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Carbohydrate biomarker recognition using synthetic lectin mimics*

Chaofeng Dai, Arpana Sagwal, Yunfeng Cheng, Hanjing Peng, Weixuan Chen, and Binghe Wang‡

Department of Chemistry, Center for Diagnostics and Therapeutics, and Center for Biotechnology and Drug Design, Georgia State University, Atlanta, GA 30302-4098, USA

Abstract: Carbohydrate biomarkers play very important roles in a wide range of biological and pathological processes. Compounds that can specifically recognize a carbohydrate biomarker are useful for targeted delivery of imaging agents and for development of new diagnostics. Furthermore, such compounds could also be candidates for the development of therapeutic agents. A tremendous amount of active work on synthetic lectin mimics has been reported in recent years. Amongst all the synthetic lectins, boronic-acid-based lectins (boronolectins) have shown great promise. Along this line, four classes of boronolectins including peptide-, nucleic-acid-, polymer-, and small-molecule-based ones are discussed with a focus on the design principles and recent advances. We hope that by presenting the potentials of this field, this review will stimulate more research in this area.

Keywords: biomarker recognition; boronic acids; boronolectins; carbohydrates; nucleic acids; peptides; polymers.

INTRODUCTION

Carbohydrate-based biomarkers are known to be correlated with a variety of diseases including cancer, Down syndrome, infectious diseases, and inflammatory responses [1], as well as numerous pathological conditions including cancer metastasis, inflammation, bacterial and viral infections, immune responses, and cardiovascular malfunctions [2–8]. Much of these are because of the versatile functions that carbohydrates play in a large number of biological processes such as fertilization, pregnancy progression, signal transduction, protein functioning and regulation, cell–cell communications, stem cell differentiation, and embryonic development. Recent advances in glycomics and glycoproteomics have contributed immensely to our understanding of glycan-associated aberrations in diseased states. For instance, most cancer types show aberrant cancer-specific glycosylations, reflecting abnormal expression of enzymes that are implicated in glycan biosynthesis such as glycosyltransferases and glycosidases. Consequently, cancer cells produce glycoproteins and glycolipids with modified glycan structures as opposed to their normal counterparts [6,9–11]. Such anomalous glycan modifications and altered glycosyltransferase and glycosidase levels provide a compelling platform for their exploitation in the improvement and development of diagnostics and therapeutics. For instance, in the case of cancer, most of the Food and Drug Administration (FDA)-approved biomarkers are carbohydrate-depend-

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‡Corresponding author: Tel.: 404-413-5545; E-mail: wang@gsu.edu

ent [12]. Even with prostate-specific antigen (PSA), it has been recognized that its glycosylation pattern is correlated with prostate cancer more so than the protein quantity itself [13–16]. In the following sections, we discuss briefly variations of carbohydrate structures in a number of pathological and physiological states (See Table 1). This lays the foundation for discussion of ways to recognize carbohydrate biomarkers and their applications with a focus on cancer.

Table 1 Selected carbohydrates implicated in various pathological changes.

Pathological/physiological states	Carbohydrate-associated antigens (CAAs)
Cancer	Tn [17], sTn, TF, di-sTF [18,19], globo H [20,21], sLe^x [22–25], sLe^a [26,27], SLX [28,29], ST-439 [30], *galectin-1 [31–33], *galectin-3 [34–37], *galectin-7 [38–40], and 9 [41–43], BCM [44,45], MUC1 [34,46–51] LPA [52–54], CEA [55–63], M344 [64], MCA [34,47,64,65], and PMA [64]
Infectious diseases	GP120/polymannose (HIV) [66–70] LPS [71]/endotoxins (bacterial infections)
Cardiovascular diseases	Glycated Hb (GlcHb) [72–74], *galectin-3 [34–37]
Inflammation and immune responses	Le^x [22–25], ABO blood group antigens [61,64,75–77], α-Gal-α-Gal [78]
Stem cell differentiation and embryo development	Stage-specific biomarkers such as Le^x [79,80], SSEA-1,3 and 4, fucose and sialic acid [81–83]
Signal transduction	Glycosphingolipids (gangliosides, cerebrosides, and globosides, etc.) [10,84]

*Galectins are a family of β-galactoside binding lectins containing homologous carbohydrate recognition domains (CRDs). Although they are not carbohydrates, their functions are often related to carbohydrate binding.

CARBOHYDRATE VARIATIONS IN DIFFERENT PATHOLOGICAL/PHYSIOLOGICAL STATES

Carbohydrate-associated antigens (CAAs) are widely recognized biomarkers for cancer diagnosis and therapeutic targets. These CAAs include Tn and TF antigens, globo H, sialyl Lewis X (sLe^x), Lewis Y (Le^y), and polysialic acids. For example, antigens Tn (CD175) and globo H are the most commonly found CAAs on cancer cells [1,85]. Specifically, over 70–90 % of solid tumors (breast, colon, lung, bladder, cervix, ovary, stomach, and prostate) possess the Tn antigen on their cell surface [86]. Tn is a mucin-type *O*-glycan composed of *N*-acetyl-D-galactoseamine bound to serine/threonine residues in glycoproteins with an α-glycosidic linkage (GalNAcα1-*O*-Ser/Thr). Other mucin-type O-linked glycans that are formed from precursor Tn include TF, sTF and sTN, and di-STF antigens. These antigens are abundant in many cancer types including bladder, colorectal, prostate, ovarian, breast, pancreas, and lung carcinomas. Sialylated carbohydrate family members such as sLe^x and sialyl Lewis A (sLe^a) are also key CAA biomarkers. Their expression levels are related to cancer metastasis [26], tumor extravasion [87], and cell adhesion [22,88,89].

