Glycoconj J (2015) 32:559–574
DOI 10.1007/s10719-015-9611-9

REVIEW

Fluorescently labelled glycans and their applications

Hongbin Yan[1] · **Ravi Shekar Yalagala**[1] · **Fengyang Yan**[2]

Received: 25 May 2015 / Revised: 13 July 2015 / Accepted: 15 July 2015 / Published online: 4 August 2015

Abstract This review summarises the literature on the synthesis and applications of fluorescently labelled carbohydrates. Due to the sensitivity of fluorescent detection, this approach provides a useful tool to study processes involving glycans. A few general categories of labelling are presented, *in situ* labelling of carbohydrates with fluorophores, fluorescently labelled glycolipids, fluorogenic glycans, pre-formed fluorescent glycans for intracellular applications, glycan-decorated fluorescent polymers, fluorescent glyconanoparticles, and other functional fluorescent glycans.

Keywords Fluorescent labelling · Glycan · Carbohydrate detection · Fluorogenic glycoside · Fluorescent polymer · Glyconanoparticle · Lectin · Fluorescently labelled glycolipid

Introduction

Fluorescent labelling has found extremely broad applications, particularly in the areas of protein and nucleic acids due to the high sensitivity and ease to work with compared with radiolabelling. In this respect, various techniques based on behaviours of fluorophores have been developed. Contrary to proteins and nucleic acids, fluorescent labelling of carbohydrates has been much less studied, probably due to a number of factors. Compared with proteins and nucleic acids, carbohydrate structures are much more complex, leading to challenges in labelling in a site-specific manner. The level of structural complexity of carbohydrates also means that interpretation of interaction is extremely difficult. In addition, concerns remain in that addition of bulk fluorescent labels to glycans tends to affect overall structures of carbohydrates and consequently their interactions with other biomolecules such as proteins.

Despite these challenges, there has been extensive literature describing attempt to establish fluorescent labelling of glycans as a versatile approach to study carbohydrate structures and their interactions in biological systems. In recent years, interest is gradually intensifying to develop chemistry for fluorescent labelling of glycans, to define their applications and to interpret results from these applications. Effort has also been directed towards the use of fluorescently labelled glycans in applications such as ion sensing and as scaffolds for functional materials.

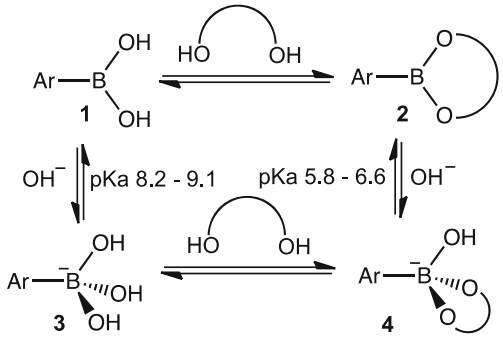
Boronic acid-based fluorescent detection of carbohydrates

Although not the main topic of this review, boronic acids have been used rather extensively in the characterization of carbohydrates, based on changes in fluorescent intensity or shift in emission profiles of boronic acids upon reactions with carbohydrates. Over the past few decades, dozens of such sensors have been explored. Some of the work has been summarized in three reviews [1–3]. It is worth noting that the vast majority of these sensors are based on the reversible reactions between boronic acid **1** and diols that are present in carbohydrates (Scheme 1) [4]. This process is pH-dependent in aqueous media. Formation of cyclic boronates **2** through the reaction

✉ Hongbin Yan
tyan@brocku.ca

[1] Department of Chemistry, Brock University, 500 Glenridge Ave., St. Catharines, ON L2S 3A1, Canada

[2] Sussex Research Laboratories Inc., 100 Sussex Drive, Ottawa, ON K1A 0R6, Canada



Scheme 1 Reactions of aromatic boronic acid fluorophores **1** with diols in aqueous solutions

of boronic acids **1** with diols renders boronate esters more acidic, as is indicated by changes in pKa (Scheme 1) [5, 6]. Subsequent reaction of the cyclic boronate with water leads to the formation of an anionic species **4**, changing boron hybridization from sp^2 to sp^3. The anionic boron centre then could act as an electron donor or participate in photoinduced charge transfer, leading to changes in fluorescent properties of the sensor, *i.e.*, the Ar group. As the diols in carbohydrates display different conformations and dissociation constants, this approach provides a means to potentially differentiate simple carbohydrates through changes in behaviour of the fluorophore.

In situ fluorescent visualization of carbohydrates

As carbohydrates do not possess chromophores, early work on fluorescent labelling of carbohydrates focused primarily on increasing the detection sensitivity of carbohydrates in various analytical techniques such as liquid chromatography and gel electrophoresis. To this end, a range of fluorescent reagents, such as dansylhydrazine **5** [7, 8], dansylamine **6** [9], 7-aminonaphthalene-l,3-disulfonic acid **7** [10], 2-aminobenzoic acid **8** [11], 2-aminopyridine **9** and 7-amino-1-naphthol **10** [12, 13], fluorescein amine **11** and 9-aminoacridine **12** [14] have been utilized (Fig. 1). Most of these labelling approaches not only provide sensitivity in detection, but also hydrophobicity that makes certain separation techniques, such as reverse phase HPLC, more readily applicable.

Most of the derivatization reactions in this regard occur at the reducing end of sugar through reductive amination (Scheme 2a), where the resulting imines or hydrazones are treated *in situ* with reagents such as sodium cyanoborohydride to generate stable amines or hydrazines. For non-reducing sugars, protocols have been developed to first oxidize vicinal diols using periodate followed by condensation of the resulting aldehyde with reactive fluorescent reagents possessing functional groups such as amine, hydrazine and aminooxy (Scheme 2b). Subsequent reduction again yields stable amines or hydrazines for detection. Alternatively, selected carbohydrates, such as galactose, can also be oxidized by enzymes, such as galactose oxidase [14], into corresponding ketones, which can be subjected to similar reductive amination process.

The labelling chemistry described above is useful for simple sugars, particularly identification and determination of composition of monosaccharides. For more complex carbohydrates, preparation of carbohydrate–fluorophore conjugates through a variety of chemistry has been developed, which is the subject of a later section of this review. There has been literature precedence, however, for successful site-specific fluorescent labelling of complex glycans, such as zwitterionic polysaccharide from *Bacteroides fragilis* [15].

While these fluorescent labelling reagents proved to be quite useful in carbohydrate analysis, the fact that both the labeling agents and products are fluorescent inevitably leads to difficulty in analysis due to background fluorescent noise.

Fluorogenic labelling agents

One obvious solution to minimize the background noise issues is to use fluorogenic labelling agents which are non-fluorescent on their own, but become fluorescent upon reactions with target carbohydrates. A number of such fluorogenic labelling agents have been demonstrated.

Early work in this area included fluorogenic labels that react with reducing sugars under strongly acidic conditions, such as 5-hydroxytetralone [16], phenylenediamine [17], and *N*-(1-naphthyl)ethylenediamine dihydrochloride [18], however, these conditions can lead to cleavages of glycosidic linkages that might be present in the reducing sugar sample. Fluorogenic reagents that react with carbohydrates under mildly basic or neutral conditions were also reported. In this respect, ethylenediamine [19], malonamide [20], 2-aminoethanesulfonic acid (Taurine) [21], ethanolamine/boric acid [22], 2-cyanoacetamide [23], and 4-methoxybenzamidine [24] have been found to be quite useful in fluorogenically labelling reducing sugars. Further, fluorogenic labelling reagents that are specific for certain monosaccharides are also known. For example, reaction of thiobarbituric acid with sialic acid that is pre-treated with periodate allows for fluorescent detection of sialic acids in the picomole range [25]. Recently, a periodate – acetoacetanilide/ammonia sequence allows for fluorogenic detection of sialic acids in glycoproteins at nmol/ml concentration [26].

