×10^3	NA

Association constants K_a for receptors **59**, **75–76** and glucopyranosides **63** and **64**

Table IV

Host-guest Complex	59–63	70–64	75–63	75–64	76–63	76–64
K_{a1} (M^{-1})	8.7×10^3	4.0×10^3	660	3.6×10^3	440	2.1×10^3
K_{a3} (M^{-1})	NA	NA	2.4×10^4	8.2×10^4	2.3×10^4	4.8×10^4

Association constants for receptors 77–85 with glucopyranosides 63 and 64

Table V

Host-guest complex	K_{a1} (M^{-1})	K_{a2} (M^{-1})	K_{a3} (M^{-1})
77–63	9.5×10^3	NA	4.8×10^3
77–64	620	NA	NA
78–63	4.9×10^4	1.3×10^3	NA
78–64	1.3×10^3	NA	NA
79–63	2.0×10^4	NA	1.5×10^4
79–64	8.4×10^3	NA	8.7×10^3
80–63	650	NA	NA
81–63	1.2×10^3	NA	NA
82–63	1.4×10^3	NA	3.9×10^3
83–63	NA	NA	NA
84–63	950	NA	NA
85–63	190	NA	NA

Binding BC₅₀^a (μM) for receptors **98–102, 105** with octylglycosides in CDCl₃**Table VI**

glycoside	98	99	100	101	102	105
αGlc	1.7 × 10 ³	NA	268	570	8.3 × 10 ³	NA
βGlc	2.0 × 10 ³	3.7 × 10 ³	4.8	24	5.4 × 10 ³	6.2 × 10 ⁴
αGal	1.5 × 10 ³	NA	368	790	NA	NA
βGal	1.2 × 10 ³	NA	120	70	NA	NA
αMan	1.6 × 10 ³	NA	262	43	NA	NA
βMan	6.0 × 10 ³	NA	660	37	NA	NA
αGlcNAc	2.0 × 10 ³	NA	1.2 × 10 ³	72	NA	NA
βMan	2.6 × 10 ³	NA	30	18	NA	NA

^aBC₅₀ was defined as the total concentration of receptor necessary for binding 50% of the ligand

Table VIIAssociation constants (K_a) between receptor **121** and carbohydrates as measured by ^1H NMR titration in D_2O

Carbohydrates	$K_a [\text{M}^{-1}]$	Carbohydrates	$K_a [\text{M}^{-1}]$
D-glucose($\alpha/\beta = 72: 28$)	4.6	2-deoxy-D-glucose	7.2
D-glucose($\alpha/\beta = 40: 60$)	9.2	D-xylose	4.6
D-galactose	2.1	D-lyxose	v.s ^a
D-mannose	v.s ^a	L-rhamnose	v.s ^a
D-arabinose	2.2	L-fucose	2.1
D-ribose	3.1	D-cellobiose	16.6
methyl- β -D-glucoside	27.3	D-lactose	v.s ^a
methyl- α -D-glucoside	6.9	D-maltose	v.s ^a

^aVery small. Minor signal movements, almost linear with substrate concentration.

Table IIx

Association constants (K_a) between receptor **121** and carbohydrates as measured by fluorescence titration in D₂O

Carbohydrates	K_a [M ⁻¹]	Carbohydrates	K_a [M ⁻¹]
D-glucose ($\alpha/\beta = 72: 28$)	9.5	methyl- α -D-glucoside	6.9
methyl- β -D-glucoside	27.3	D-xylose	4.6

Table IXCarbohydrate substrates used with receptor **119**

Carbohydrates		K_a (M^{-1})	
	1H NMR	ICD	Fluorescence
D-cellobiose (1 β -OH: 1 α -OH = 3:2)	600 ^a	580	560
Methyl β -D-cellobioside	B	910	850
D-Xylobiose	B	250	270
D-N, N'-diacetylchitobiose	120	NA	120
D-lactose	B	11	14
D-Mannobiose	B	13	9
D-Maltose	B	15	11
D-Gentibiose	NA	12	5
D-Trehalose	C	c	NA
D-Sucrose	NA	c	c
D-Glucose	11 ^{a,d}	12	c
D-Ribose	NA	c	c
D-N-acetylglucosamine	24 ^d	NA	19

^aT = 298 K,^bIntermediate exchanges led to peak broadening and prevented the determination of K_a .^cNo change in spectra upon addition of carbohydrate,^dFast exchanges on the NMR time scale