In addition to cancer, carbohydrate-dependent biomarkers also play important roles in other pathological or physiological processes such as bacterial and viral infection, immune responses and inflammation, and cardiovascular disease (CVD) development. For example, in the case of Gram-negative bacteria, the structural difference in the saccharide portion (O-antigen) of the lipopolysaccharide (LPS) forms the basis for serotyping [90]. In addition, LPSs often are endotoxins and prolonged exposure to LPS in the case of severe infections can lead to production of immune response mediators that can cause tissue damage, septic shock, and organ failure [91]. It is also known that structure modifications in LPS affect the pathogenicity of bacteria such as *P. multocida* [91]. The involvement of carbo-

hydrate interactions in viral infections can be best understood by the example of human influenza virus. The infection is caused by binding of viral hemagglutinin to sialic acid on the surface of epithelial cells [92]. Another example comes from human immunodeficiency virus (HIV), in which case a viral exterior polymannose glycoprotein gp120 binds to human $CD4^+$ cells [93,94]. Several lectins such as cyanovirin-N (CV-N) have been shown to possess anti-HIV activity through binding to parts of gp120, and thus blocking viral entry into cells [95,96].

Carbohydrate recognition also plays an important role in immune responses and inflammation processes. sLe^x interaction with L-selectin is widely known to be crucial during the adhesion and migration of white blood cells to infection sites [97,98]. The ABO blood group antigens are entirely carbohydrate-based and differ only in the presence of one terminal sugar unit on the surface of red blood cells [99]. Immune responses due to exposure to different blood types are largely mediated by recognition of the relevant sugar [100].

In the case of CVDs, two major glycoprotein biomarkers, glycated hemoglobin (GlcHb) and fibrinogen, are widely recognized. For instance, diabetic patients show considerably higher GlcHb (glycated valine HbA_{1c}) than normoglycemic individuals. In these patients, nearly 24 % of total Hb is GlcHb toward the end of the erythrocyte lifespan [101–103]. There is a significant increase of CVDs associated with increased levels of GlcHb, suggesting the importance of GlcHb levels as biomarkers for disease prediction and progression. Fibrinogen is a soluble plasma glycoprotein with a molecular weight of 340 kDa and a precursor of fibrin, the principal protein for vertebrate blood clotting [104,105]. The carbohydrate composition of fibrinogen is approximately 3 % and comprises NeuAc, Gal, Man, and GlcNAc linked to the γ-chain on Asn52 and the β-chain on Asn364 [106,107]. Glycosylation of fibrinogen has regulatory effects on its functioning. For instance, deglycosylated fibrinogen accelerates polymerization and increases the aggregation of fibrin fibers [108]; increased negative charge on sialic acids linked to fibrinogen adversely affects fibrin polymerization [109]; and removal of the sialyl moiety from fibrinogen mediates plasma clearance via the hepatic galactose/galactosamine binding lectin [110]. Fibrinogen-related irregularities are also involved in a large number of pathologic conditions, such as hepatoma [111], pancreatic and other cancers [112–116], tumor metastasis [115,117–119], human hemopoietic cell proliferation [120], and embryogenesis and reproduction [121].

Moreover, in many biological processes such as embryo formation, stem cell differentiation, and signal transduction, glycoproteins are of crucial importance [81–83]. A recent breakthrough reported the key role played by sLe^x in spermatozoa-egg binding interactions leading to human gamete formation [122]. It has also been reported that Lewis X (Le^x) is a stage-specific embryonic antigen, which can be used for identifying and isolating specific cell types from heterogeneous populations [79,80]. Carbohydrates are also observed to show changes in expression levels at various developmental stages [123–125].

The discussion provided above is a mere glimpse of the vast potential that carbohydrates possess as biomarkers. Conceivably, small and macromolecules capable of carbohydrate recognition can act as "binders" and thus can be explored for their diagnostic and therapeutic applications. In the search for highly selective and specific "sugar binders", antibodies/lectins have stood out as the obvious. However, the success so far has been limited due to issues associated with cross-reactivity, cost-effectiveness, stability, and availability of antibodies. Thus, efforts in developing antibodies/lectins for diagnostic and therapeutic applications have been hampered. In view of this, small molecule synthetic alternatives for carbohydrate recognition offer many advantages in terms of low cost, high stability, and easy storage. These compounds can be tailored for a specific purpose [17,126–128].

Currently, extensive efforts are directed toward developing synthetic sugar binders as diagnostics, cell imaging agents, and therapeutics. This review will not attempt to comprehensively cover this area. Readers are referred to earlier publications for more details [85,129]. Instead, our focus here is to bring back to light the importance of carbohydrate recognition by discussing the latest advances made in the field.

Boronic acids have been widely used in carbohydrate recognition due to their intrinsic affinity for the hydroxyl group, which are commonly found on carbohydrates. We have termed those boron-containing compounds that can specifically recognize biologically important targets as "boronolectins" [130]. At present, available boronolectins generally belong to four different classes: (a) peptide/protein-based boronolectins, (b) nucleic-acid-based boronolectins, (c) polymer-based boronolectins, and (d) small-molecule-based boronolectins. For the following sections, we briefly discuss some recent progress in these classes. For earlier work, readers are referred to previous publications [1,85,129,130].

PEPTIDE/PROTEIN-BASED BORONOLECTINS

In principle, designing highly selective "sugar-binder" sensors invariably involves constructing three-dimensional scaffolds complementary to the target carbohydrates. However, the de novo synthesis of such three-dimensionally well-controlled boronic acid scaffolds is a major challenge. In this regard, an alternative is the utilization of peptide-based boronolectins (PBLs), which offers structural diversity and the possibility of building combinatorial libraries (Fig. 1). The employment of amino acid backbones also provides the potential advantages of easy synthesis, good solubility, and biocompatibility. In previous reviews [85,129], the significant contributions made by the Anslyn, Hall, Lavigne, Kubik, and Duggan labs toward developing PBLs have been discussed. Here, our attempt is to largely focus on the new developments in this field.