A recently reported labelling chemistry involves the reaction of reducing sugars, *e.g.*, D-galactose **13**, with 1,3-di(2-pyridyl)-1,3-propanedione **14** in the presence of sodium bicarbonate to produce a fluorescent 2-pyridylfuran **15** (Scheme 3) [27]. Compared with chemistry described previously, this fluorogenic labelling chemistry is carried out under mild

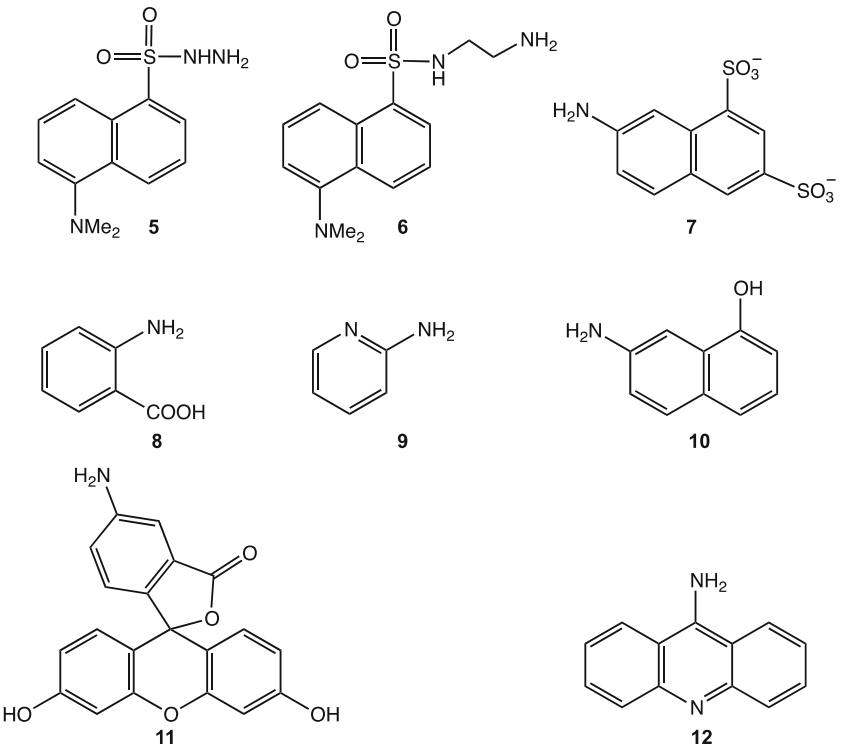


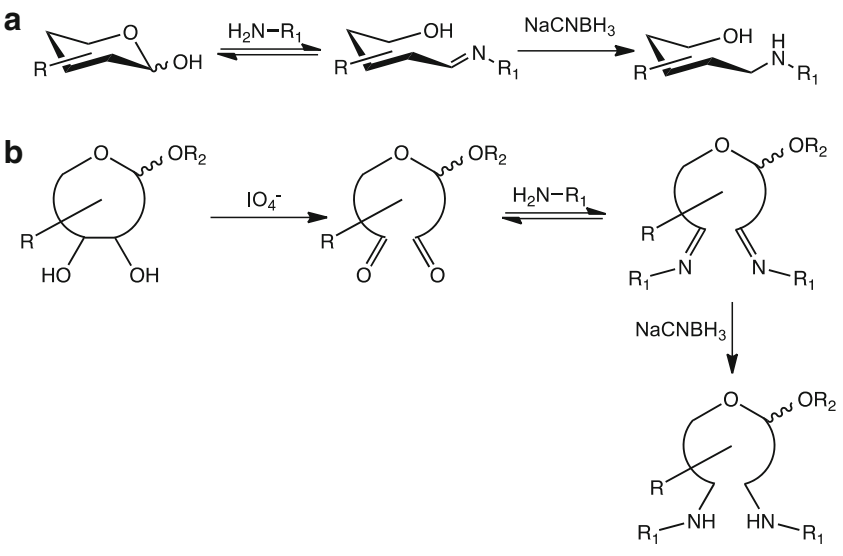
Fig. 1 Some commonly used fluorescent probes in carbohydrate labeling

conditions, and is quite efficient, with an analysis time of 10 min. Methods were also reported for the separation of reaction products by HPLC from a rather large number of monosaccharides.

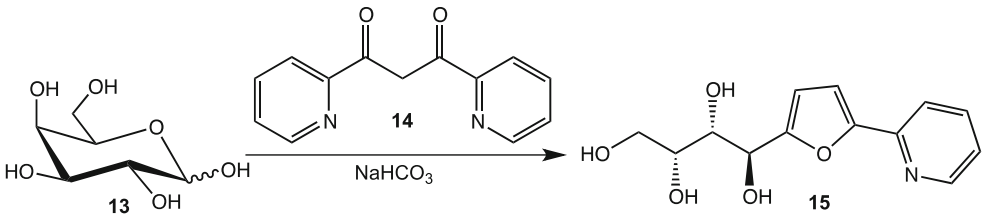
Fluorescently labelled glycolipids

Glycolipids are important cellular components that play varying roles in biological processes. It is of importance to detect and identify these species, to monitor their metabolism, to track intracellular distribution and to study the biological activities of both endogenous and synthetic glycolipids. Fluorescent labelling provides sensitivity to these challenging tasks. A number of fluorescently labelled glycolipids have been synthesized.

One of the earliest applications of 4,4-difluoro-4-bora-3a, 4a-diaza-*s*-indacene (BODIPY) fluorophores in labelling biological samples was found in lipids such as sphingolipids [28–30]. With the advantages that BODIPY fluorophores possess, their applications have been extended towards labelling glycolipids. In fact, some of these BODIPY labelled



Scheme 2 Labelling of carbohydrates with fluorophores **a** through reductive amination of reducing sugar, and **b** through a periodate oxidation–reductive amination procedure



Scheme 3 Formation of fluorescent 2-pyridylfuran **15** by the treatment of reducing sugars, such as D-galactose **13**, with 1,3-di(2-pyridyl)-1,3-propanedione **14**

sphingolipids, such as BODIPY conjugates with ceramide, sphingomyelin, glucosylceramide, lactosylceramide, and GM1 ganglioside (Fig. 2), are commercially available from Molecular Probes (now Life Technologies). These BODIPY-sphingolipid conjugates are useful in studying transport and metabolism of sphingolipids [28], and as structural markers for the Golgi complex [31].

Other BODIPY glycolipids have also been prepared [32]. While the stereochemistry in the BODIPY ceramide conjugates from Life Technologies is unknown, a saturated BODIPY α-galactosylceramide analogue **16** (Fig. 3) with defined stereochemistry was synthesized to study the *in vivo* behaviour of α-galactosylceramide in mice by fluorescent techniques. This compound was also found to be active in inducing apoptosis of liver derived natural killer T cells [33]. In order to investigate the importance of the stereochemistry of the long chain of ceramide in the uptake of lactosylceramide through endocytosis [34], Bittman's laboratory synthesized BODIPY conjugates with maltosylceramide [35] and three stereoisomers of the naturally occurring lactosylceramide **17**–**19** [36] (Fig. 3).

While BODIPY fluorophores are attached to the hydrophobic portions of glycosphingolipids in the examples described above, BODIPY has also been covalently linked to sialic acid in gangliosides [37]. These constructs could potentially perturb interactions between glycans with sugar-binding proteins; however, they can be useful in the investigation of the biological fate or activity of the lipid head of glycolipids.

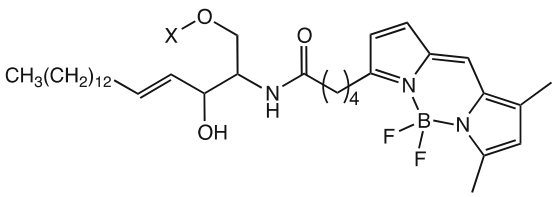
Another commonly used fluorophore, 7-nitrobenz-2-oxa-1,3-diazole (NBD), has also been conjugated to the hydrophobic tail of GM1 and GM2 gangliosides to investigate the transfer activity of GM2-activator proteins [38].