Table X

A summary of non-boronic acid-based sensors

Sensor type	Sensor target	Binding affinities
RNA aptamers	sialyl Lewis X (sLe ^x , 53)	K_d values were around 10^{-9} to 10^{-11} M, 100 fold selectivity over lactose, 5–10 fold over other similar Lewis group sugars.
aptamer-based assay development	neomycin B (54)	Showed a linear range between 25 and 2.5×10^3 μ M in milk, 100-fold binding selectivity over paromomycin,
Cation-charged modified DNA aptamers	Sialyllactose	The best K_d was 4.9 μ M.
2'-aminopyrimidine RNA aptamers	moenomycin A	The K_d values of the aptamers were 350 and 320 nM.
boronic acid-modified DNA aptamers	Fibrinogen was used as a model glycoprotein	K_d in the range of low to mid nM
compound 66	octyl- β -D-glucopyranoside (63)	The association constant was 2.7×10^4 M^{-1} in chloroform at 25 °C as determined by ¹ H NMR.
compound 87	glucopyranosides (63 and 64)	$K_a = 1.2 \times 10^5$ M^{-1} for 63 , 2.2×10^4 M^{-1} for 64 .
compound 88 and 89	glucopyranosides (63 and 64)	K_{a1} (1:1 binding) of 5.8×10^5 M^{-1} and K_{a3} (2:1 binding) of 1.4×10^4 M^{-1} for receptor 88 with 63 ; K_{a1} of 1.1×10^4 and K_{a3} of 4.8×10^4 M^{-1} for receptor 89 .
biphenyl-based receptor 90	disaccharides (91β)	Binding affinity was determined by ¹ H NMR with $K_a > 10^6$ M^{-1} for 91β in water-containing chloroform solutions (1:10, 1:20)
a self-assembled cage receptor containing pyrrole residues (95)	Oct β Glc	An association constant of 4.8×10^4 M^{-1} with Oct β Glc was determined by using ¹ H NMR in CDCl ₃ assuming a 1:1 stoichiometry
β -mannoside- selective pyrrolic tripodal receptor 96	β -mannose	The BC ₅₀ with β -mannose is less than 1 μ M in CDCl ₃ with selectivity of about 800 fold over α -mannose or α -galactose, and 680 μ M in CD ₃ CN solution with selectivity of about 40 fold over α -galactose.
tricatecholic receptor for carbohydrate recognition (97 , Scheme 16)	octyl glycosides (α -mannoside)	BC ₅₀ of 0.87 5.2 mM and 6-fold selectivity over α glucoside.
a carbohydrate receptor 108	β -glucoside 109	binding constant was determined to be 980 M^{-1} by using ¹ H NMR in CDCl ₃ /CD ₃ OH (92:8)
receptor 112	n-octyl- β -D- cellobioside 113	In CHCl ₃ /MeOH (98:2), the binding constant was 6.4×10^4 M^{-1} .
Metal chelator- based sensors: Zn(II) complex of new receptors 130 and 131	D- galacturonic acid	The log K_a for binding with D-galacturonic acid is 3.1 for 130 and 1.9 for 131 as determined in water:MeOH = 1:2 at pH = 8.

Table XI

Common lectins396·397

Abbreviation	Common name/Species	Specificity
AAA	Fresh water eel/ <i>Anguilla anguilla</i>	α -Fucose
AHA	Actinohivin/ <i>Longispora albida</i>	Man
AIA	Jack fruit, jaca/ <i>Artocarpus integrifolia</i>	Galactose
APA	Leek/ <i>Allium porrum</i>	Man
AUA	Ramsons lectin/ <i>Allium ursinum</i>	Man
BPA	Camels foot tree/ <i>Bauhinia purpurea</i>	GalNAc/Gal
CHA	None/ <i>Cymbidium hybrid</i>	Man
Con A	Jack bean/ <i>Canavalia ensiformis</i>	Man, Glc, GlcNAc
CVL	None/ <i>Chaetopterus variopedatus</i>	β -Gal
CV-N	Cyanovirin-N/ <i>Nostoc elliposporum</i>	α (1,2)Man
DBA	Horse gram/ <i>Dolichos biflorus</i>	GalNAc
DSA	Jimson weed/ <i>Datura stramonium</i>	GalNac
ECA	Cocks comb coral tree/ <i>Erythrina cristagalli</i>	Gal β -1, 4GlcNAc olig
EHA	Broad-leaved helleborine/ <i>Epipactis helleborine</i>	Man
GNA	Snowdrop/ <i>Galanthus nivalis</i>	α (1,3)Man
GRFT	Griffithsin/ <i>Griffithsia spp.</i>	Man, Glc, GlcNAc
GSI	None/ <i>Griffonia simplicifolia -I</i>	α -galactose
GSII	None/ <i>Griffonia simplicifolia -II</i>	GlcNAc
GSL	None/ <i>Gerardia savaglia</i>	D-Mann
HBA	Rubber tree/ <i>Hevea brasiliensis</i>	GlcNAc
HHA	Amaryllis/ <i>Hippeastrum hybrid</i>	α (1,3)- α (1,6)Man
HPA	Roman snail/ <i>Helix pomatia</i>	α -GalNAc
LCA	Lentil/ <i>Lens culinaris</i>	Man, Glc, GlcNAc
LOA	Twayblade/ <i>Listera ovata</i>	α -(1,3)Man
Lotus A	Lotus, asparagus pea/ <i>Lotus tetragonolobus</i>	α -Fucose
MAA	Maackia/ <i>Maackia amurensis</i>	α -2.3 sialic acid
MHA	Myrianthin/ <i>Myrianthus holstii</i>	GlcNAc
MVL	None/ <i>Microcystis viridis</i>	Man β (1,4) GlcNAc
None	Mermaid/ <i>Laxus oneistus</i>	Man
None	Sweet pea/ <i>Lathyrus odoratus</i>	Man, Glc, GlcNAc
NPA	Daffodil/ <i>Narcissus pseudonarcissus</i>	α (1,6)Man
PAA	Avocado/ <i>Persea Americana</i>	unknown
PNA	Peanut/ <i>Arachis hypogaea</i>	Gal-B-OR
PSA	Garden pea/ <i>Pisum sativum</i>	Man, Glc/GlNAc
SBA	Soya bean/ <i>Glycine max</i>	GalNAc
SNA	Elderberry/ <i>Sambucus nigra</i>	α -2,6 sialic acid

Abbreviation	Common name/Species	Specificity
STA	Potato/ <i>Solanum tuberosum</i>	GlcNAc oligomers
SVN	Scytovirin/ <i>Scytonema varium</i>	α (1,2)- α (1,6)Man
UDA	Stinging nettle/ <i>Urtica dioica</i>	GlcNAc oligomers
UEA-I	Gorse, Furze/ <i>Ulex europaeus-I</i>	α -Fucose
VFA	Broad bean, faba bean/ <i>Vicia faba</i>	Man, Glc, GlcNAc