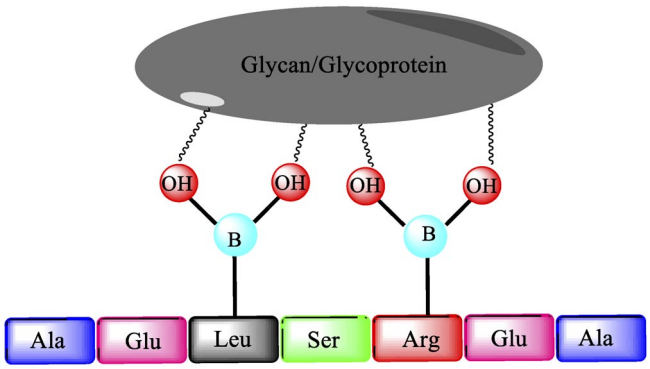


Fig. 1 A schematic representation of PBLs.

The selective recognition of carbohydrates in water at physiological pH has been a long-standing challenge in the field of chemical biology and medicine [131]. Although a number of boronic acids are known to show strong binding affinity to complex carbohydrates in organic solvents [52,132–135], only a handful have been reported to work in water at physiological pH [23,136–145]. The key obstacle lays in the competition from solvent molecules. In a recent effort, by mimicking nature's molecular recognition principles, the Hall group designed a series of PBL receptors for selective recognition of cancer-associated Thomsen–Friedenreich (TF) antigen under physiological conditions [146]. Specifically, the strategy involved building combinatorial libraries encompassing functionally and structurally diverse peptide backbones with enhanced H-bonding capabilities, and carrying benzoboroxoles, an arylboronic acid capable of binding to complex glycopyranosides (six-membered-ring sugars) as key recognition moiety. The latter is important since cell-surface glycoconjugates are comprised mostly of hexopyranosides, while most boronic acids display a marked preference for binding furanose sugars. Free amine-linked poly(ethylene glycol) (PEG) was tethered for improved solubility and further conjugation. Aromatic residues were rationally placed on another terminal for effective hydrophobic CH-π interactions with nonpolar face of saccharides. The most potent receptor obtained from the screening of a

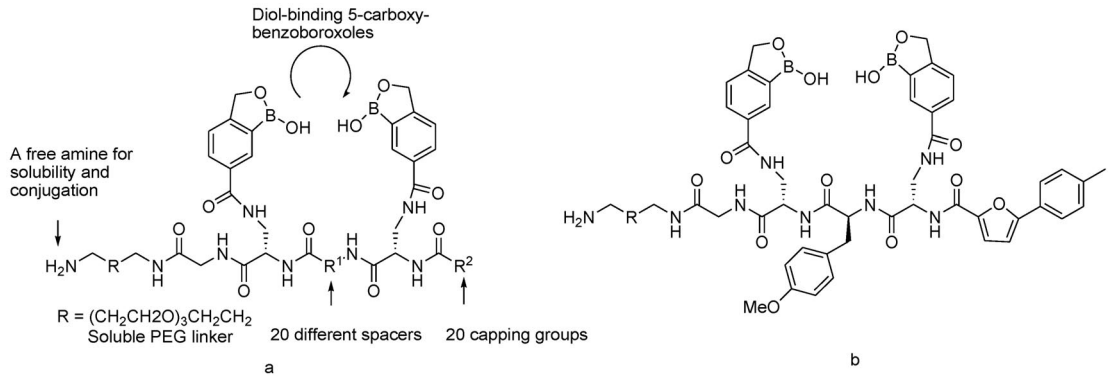


Fig. 2 (a) Design of peptidyl bis(boroxoles) library; (b) structure of most potent inhibitor.

library of 400 showed an IC_{50} of 20 μM against the TF antigen at physiological condition (Fig. 2b). This approach successfully demonstrated the feasibility of specific targeting of a tumor-associated carbohydrate antigen (TACA) using a combination of peptide backbone and benzoboroxoles as hexopyranoside-binding agents. Using a similar strategy, PBLs for other TACAs can also be developed.

As another example, the Lavigne and Thompson labs developed a methodology to synthesize and screen bead-based PBL libraries. Selective PBLs exhibiting high selectivity for glycoprotein OVA (ovalbumin) were obtained [147]. This approach involved design, synthesis, and screening of several structurally and functionally diverse PBLs using proof-of-concept glycoproteins. A synthetic lectin, SL2 (Ac-RTD*RFLD*V-BBRM, where D* refers to diaminobutanoic acid functionalized with phenylboronic acid (PBA) moiety), was obtained with higher selectivity for glycoprotein OVA over PSM (porcine stomach mucin) and BSM (bovine submaxillary mucin). Specifically, the percent change in luminosity upon binding with OVA was four and three times stronger than that resulting from binding with BSM and PSM, respectively. Similar efforts can be extended to the development of boronic-acid-based boronolectins for specific recognition of cancer-related glycans and glycoproteins (e.g., sLe^x, sLe^a, and CEA) and to obtain highly selective diagnostic tools and possible therapeutics.

Considering their unique chemical properties, genetic encoding of boronic acids into biomacromolecules using unnatural amino acids provide the possibility for chemical modification and biomolecular recognition at the same time [144,148,149]. Working along this line, the Schultz lab significantly advanced the field of PBLs by incorporating boronic acids into the genetic code of *Escherichia coli* [150]. Specifically, the authors introduced a general methodology for the incorporation of *p*-boronophenylalanine directly into the *E. coli* proteins using a pair of mutagenic amber suppressor tyrosyl tRNA ($Mj\text{tRNA}^{\text{Tyr}}_{\text{CUA}}$)/tyrosyl-tRNA synthetase (*Mj*TyrRS) derived from *Methanococcusjannaschii* (*Mj*) in response to amber stop codon TAG. In addition to direct carbohydrate recognition applications, the ability for chemical modifications such as Suzuki coupling, oxidation, and reduction could make boronic acid moieties as orthogonal handles for selective modification of proteins.