Dovichi and co-workers recently demonstrated synthesis and electrophoretic separation of various BODIPY [39] and tetramethylrhodamine [40] labelled glycosphingolipids as a means to monitor metabolism of glycosphingolipids. When coupled with laser-induced fluorescence or mass spectroscopic detection, this system allowed for the study of glycosphingolipid metabolites at a very broad dynamic range from mM to pM for laser-induced fluorescence detection and down to yotomoles for mass spectroscopic detection [41–43]. The extremely high sensitivity in detecting BODIPY labeled lactosylceramide (amol) involved in glycan processing was also demonstrated by a fluorescence monitored mass spectrometry setup using nanoLC-microelectrospray-quadrupole ion trap-time of flight analysis [44].

Fluorescently labelled glycans for intracellular applications

While carbohydrates can be fluorescently labelled *in situ* for detection purposes, glycans bearing fluorophores at specific linkages can serve as sensitive markers when applied to *in vitro* and potentially *in vivo* experiments. A major consideration in using fluorescently labelled glycans in these studies rests in the concern that covalently linked fluorophores can lead to changes in the overall property of the glycan. Therefore, results from these studies must be interpreted with great care. Nevertheless, the utility of fluorescently labelled glycans has found interesting applications in a range of scenarios. Development of suitably fluorescently labelled glycans represents a fast evolving area of research in carbohydrate chemistry.

Understanding sialic acid processing is an important aspect of research in glycobiology. As such, use of fluorescently labelled sialic acid and analogues offers the ability to study sialic acid-processing enzymes such as cytidine monophosphate (CMP)-sialic acid synthase, sialyltransferase and neuraminidase. In this respect, a sialic acid conjugated with fluorescein isothiocyanate (FTIC) at *N*-9 position, as in **20**, was found to be a good substrate for GalNAc α2,6-sialyltransferase, allowing for transferring of a



BODIPY - ceramide: X = H;
BODIPY - GM1: X = GM1 ganglioside glycan;
BODIPY - lactosylceramide: X = lactoside

Fig. 2 Some BODIPY labeled sphingolipids that are commercially available from life technologies

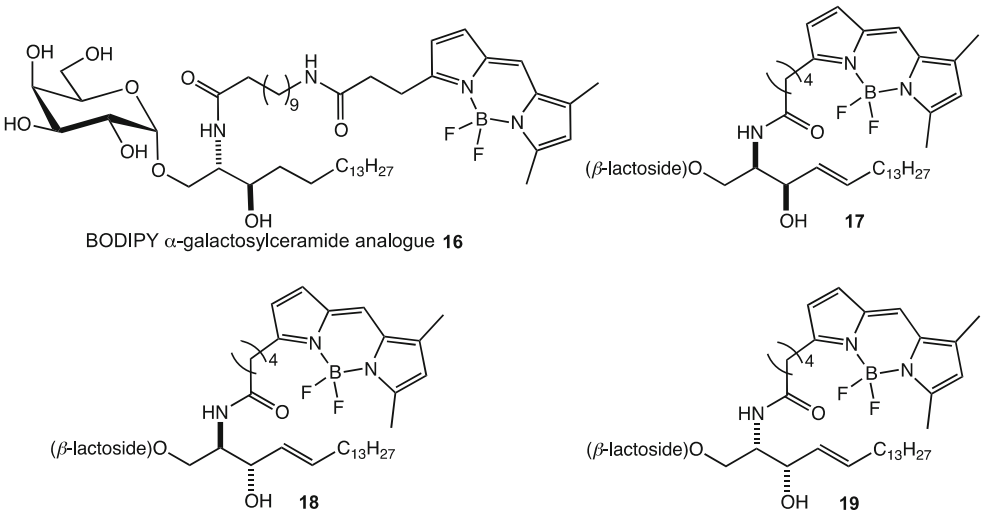
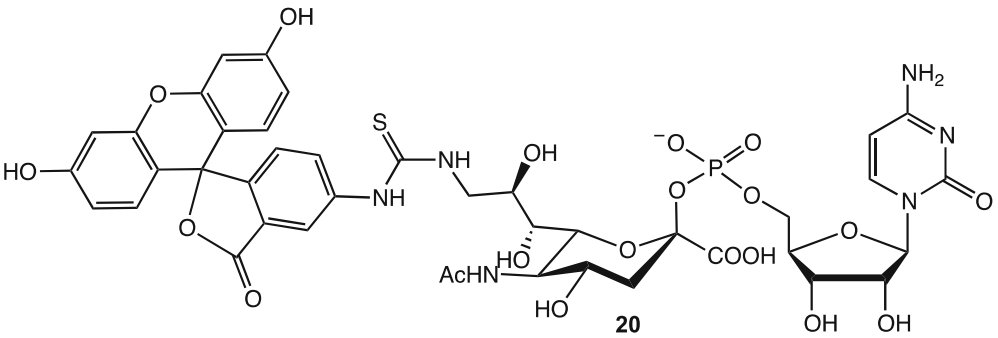
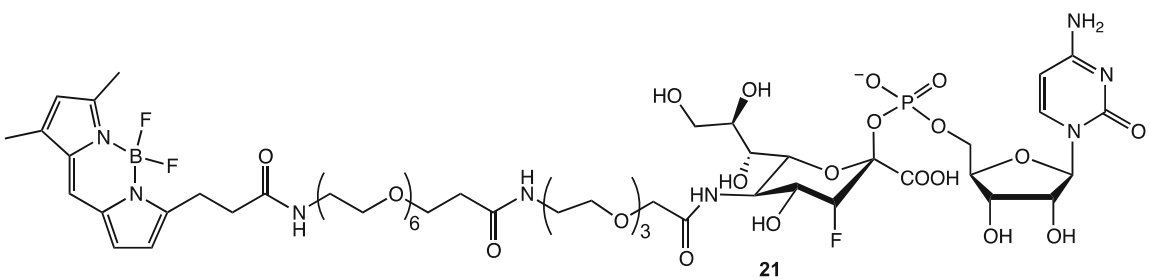


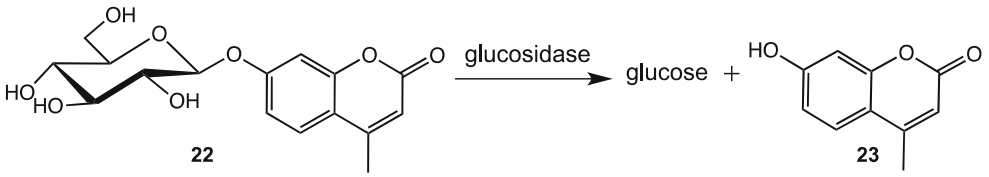
Fig. 3 BODIPY-glycosylceramide conjugates with defined stereochemistry

fluorescently labelled sialic acid analogue to an antifreeze protein [45]. The fluorescent sialic acid donor **20** was also used in a sensitive assay to determine the enzyme kinetics of rat liver α2,6-sialyltransferase [46] and to characterize a human plasma sialyltransferase [47]. This fluorescent CMP-sialic acid and another sialic acid analogue derived with fluorescein at *N*-5 were subsequently shown to be good markers for intracellular sialylation, allowing for the visualization of cellular sialic acid processing by fluorescent imaging [48]. *N*-Acetylneuraminic acid (NAcNeu) labelled with FITC at *N*-9 position has also been shown in a recent application to preferentially label subcutaneous and liver tumors in mice, allowing for precise tumor location in surgical procedures [49]. Thus, when FITC-NAcNeu was administered to mice intravenously, this modified sialic acid was metabolized and displayed on the tumor cell surfaces.



In a similar approach, sialic acid modified at *N*-5 with BODIPY, as in **21**, was synthesized. The C3-position is also fluorinated so that the modified BODIPY-NAcNeu does not serve as a substrate for sialyltransferase, however, the conjugate is still recognized by CMP-sialic acid transporter [50]. When this conjugate was fed to a PC-12D cell culture, it was possible to visualize specific Golgi vesicles through fluorescence confocal imaging.





Scheme 4 Hydrolysis of 4-methylumbelliferyl-β-D-glucopyranoside **22** by glucosidase, leading to the formation of fluorescent 4-methylumbelliferone **23**

Fluorescently labelled glycans were also shown to be useful in tracking the internalization and cell activation of inositol phosphate glycans [51], to profile cytoplasmic peptide:*N*-glycanase [52] or other glycosidases [53], and cell targeting and uptake of glycans [54–56].