NUCLEIC-ACID-BASED BORONOLECTINS (NBLs)

In addition to peptides and proteins, nucleic acids can also serve as important structural scaffolds. Such a concept has been demonstrated by the pioneering work of several labs in aptamer selection [151–153] and DNA modification [154,155]. The Wang lab has been working on constructing boronic-acid-modified DNA as potential boronolectins [156–159]. Along this line, a series of boronic-acid-modified thymidine nucleotides (BTTPs) (Fig. 3) have been designed and synthesized. These BTTPS can be recognized by DNA polymerases and successfully incorporated into DNA. A key focus in the recent effort is postsynthesis or post-PCR (polymerase chain reaction) modification of DNA. Therefore, thymidine

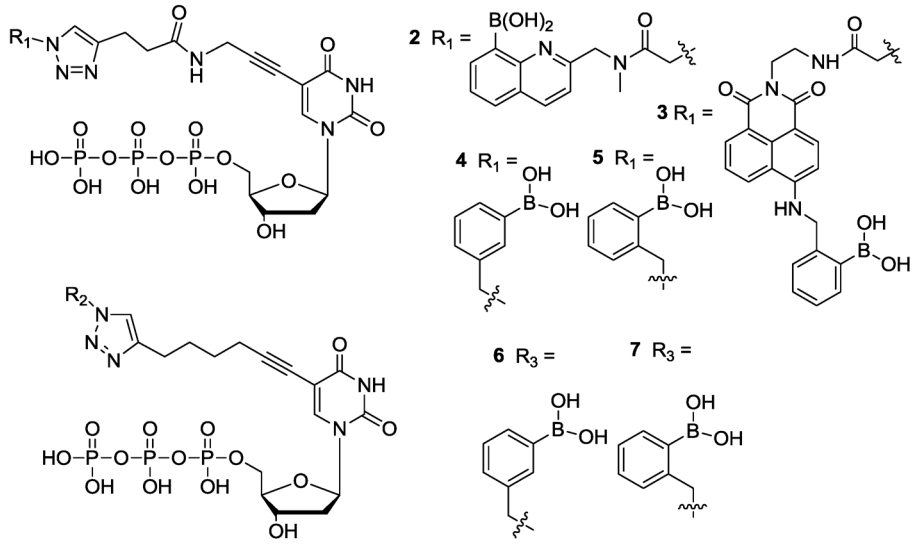


Fig. 3 Structures of BTTPs.

analogs with a pre-installed handle were incorporated into DNA either chemically or enzymatically. Further decoration with boronic acid was accomplished [157,160]. The advantages of postsynthesis decoration include: (1) convenience in creating diversity, (2) avoidance of boronic acid stability issues during synthesis or PCR, (3) ease in synthetic chemistry, and (4) ease in controlling PCR-based incorporation of a modified base (T). This platform approach not only allows for chemical modification on DNA scaffolds, but also demonstrated a viable way to produce boronic-acid-modified DNA libraries with structural diversities. This offers the potential for high-throughput selection of aptamers that are capable of differentiating glycosylation patterns on the glycoproteins.

Anslyn and co-workers have previously introduced a method of fine-tuning the selectivity of synthetic small-molecule bis-boronic-acid-based receptors for tartrate using aptamer selection [161]. Their recent study explored the binding efficiency of boronic-acid-functionalized DNA duplexes as sugar recognition units [162]. Specifically, multivalent (bis- and tetra-) boronic acid-DNA conjugates were synthesized as sugar hosts, which are capable of duplex formation. Based upon cellulose paper chromatography and gel mobility analysis, it was found that tetra-boronic-acid-functionalized DNA show enhanced sugar binding and selectivity as compared to bis-boronic-acid-modified and native DNA duplexes (Fig. 4). The lower binding affinity shown by bis-boronic acid DNA duplex reinforces the need for multivalent boronic acid moieties for stronger conjugate–sugar interactions. The approach brings new prospects to the application of DNA-based boronolectins in sugar recognition.

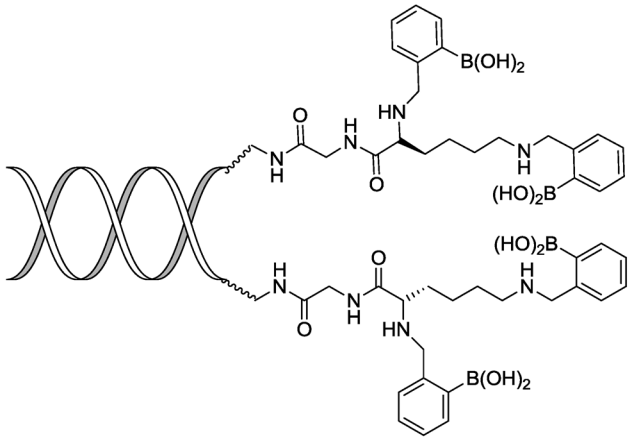


Fig. 4 Structure of a tetravalent boronic acid-DNA conjugate.

POLYMER-BASED BORONOLECTINS

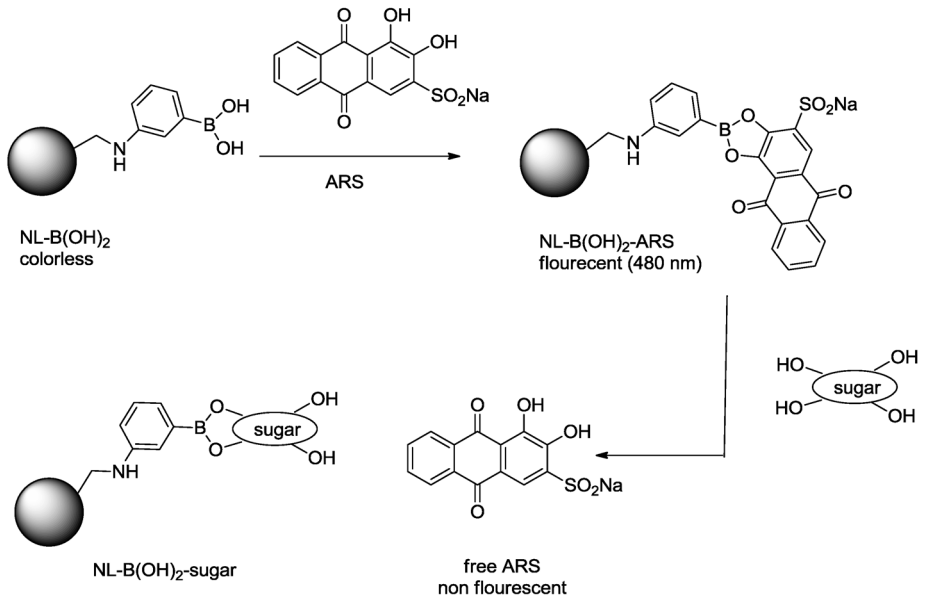
Functionalized polymers and nanoparticles have drawn great research interest during the past decades, particularly in the development of diagnostics and drug delivery approaches. The easy access to multivalent modification of polymers and nanoparticles can lead to improved functional diversity. Compared to the well-known benefits of functionalized polymers in the biomedical area, boronic-acid-containing polymers had remained underutilized until recently. Conceivably, all the design strategies that have proven successful in other boronolectin areas could also be applied in boronic-acid-containing polymer design. Boronic-acid-containing (co)polymers for specific carbohydrate targeting are also termed "polymer-based boronolectins" [163–165]. In the following section, we will highlight some recent developments in this area.

Diabetes is a well-known chronic metabolic disorder characterized with high blood glucose levels either due to insulin deficiency or the inability of the cells to respond to insulin. The prevailing therapeutic method for blood glucose control is subcutaneous insulin injection. However, for optimal results insulin injection should be coupled with glucose monitoring. Continuous glucose monitoring is especially useful. In this respect, boronopolymers could be used as sugar sensors. The sensing strategy can rely on either optical property changes or conductivity changes upon binding [165].

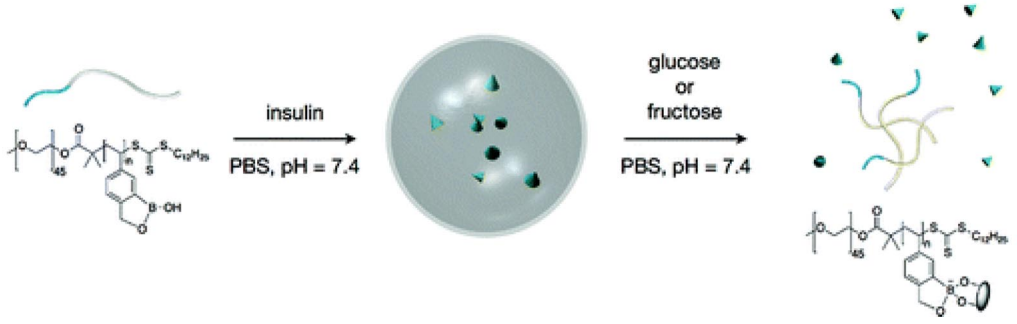
In 2010, the Clark group [166] reported glucose-sensitive nanosensors by using an indicator displacement assay [167] based on the widely used ARS method [168]. This method is based on competitive binding properties of aryl and alkyl diols with boronic acids (Scheme 1).

These nanosensors were able to monitor dynamic changes in blood glucose concentrations in a mouse model. The results obtained using the nanosensors were comparable to measurements taken using a glucometer. The development of these nanosensors offers an alternative and minimally invasive tool for monitoring glucose levels. Recently [169], this nanosensor has been improved with regard to sensor lifetime by modifications to the plasticized polymeric membrane.

The Kim group recently reported a strategy that involves a combination approach for glucose sensing (diagnosis) and insulin release (therapeutics). The strategy involved synthesis of a boroxole-based styrenic monomer and its controlled polymerization via a reversible addition-fragmentation and chain transfer (RAFT) method [170]. The resulting poly(styreneboroxole) (PBOx) was able to self-assemble to form nanostructures that exhibited monosaccharide-responsive disassembly to release encapsulated insulin in the presence of sugars. Controlled release was demonstrated with fluorescein isothiocyanate (FITC)-labeled insulin (Scheme 2). Compared with previously reported phenylboronic-



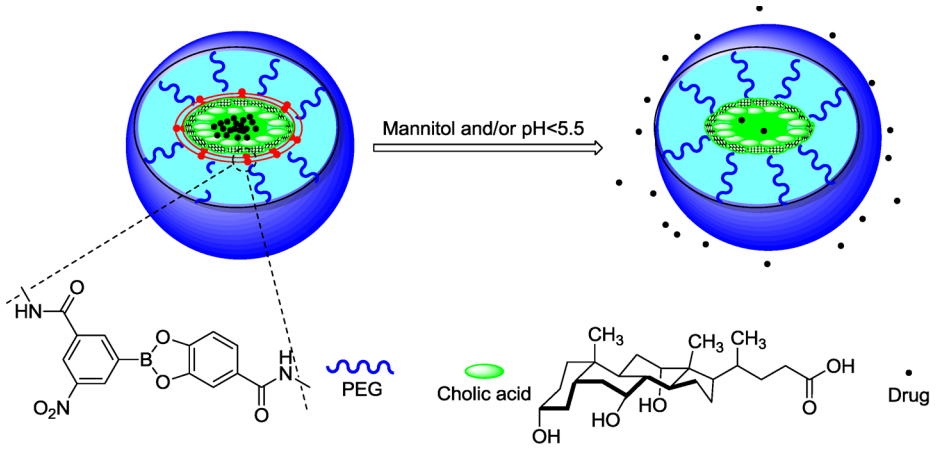
Scheme 1 The general concept of using a displacement assay in determining sugar concentration using PBLs and ARS.