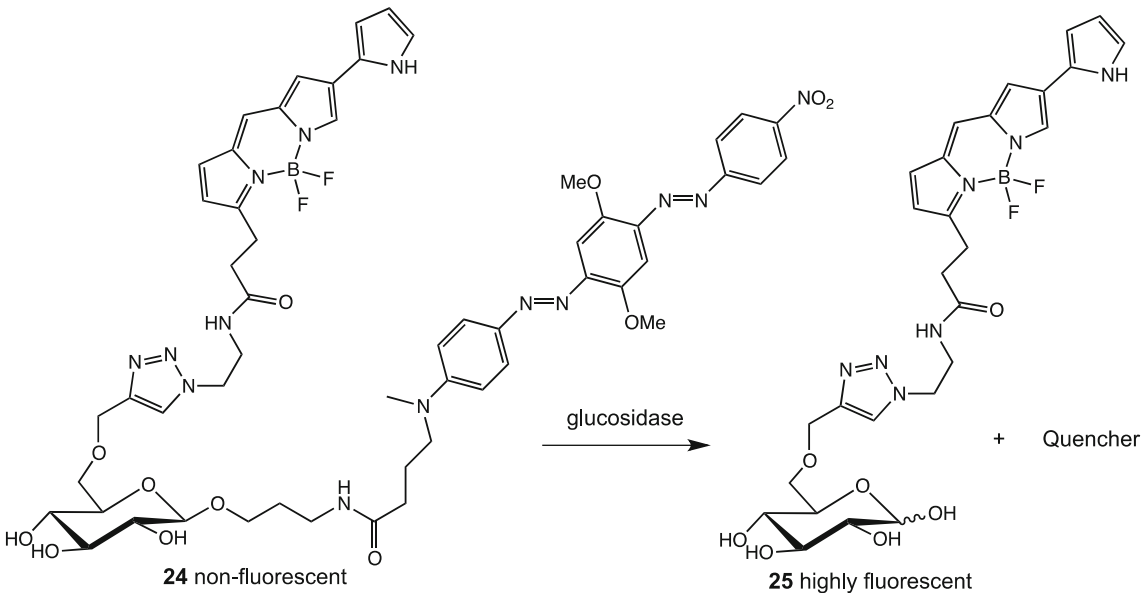
Fluorogenic glycosidase substrates

Fluorogenic glycosides have been widely used in the study of activities of glycosidases [57] due to the sensitivity of fluorescent detection; some of these fluorogenic glycosides are also commercially available.

Among these, 4-methylumbelliferyl glycosides represent one of the most commonly used fluorogenic probes in studying glycosidase activities. Commercially available from the 1960s, 4-methylumbelliferyl glycosides, such as 4-methylumbelliferyl-α/β-D-glucopyranoside, 4-methylumbelliferyl-β-D-cellobioside, 4-methylumbelliferyl-β-D-galactopyranoside, and 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide, have been used for the identification, characterization and kinetic study of glycosidases [58–61]. Cleavages of the glyco linkage in these glycosides, such as 4-methylumbelliferyl-β-D-glucopyranoside **22**, by glycosidases lead to the formation of fluorescent 4-methylumbelliferone **23** (Scheme 4).

This approach was extended to more complex 4-methylumbelliferyl fluorogenic glycosides, such as di-, tri- and oligosaccharides [62–67], and challenging monosaccharides such as sialic acids [68–70]. 4-Methylumbelliferyl aglycone analogues were also explored to better suit the properties of glycosidases [71–74]. A number of other fluorenegic glycosides based on aglycons other than 4-methylumbelliferyl were shown to be useful glycosidase substrates, possessing properties that could be more suitable for their respective applications [75–77].

A recent design of a fluoregenic glycoside substrate for glucocerebrosidase allowed for live cell imaging enabled by Fluorescent Resonance Energy Transfer (FRET) that depends on whether the glycoside substrate is processed by the hydrolase or not [78]. As shown in Scheme 5, the BODIPY fluorescence is quenched by the blackhole quencher in the glycosidase substrate **24**, however, cleavage of the glycosidic linkage leads to the depart of the quencher, restoring fluorescence of the sugar-BODIPY conjugate **25**. This probe is also presumed to be suitable for investigating intracellular glucocerebrosidase activity due to its weakly basic quencher, allowing for its accumulation in the slightly acidic lysosome.

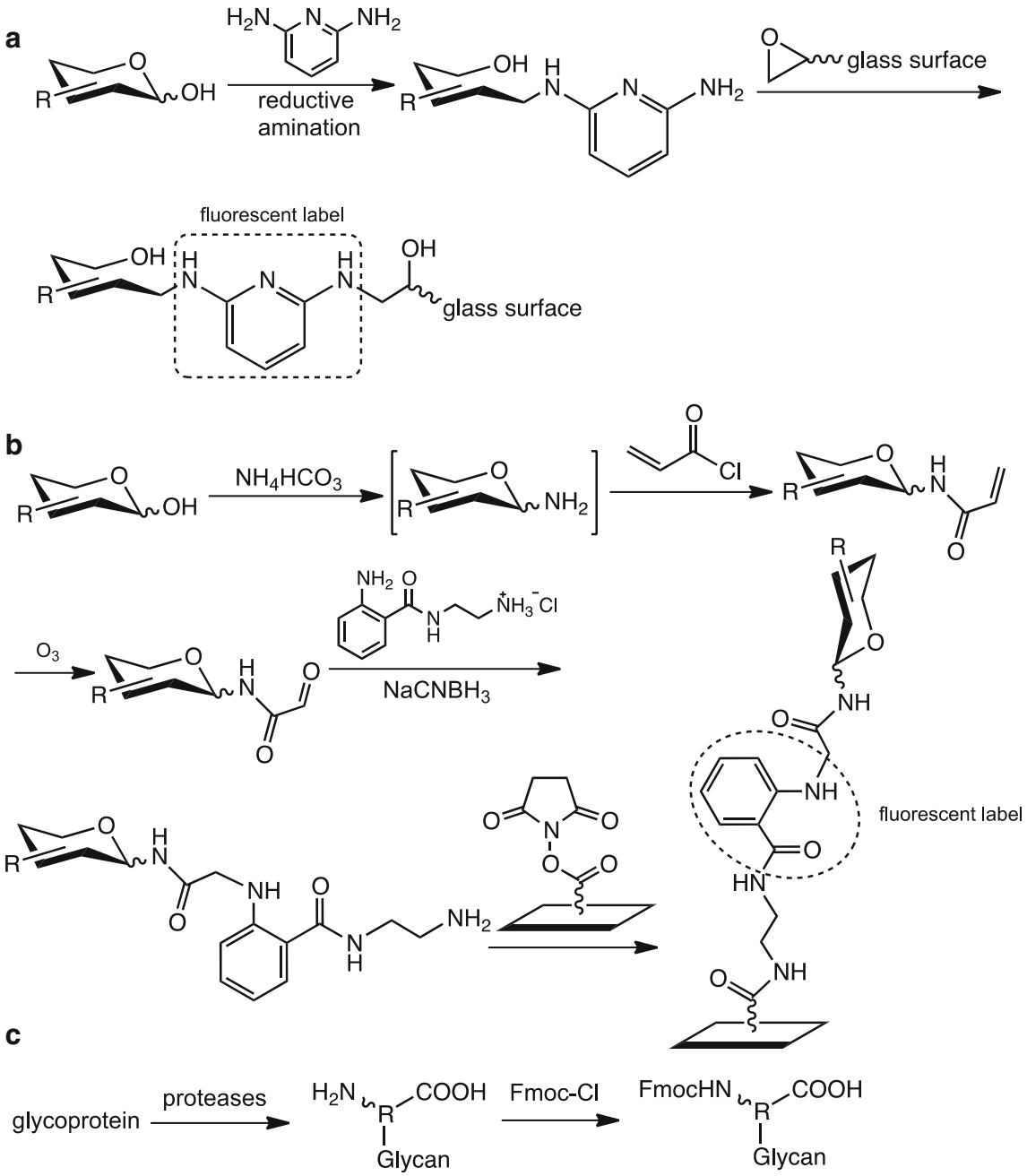


Scheme 5 Glucoside decorated with a BODIPY-block hole quencher FRET pair **24**. Cleavage of the glycosidic linkage by glycosidase leads to the restoration of BODIPY fluorescence

Fluorescent glycan microarray

With the increasing interest in glycogenomics, the glycobiology community started to pick up interest in glycan arrays [79–81]. Despite of the challenges in access to complex sugar and appropriate immobilization chemistry, this approach has the potential to allow for the studies of protein – carbohydrate interactions on large scales. To date, glycan microarrays are prepared in more than a dozen laboratories. While fluorescently labelled proteins have been widely used in glycan arrays [82], fluorescently labelled glycans are starting to find applications in microarrays [83]. In this application, fluorescently labelled glycans can be generated through chemistry such as reductive amination at the reducing end of sugar followed by immobilization (*e.g.*, Scheme 6a) [84–86], however, this approach is often not applicable to study intact glycans as it leads to the formation of acyclic sugar at the reducing end, which likely changes the binding behaviour of glycans.