Scheme 2 Self-assembly of PEG-b-PBOx and its disassembly in the presence of monosaccharides. Reprinted with permission from *J. Am. Chem. Soc.* **134**, 4030 (2012), copyright © (2012) American Chemical Society.

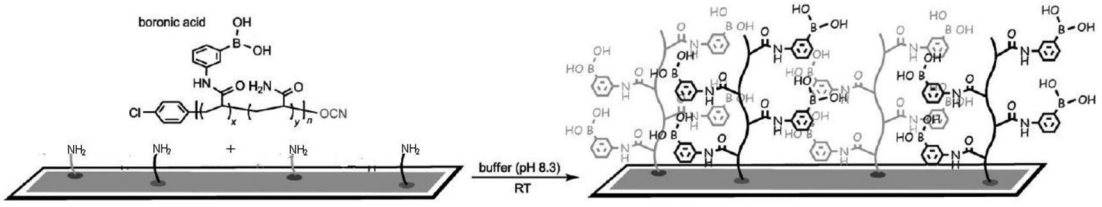
acid-containing polymers [171,172], the PBOx offers the possibility of insulin release at physiological pH with a tunable release rate.

Besides diabetes, cancer claims many lives worldwide. The need to improve specificity and bioavailability of chemotherapeutic agents and reduce drug cytotoxicity has drawn significant efforts to the development of stimuli-responsive drug-delivery nanoplatforms for controlled drug release in response to specific cellular signals [173–176]. Lam and co-workers developed dual-responsive boronate cross-linked micelles (BCMs) [177] based on previously reported telodendrimer systems [178–182] for efficient anticancer drug delivery. In their study, reversible boronic acid/catechol complexation was utilized to cross-link the core-shell polymer of BCMs and paclitaxel as a model payload. Since the boronic acid/catechol pair was sensitive to pH and diols (Scheme 3), targeted drug delivery was achieved by weakening the cross-linking at low pH microenvironment of tumor and exogenous competing mannitol administration.



Scheme 3 Structure of the BCMs and drug release in response to acidic pH or mannitol.

Another application of boronopolymer is capturing. For example, in 2010, the Sun group developed an *O*-cyanate chain-end-functionalized boropolymer [183], which is easily immobilized onto an amine surface via isourea bond formation (Scheme 4). This could be used for affinity purification of carbohydrates and glycoconjugates for their identification and subsequent functional investigation.



Scheme 4 *O*-Cyanate chain-end-functionalized boropolymer and its oriented immobilization onto amine surface via isourea bond formation as a 3D carbohydrate receptor expression. Adapted with permission from *ChemBioChem* **11**, 2018 (2010), copyright © (2010) John Wiley.

By changing the "chain end" to biotin, biotin-boropolymer was developed [184]. Such a polymer was immobilized onto streptavidin-derivatized magnetic beads for bead-based glyco-capturing and assays (Fig. 5).

Another example that boronic-acid-based polymers are used as capturing ligands was published in 2010 by the Ye lab [182]. Specifically, boronic acid was tethered to Sepharose through either click chemistry or addition to an epoxide (Scheme 5). The conjugate was then used as affinity matrix in a column to isolate OVA from a mixture of crude *E. coli* protein mixture. The affinity of boronic-acid-modified matrix to OVA over other closely related proteins resulted in superior effectiveness over the existing commercial immobilized boronic acid gel.

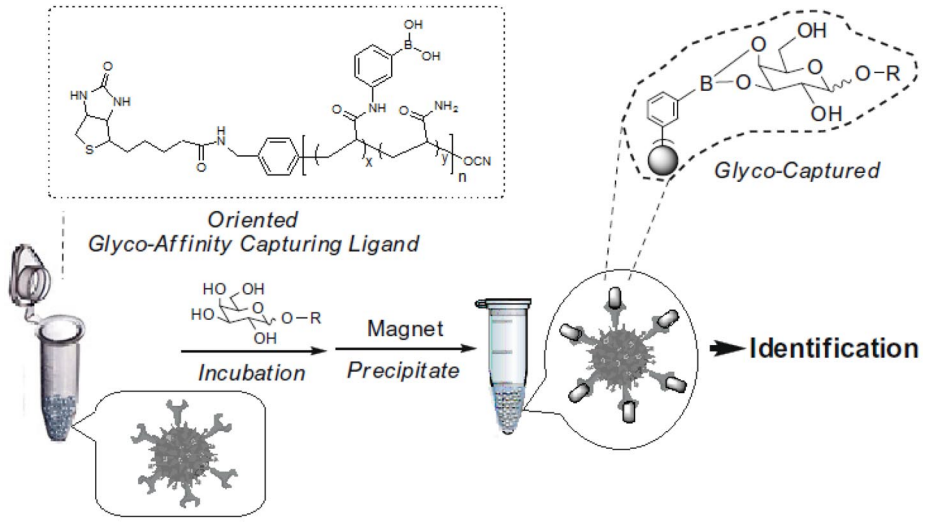
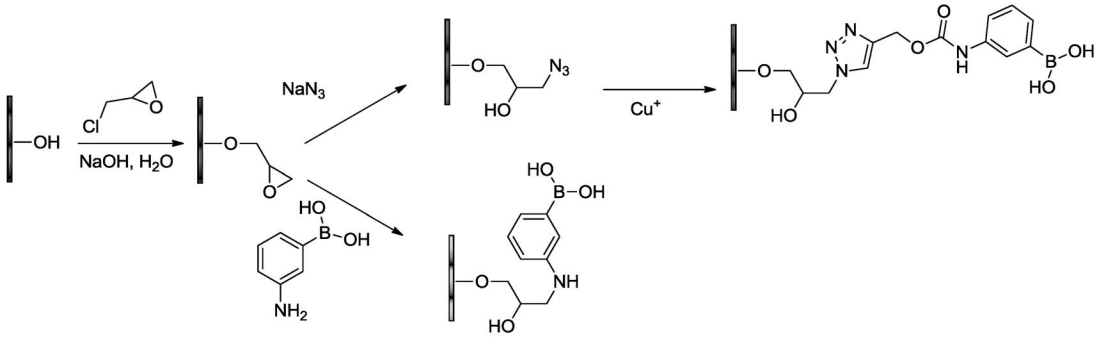


Fig. 5 Structure of biotin boropolymer as functional glyco-affinity capturing ligand for efficient carbohydrate and glycoconjugate purification and identification. Adapted with permission from *React. Funct. Polym.* **70**, 471 (2010), copyright © (2010) Elsevier.



Scheme 5 Immobilization of boronic acid ligands using click reaction and direct ring opening of epoxide.

SMALL-MOLECULE-BASED BORONOLECTINS (SBLs)

Another important family of boronlectins is SBLs [130,185]. A major challenge in this field hindering the development of diagnostic and therapeutic agents for biologically important carbohydrate biomarkers is the difficulty in rational design and synthesis of highly specific and tight binders for complicated carbohydrates with subtle structural differences. Since there have been several reviews in this area [85,129,145,186], herein we will focus on recent development including some very successful applications of SBLs.

A series of bis-boronic acids with different linkers were reported by the Wang lab. Some of them are shown in Fig. 6 [23,187]. Among all the compounds synthesized, compound **8a** show selective binding to sLe^x and **8d** showed selective binding to Le^x [188]. Concentration-dependent fluorescent labeling was observed in CHOFUT4 cells, which express the Le^x antigen, but not COLO205 (sLe^a and sLe^x) and CHO (negative control) cells (Fig. 7).

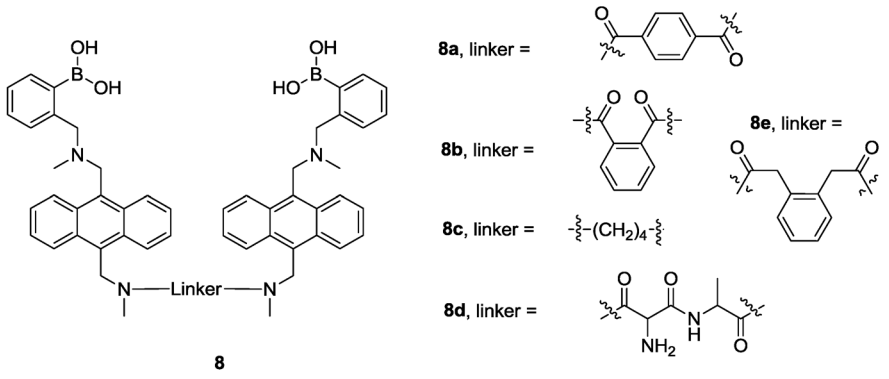


Fig. 6 Bis-boronic acids (boronolectins) capable of recognizing carbohydrates.

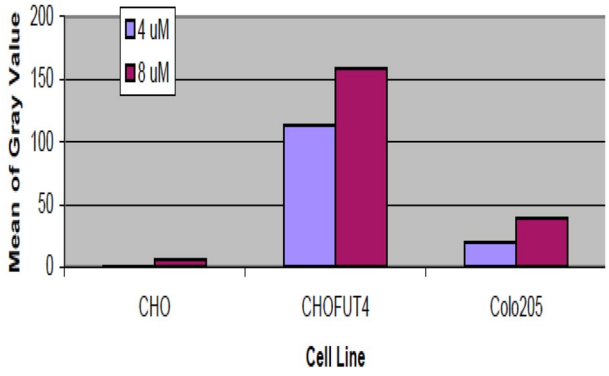


Fig. 7 8d staining of CHO, CHOFUT4, and COLO205 cells at 4 (left) and 8 (right) μM.

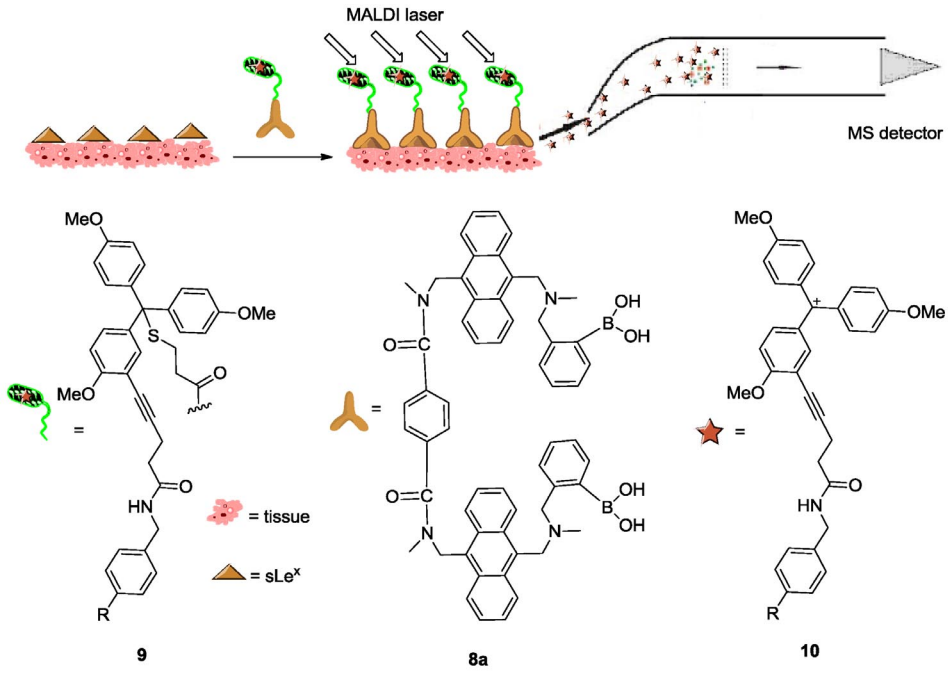
Fluorescent labeling using compound **8a** has been extended to in vivo studies. Mouse models with HEPG2 and COLO205 cancer cells implanted were tested with flank tumor injections. Specific labeling of the tumor over other tissues such as liver and colon was obtained at 24 h post-injection time point (Table 2). In contrast, control compound **8b** did not label the tumor or the other organs. Such results clearly show that selective targeting can be achieved in vivo.