Alternative approaches require attachment of spacers to the reducing end of sugar that not only allows for incorporation of



Scheme 6 Approaches for the preparation of fluorescent glycan arrays. **a** Fluorescent glycan array fabricated through reductive amination using 2,6-diaminopyridine as a bifunctional linker and fluorophore [85]; **b** Immobilization via derivatized fluorescent glycosylamides [83]; and **c** release of glycopeptide from glycoproteins by the treatment with proteases [87]

fluorophores but also immobilization, however, this approach adds complexity to the synthesis of glycan libraries. A number of spacer designs have been reported for general conjugation purposes, especially for the preparation of glycoproteins [88], some are developed particularly for the generation of glycan arrays or immobilization [82, 89–92]. Strategies have also been developed to release glycans from glycoconjugates through enzymatic [93] or chemical cleavages [94]. In order to incorporate a fluorophore and to attach to a solid support in glycan microarrays, a bifunctional linker is required. As demonstrated in Scheme 6b [83], natural glycans can be treated with ammonium bicarbonate to first yield corresponding glycosylamines that subsequently react with acryloyl chloride. The resulting glycosylacrylamides are then subjected to ozonolysis. The aldehyde functionality of the product reacts with a fluorescent linker *N*-aminoethyl-2-aminobenzamide to give a product that can readily be immobilized to surfaces that are derivatized with *N*-hydroxysuccinamide.

In a different strategy (Scheme 6c), glycoproteins are first treated with proteases to give glycopeptides with *O*- or *N*-glycans. The amino functionality in the glycopeptide products is then protected by treatment with 9-fluorenylmethyl chloroformate. The Fmoc protected glycopeptides are fluorescent, yet cleavable, and can be subsequently used for immobilization [87].

Glycan decorated fluorescent polymers

Fluorescent materials modified with carbohydrates have found some interesting properties and applications, as glycans can serve as structural scaffolds, provide polarity or solubility in aqueous media, and perhaps most importantly, a structural recognition element for these materials.

Some of the early work in the use of glycan-decorated fluorescent polymers showed that these conjugates are useful in studying lectins through fluorescent imaging. As demonstrated by Bovin and co-workers [95], glycan-polyacrylamide-fluorescein conjugates were found to be useful tools in this respect. One drawback from this probe, however, derives from the relatively weak interaction between lectins and simple glycans.

Bunz and co-workers first introduced glucose to fluorescent poly(*p*-phenyleneethynylene) (PPE) **26** to guide organization of PPE on nanoscales (Fig. 4, R = Ethex) [96]. These glucose-coated PPE polymers were found to form fibrous mats of good mechanical strength, and the interchain interactions are modulated by the presence of glucose. The same laboratory later demonstrated [97] that fluorescent emission profiles of glucose-coated PPE (**26**, Fig. 4, R=−$OC_{12}H_{23}$) can be modulated by addition of surfactants.

This polymer design was later on utilized by Seeberger and co-workers for the detection of bacteria [98]. In the presence

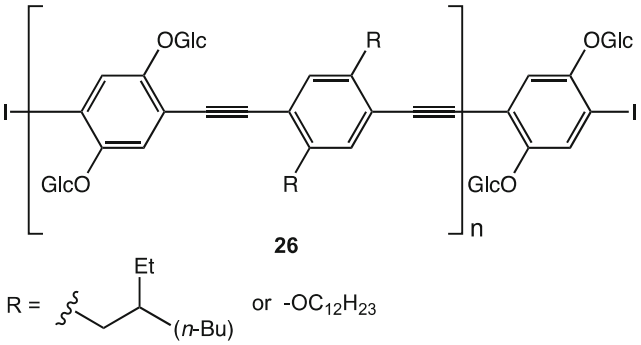


Fig. 4 Glucose decorated PPE

of concanavalin A (Con A), fluorescence of PPEs coated with mannose (**27**, Fig. 5, sugar = mannose) is quenched in a manner that is dependent on the concentration of Con A, while PPEs decorated with galactose did not show any significant binding with Con A. Furthermore, mannose-coated PPEs were shown to fluorescently stain wild type bacterium *E. coli* that bind to mannose at 10^4 clustered cells, while the ability of mannose-PPE to stain is abolished in an *E. coli* mutant strain that does not bind to mannose. It is worth noting that the detection limit for *E. coli* shown in this work is comparable to that of fluorescently labeled antibodies [98]. This detection concept is interesting as the polymer can be readily tailored to target pathogens that possess varying carbohydrate binding properties.

The success demonstrated by Seeberger's laboratory in utilizing fluorescent glycan-decorated polymers to study glycan-lectin interactions and to detect pathogens initiated significant interest to further develop this concept. Indeed, polymers of varying structures have been modified with glycans to study the glycan-lectin interactions, some have also been investigated towards pathogen detections [99–106]. Work reported by Tolbert, Bunz and co-workers [107] provided a system where fluorescence of mannose-decorated PPE is quenched through the formation of non-fluorescent aggregates with Con A (Schemes 6 and 7). This system is of particular interest as the fluorescent quenching becomes more pronounced with the increase in PPE concentration, which approaches the apparent binding constant of streptavidine and biotin.

Wolf and co-workers prepared micrometer-sized microparticles using glycosylated PPE and showed that these microparticles bind and agglutinate corresponding lectins [99]. These PPE particles could have potential in applications such as affinity chromatography to "catch" glycan binding proteins.

An interesting type of mannosylated polymer **28** consisting of amidoamine building blocks was also shown to be useful in bacterial detection [108]. These unique polymers do not possess conventional fluorophores, yet display fluorescence that

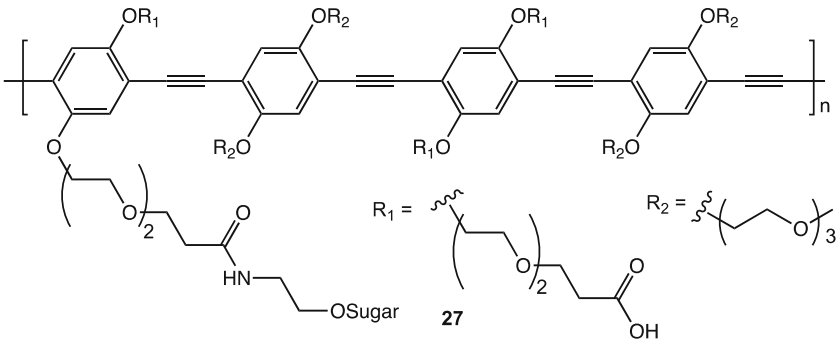
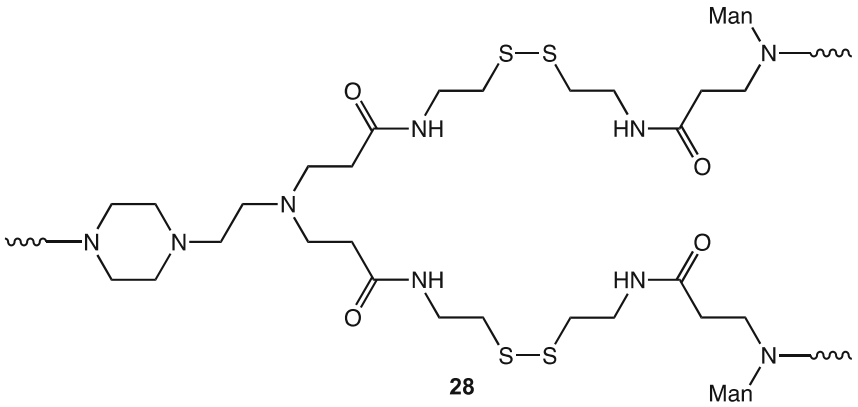