Table 2 Tumor staining with **8a** and **8b**.

	Intensity		
	Tumor	Liver	Colon
8a	95 (+/–12)	27 (+/–16)	23 (+/–6)
8b	12 (+/–10)	8 (+/–11)	14 (+/–9)

Traditional cancer histological work relies on fluorescent/color staining of tissues either using nonspecific dyes or dyes conjugated to a targeting molecule such as an antibody. The application is limited by stability, cost, and availability of the related antibodies. Such approaches also suffer from difficulties in multiplexing due to spectral resolution/overlap issues and in quantitation. Recently, laser reactive reporter ions have been used with antibodies to provide a matrix-free, targeted approach to identify the location and concentrations of specific proteins in tissues by matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) [189–194].

The Wang lab has combined MALDI-IMS with boronolectin applications. Specifically, compound **8a** conjugated to a matrix-free mass spectrometric tag (a trityl group) (Scheme 6) was synthesized and tested with fresh-frozen renal tissues containing both tumor and normal regions [195]. After incubation with sLe^x-trityl probe and washing, the tissue was systematically analyzed by a grid of MALDI laser shots. The peak of *m*/*z* 518 observed from the area of tissue as shown in red pixel intensities was coincident with immunostaining of the sLe^x expressing regions, which marked out the tumor area (dark stained bottom tip area) (Fig. 10). This same probe also showed binding to alcohol fixed renal tissues. Thus, MALDI-IMS results tracked with the immuno-staining results and tumor tissue location, demonstrating feasibility of using a boronolectins-MS tag conjugates such as **11** for MALDI-IMS/histological work.



Scheme 6 The general concept of boronolectin-MS tag conjugation for imaging applications.

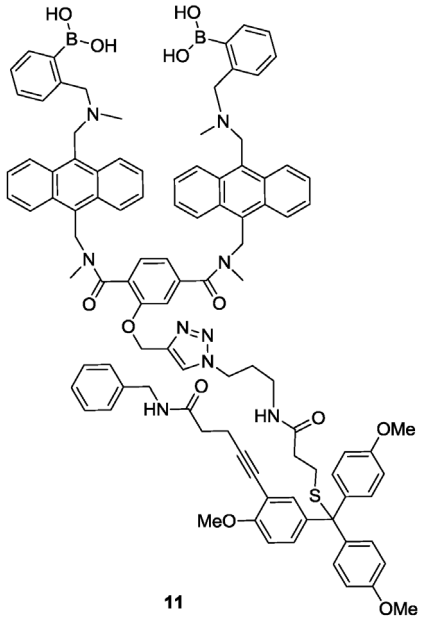


Fig. 8 Structure of a boronoletin MS tag conjugate.

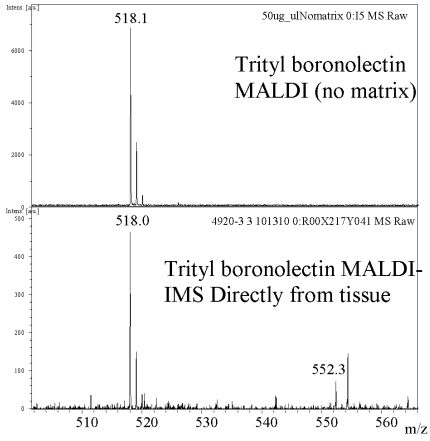


Fig. 9 MALDI-IMS trityl peak using frozen kidney tissue cut and stored at –80 °C. Other peaks track to the RCCs (renal cell carcinomas). Reprinted with permission from *Chem. Commun.* **47**, 10338 (2011), copyright © (2011) Royal Society of Chemistry.

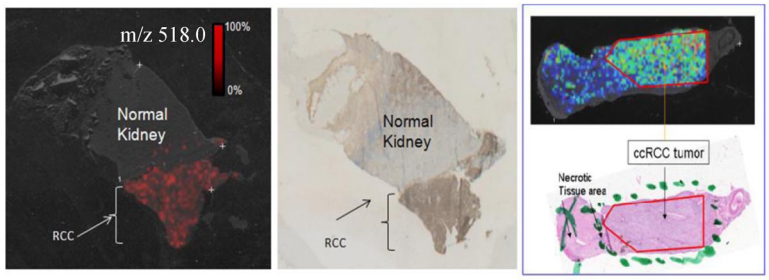


Fig. 10 MALDI-IMS (left) and immuno staining (middle) images of kidney tissue described in Fig. 9. A pathologist confirmed that immunostaining and MALDI-IMS-boronolectin signal results overlap in the tumor region, and not in normal cell areas. The third panel (right) shows boronolectin staining of a Sakura/UMFix alcohol fixed renal tumor tissue (top). Reprinted with permission from *Chem. Commun.* **47**, 10338 (2011), copyright © (2011) Royal Society of Chemistry.

CONCLUSIONS

In conclusion, carbohydrates play very important roles in a wide range of biological and pathological processes. Compounds that can specifically recognize carbohydrate biomarkers can be used for specific delivery of imaging and therapeutic agents. In this regard, boronic-acid-based binders/sensors play a very important role. A tremendous amount of work on synthetic lectin mimics has been reported in recent years to demonstrate their application potential. However, compared to the vast opportunities in this field, the amount of work in this area is very limited. A key difficulty is in the development of selective boronolectins, although very promising results have been generated in several labs. We hope that this review will trigger more work in this area so that this tremendously underexplored area will become much more active.

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