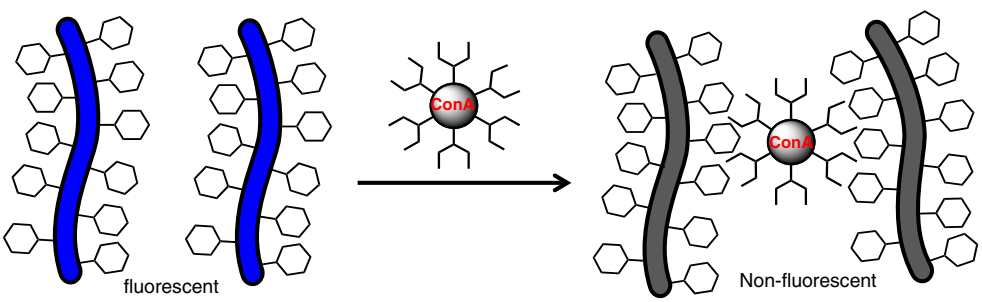
Fig. 5 Galactose and mannose coated PPE for detection of bacterial pathogens

is dependent on the size of the polymer. Further, fluorescence is significantly enhanced when mannose is covalently attached to the polymer. Binding of the mannosylated poly(amidoamine) to bacterial *E. coli* leads to fluorescence quenching, which allows for the detection of the bacterial species at 100 cfu/ml.



Lehn and co-workers also coined the term "glycodynamers" to describe polymers bearing oligosaccharides that demonstrate different fluorescent properties based on the constitution of the polymer that can be readily tuned through reversible reactions [109, 110]. Thus, a small library of glycan-decorated hydrazides **29**, **30** and aldehydes **31**–**33** were generated (Fig. 6). These hydrazides and aldehydes undergo condensation reactions to form fluorescent polymers that display different fluorescent and lectin-binding properties upon changes in conditions such as temperature and solvent.

Other carbohydrate-decorated fluorescent systems, such as dendrimers [111], peptide-based glycoclusters [112, 113], glycosylated pyrene-graphene assembly [114], supramolecular scaffolds [115], and chitin nanocrystals [116] were also developed towards lectin binding and bacterial detection. These designs allow for the glycocluster effect to maximize the interaction between glycans and lectins as well as bacterial surface glycan-binding proteins. Glycoclusters based on small molecular core structures were also shown to be useful in these applications [117–120].



Scheme 7 Fluorescence quenching of glycopolymers through binding to ConA. Figure reproduced from the Journal of American Chemical Society with permission

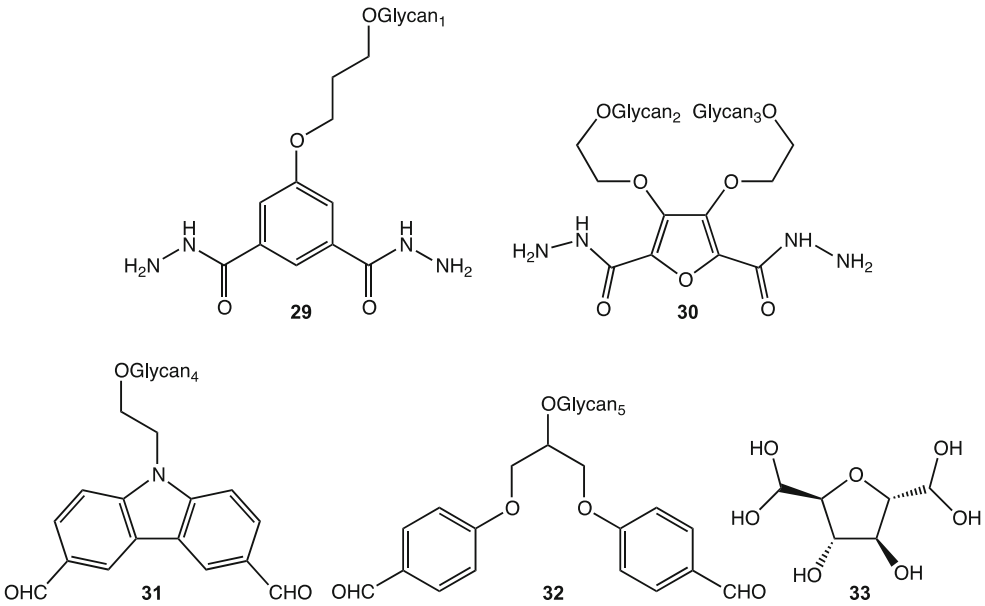


Fig. 6 Building blocks used in the reversible glycodynamer formed through condensation reactions

Fluorescent glyconanoparticles

One recent development in harnessing glycan-carbohydrate binding proteins involves fluorescent glyconanoparticles. Two recent reviews summarized the preparation of glyconanoparticles and their applications in colorimetric assays and biomedical research [121, 122]. Assembly of glycan-decorated nanoparticles introduces the ability to tune the behaviour of the conjugates through the unique optical, magnetic, and electronic properties of nanoparticles, and modulation of the size and shape of the nanoparticles.

Quantum dots (QDs) represent one of the most frequently used nanoparticles, partly due to the ease in modulating fluorescent properties through their sizes. QDs can also be readily modified on the surface to allow for the immobilization of multiple ligands, which is typically required in studying glycan-protein interactions. In this respect, *N*-acetylglucosamine bearing a sulfhydryl group at the reducing end through an alkyl linkage is readily attached to 5 nm CdSe/ZnS core-shell QDs [123]. It was determined that approximately 210 GlcNAc molecules were incorporated into each QD, and the fluorescence of the QDs is quenched in the presence of WGA lectin in a concentration-dependent fashion. In a similar approach, CdSe/ZnS QDs were decorated with glycans derived from di- or trisaccharides, *e.g.*, D-lactose, D-melibiose, or D-maltotriose, through reductive amination, and investigated for their ability to agglutinate soybean agglutinin, jacalin, and Con A [124]. These glycan-decorated QDs can selectively agglutinate corresponding lectins and the resulting aggregates can be deglutinated with free glycans selectively. QDs were also derivatized with β-cyclodextrin (CD), β-mannosylated or β-galactosylated CD [125]. These QDs selectively aggregated Con A, peanut agglutinin, and galanthus nivalis lectins, respectively. In a separate study, mannosylated QDs were also shown to be useful in detecting bacterial lectin in *E. coli* at 10^4 cfu/ml as results of the interaction between mannose and bacterial surface FimH mannose-specific lectin [126]. Metal nanoparticles, such as gold or iron oxide nanoparticles, were also found to be useful "carriers" of glycans for the detection of glycan-binding proteins and microorganisms, however, these nanoparticles are either non-fluorescent or weakly fluorescent on their own [127], unless modified on the surface with fluorophores [128].

Modification of liposomes by glycans and fluorophores has also been explored. As liposomes of varying sizes and structures are quite readily accessible, such as unilamellar and multilamellar vesicles, liposomes can accommodate rather different glycans and fluorophores. Lee and co-workers prepared an amphiphilic tetra(*p*-phenylene) – mannoside conjugate linked by oligo(ethylene oxide) **34** that readily assemble into liposomes of about 40 nm diameters [129]. These vesicles were shown to bind to the pili of bacterial *E. coli* ORN 178, presumably through interactions with the surface FimH. The authors also demonstrated in a subsequent report that a triblock mannoside – aromatic conjugate **35** assembles into rod shape structures, which can be transformed into spherically shaped particles upon addition of Nile Red [130]. Both structures can precipitate Con A and bind to the pili of bacterial *E. coli* ORN 178 (Fig. 7).

Fluorescently labeled glycans in material sciences and as ion sensors

Due to a number of unique structural features that carbohydrates possess, such as the well-defined stereochemistry and the hydroxyl functionalities that are uniquely positioned in the

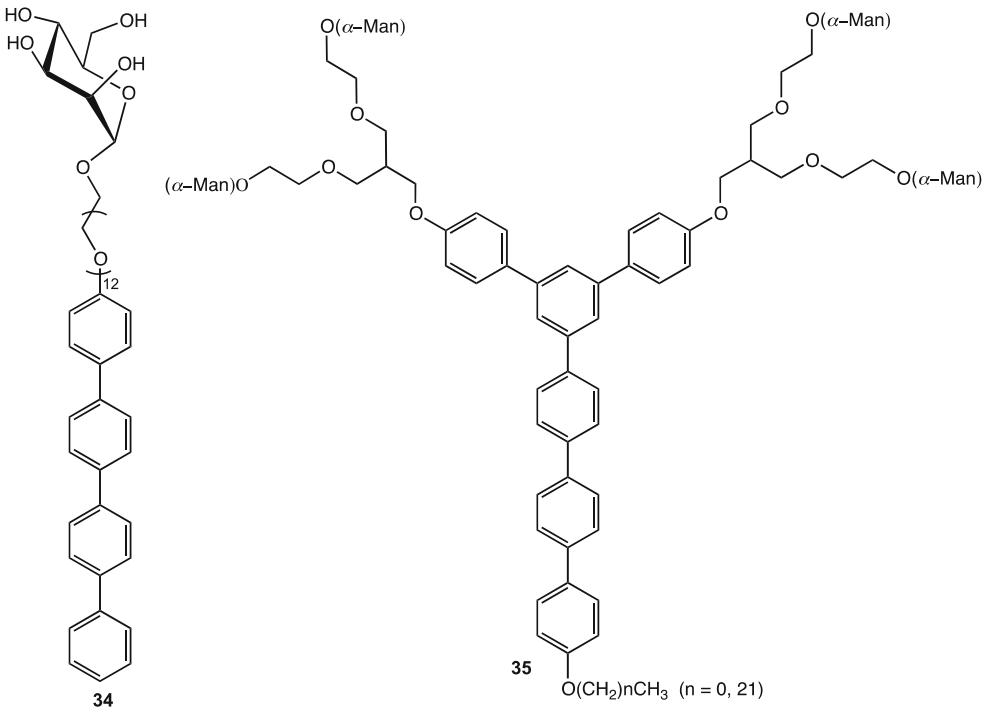
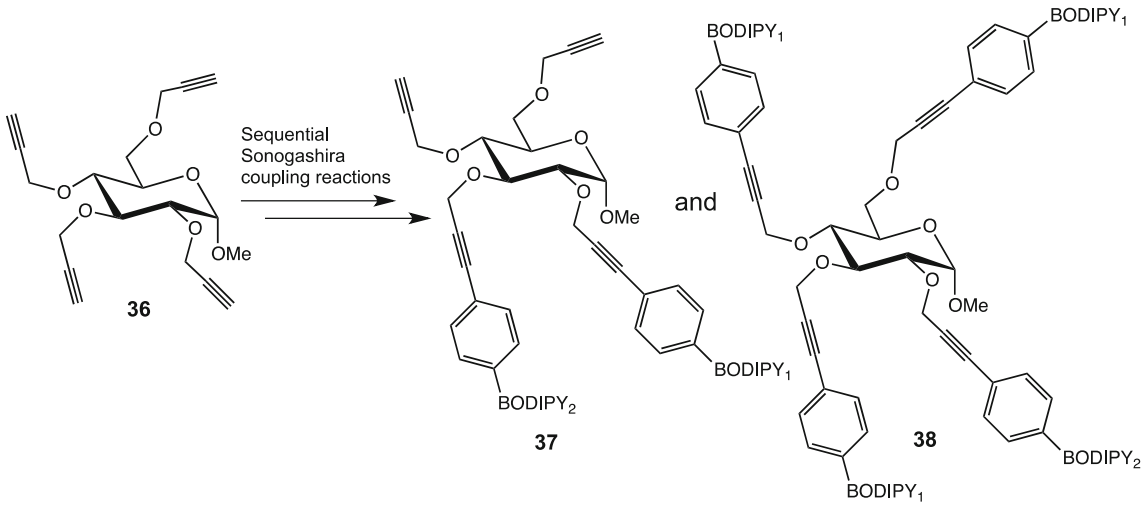


Fig. 7 Fluorophore-decorated glycosides that assemble into defined structures

scaffold, carbohydrates have been introduced in various molecular designs.

In this respect, α-methylglucoside was used as a "biologically-relevant platform" to assemble artificial light-harvesting antenna system [131]. Sequential Sonogashira coupling reactions of tetrapropargyl α-methylglucoside **36** with suitably substituted BODIPY fluorophores (Scheme 8) led to the formation of multifluorophore-cassettes **37** and **38** that allow for ultrafast and efficient energy transfer. It was argued that this construct could be applied to biological systems, as carbohydrates are indispensible components of the living system. Similarly, this concept was also extended to the synthesis of multifluorophore sugar conjugates that allow for effective energy transfer through FRET, due to the proximity of adjacent fluorophores attached to the sugar scaffold [132].

Work from a few research groups have demonstrated the assembly of sugar-fluorophore conjugate, mostly macrocycles [133–140]; some have shown interesting photochemical properties in response to the presence of specific ions, such as Cu^{2+}, Hg^{2+} and Pb^{2+}. BODIPY was also incorporated in some of these designs, and found to show binding to a selection of ions including Cu^{2+}, leading to fluorescent quenching [138]. Based on recent findings in our laboratory [141], however, caution needs to be exercised in the use of BODIPY fluorophores in Cu^{2+} sensing, as BODIPY can undergo reactions with various copper (II) salts.



Scheme 8 Synthesis of multifluorophore-cassettes using glucoside as a platform

Conclusions and perspectives

Among the reasons for the delay in applying fluorescently labelled carbohydrates in glycobiology research is the concern that attachment of fluorophores can change the conformations of glycans and consequently the way they interact with their binding partners, such as proteins. Zhu and co-workers [142] recently compared the binding constants of 24 glycans with seven plant lectins that are either label-free or fluorescently labeled. It was determined that the interaction profiles are different for label-free and fluorescently labeled lectins, in some cases a very significant difference was observed. While similar perturbation to glycan conformation will likely occur when fluorophores are covalently attached, with careful choice of fluorophores and design of attachment chemistry, fluorescently labelled glycans will find interesting applications in glycobiology research. In this respect, a number of considerations should be accounted for. Size of the fluorophore will be an important consideration in selecting labelling reagent. Typically, relatively small fluorophores are more desirable. Hydrophobicity of the fluorophore needs to be taken into consideration. For intracellular applications, hydrophobic fluorophores could potentially enhance internalization of the probe, however, non-specific hydrophobic interaction with non-target molecules will need to be considered. A tendency for the probe to reside within the cell and stability in the target cellular compartment are required for these applications as well. Some of the issues with fluorescently labelled glycans can be addressed with the choice of appropriate conjugation chemistry and linkers.

An alternative approach to fluorescently track glycan processing has been explored widely in the literature. This approach entails introduction of functional groups into glycans, such as azido- and alkynyl- that represent small changes to glycan structures, followed by visualization with fluorophores bearing compatible reactive groups. This approach, which is not the topic of this review, however, is often challenged with background fluorescence from the staining process.

As outlined in this review, the structural complexity of glycans poses challenges in many aspects; however, it also provides opportunities to explore these moieties in a wide range of applications. Glycans can be explored as structural scaffolds for fluorescent characterization of lectins and carbohydrate-binding proteins, and potentially in the detection of whole organisms. They will also find utility in functional materials, such as fluorescent sensing of specific analytes.

Acknowledgments Work in Yan's laboratory was funded by the Natural Science and Engineering Research Council of Canada. The authors wish to thank Dr. Dennis Whitefield for helpful suggestions.

References

1. Cao, H.S., Heagy, M.D.: Fluorescent chemosensors for carbohydrates: a decade's worth of bright spies for saccharides in review. J. Fluoresc. **14**, 569–584 (2004)
2. Mader, H.S., Wolfbeis, O.S.: Boronic acid based probes for microdetermination of saccharides and glycosylated biomolecules. Microchim. Acta **162**, 1–34 (2008)
3. Sinkeldam, R.W., Greco, N.J., Tor, Y.: Fluorescent analogs of biomolecular building blocks: design, properties, and applications. Chem. Rev. **110**, 2579–2619 (2010)
4. Wang, W., Gao, X.M., Wang, B.H.: Boronic acid-based sensors. Curr. Org. Chem. **6**, 1285–1317 (2002)
5. Yoon, J., Czarnik, A.W.: Fluorescent chemosensors of carbohydrates - a means of chemically communicating the binding of polyols in water based on chelation-enhanced quenching. J. Am. Chem. Soc. **114**, 5874–5875 (1992)
6. DiCesare, N., Lakowicz, J.R.: Spectral properties of fluorophores combining the boronic acid group with electron donor or withdrawing groups. Implication in the development of fluorescence probes for saccharides. J. Phys. Chem. A **105**, 6834–6840 (2001)
7. Weber, P., Harrison, F.W., Hof, L.: Histochemical application of dansylhydrazine as a fluorescent labeling reagent for sialic-acid in cellular glycoconjugates. Histochemistry **45**, 271–277 (1975)
8. Weber, P., Hof, L.: Introduction of a fluorescent label into carbohydrate moiety of glycoconjugates. Biochem. Biophys. Res. Commun. **65**, 1298–1302 (1975)
9. Ingham, K.C., Brew, S.A.: Fluorescent labeling of the carbohydrate moieties of human chorionic-gonadotropin and α-1-acid glycoprotein. Biochim. Biophys. Acta **670**, 181–189 (1981)
10. Mechref, Y., Ostrander, G.K., El Rassi, Z.: Capillary electrophoresis of carboxylated carbohydrates.1. Selective precolumn derivatization of gangliosides with UV absorbing and fluorescent tags. J. Chromatogr. A **695**, 83–95 (1995)
11. Anumula, K.R., Du, P.: Characterization of carbohydrates using highly fluorescent 2-aminobenzoic acid tag following gel electrophoresis of glycoproteins. Anal. Biochem. **275**, 236–242 (1999)
12. Coles, E., Reinhold, V.N., Carr, S.A.: Fluorescent labeling of carbohydrates and analysis by liquid-chromatography - comparison of derivatives using mannosidosis oligosaccharides. Carbohydr. Res. **139**, 1–11 (1985)
13. Her, G.R., Santikarn, S., Reinhold, V.N., Williams, J.C.: Simplified approach to HPLC precolumn fluorescent labeling of carbohydrates - *N*-(2-pyridinyl)-glycosylamines. J. Carbohydr. Chem. **6**, 129–139 (1987)
14. Abraham, G., Low, P.S.: Covalent labeling of specific membrane carbohydrate residues with fluorescent-probes. Biochim. Biophys. Acta **597**, 285–291 (1980)
15. Trabbic, K.R., De Silva, R.A., Andreana, P.R.: Elucidating structural features of an entirely carbohydrate cancer vaccine construct employing circular dichroism and fluorescent labeling. MedChemComm **5**, 1143–1149 (2014)
16. Momose, T., Ohkura, Y.: Organic analysis. X. Reaction mechanism of 5-hydroxytetralone with glucose. Chem. Pharm. Bull. **6**, 412–415 (1958)
17. Towne, J.C., Spikner, J.E.: Fluorometric microdetermination of carbohydrates. Anal. Chem. **35**, 211–214 (1963)
18. Bounias, M.: *N*-(1-Naphthyl)ethylenediamine dihydrochloride as a new reagent for nanomole quantification of sugars on thin-layer plates by a mathematical calibration process. Anal. Biochem. **106**, 291–295 (1980)
19. Honda, S., Kakimoto, K., Sudo, K., Kakehi, K., Takiura, K.: Fluorimetric determination of reducing sugars with ethylenediamine sulfate. Anal. Chim. Acta **70**, 133–139 (1974)

20. Honda, S., Matsuda, Y., Terao, M., Kakehi, K.: Fluorimetric determination of reducing carbohydrates with malonamide. Anal. Chim. Acta **108**, 421–423 (1979)
21. Kato, T., Kinoshita, T.: Fluorometric analysis of biological-materials. 1. Fluorophotometric determination of carbohydrates using taurine and borate. Chem. Pharm. Bull. **26**, 1291–1294 (1978)
22. Kato, T., Kinoshita, T.: Fluorometric analysis of biological-materials. 2. Fluorometric detection and determination of carbohydrates by high-performance liquid-chromatography using ethanolamine. Anal. Biochem. **106**, 238–243 (1980)
23. Honda, S., Matsuda, Y., Takahashi, M., Kakehi, K., Ganno, S.: Fluorimetric determination of reducing carbohydrates with 2-cyanoacetamide and application to automated-analysis of carbohydrates as borate complexes. Anal. Chem. **52**, 1079–1082 (1980)
24. Kai, M., Tamura, K., Yamaguchi, M., Ohkura, Y.: Aromatic amidines as fluorogenic reagents, for reducing carbohydrates. Anal. Sci. **1**, 59–63 (1985)
25. Hammond, K.S., Papermaster, D.S.: Fluorometric assay of sialic-acid in picomole range - modification of thiobarbituric acid assay. Anal. Biochem. **74**, 292–297 (1976)
26. Matsuno, K., Suzuki, S.: Simple fluorimetric method for quantification of sialic acids in glycoproteins. Anal. Biochem. **375**, 53–59 (2008)
27. Cai, Z.P., Hagan, A.K., Wang, M.M., Flitsch, S.L., Liu, L., Voglmeir, J.: 2-Pyridylfuran: a new fluorescent tag for the analysis of carbohydrates. Anal. Chem. **86**, 5179–5186 (2014)
28. Pagano, R.E., Watanabe, R., Wheatley, C., Dominguez, M.: Applications of BODIPY-sphingolipid analogs to study lipid traffic and metabolism in cells. Methods Enzymol. **312**, 523–534 (2000)
29. Rasmussen, J.-A.M., Hermetter, A.: Chemical synthesis of fluorescent glycero- and sphingolipids. Prog. Lipid Res. **47**, 436–460 (2008)
30. Bittman, R.: The 2003 ASBMB-avanti award in lipids address: applications of novel synthetic lipids to biological problems. Chem. Phys. Lipids **129**, 111–131 (2004)
31. Pagano, R.E., Martin, O.C., Kang, H.C., Haugland, R.P.: A novel fluorescent ceramide analog for studying membrane traffic in animal-cells - accumulation at the Golgi-apparatus results in altered spectral properties of the sphingolipid precursor. J. Cell Biol. **113**, 1267–1279 (1991)
32. Gretskaya, N.M., Bezuglov, V.V.: Synthesis of BODIPYA® FL C_5-Labeled D-erythro- and L-threo-lactosylceramides. Chem. Nat. Compd. **49**, 17–20 (2013)
33. Vo-Hoang, Y., Micouin, L., Ronet, C., Gachelin, G., Bonin, M.: Total enantioselective synthesis and *in vivo* biological evaluation of a novel fluorescent BODIPY α-galactosylceramide. Chembiochem **4**, 27–33 (2003)
34. Singh, R.D., Liu, Y.D., Wheatley, C.L., Holicky, E.L., Makino, A., Marks, D.L., Kobayashi, T., Subramaniam, G., Bittman, R., Pagano, R.E.: Caveolar endocytosis and microdomain association of a glycosphingolipid analog is dependent on its sphingosine stereochemistry. J. Biol. Chem. **281**, 30660–30668 (2006)
35. Singh, R.D., Puri, V., Valiyaveettil, J.T., Marks, D.L., Bittman, R., Pagano, R.E.: Selective caveolin-1-dependent endocytosis of glycosphingolipids. Mol. Biol. Cell **14**, 3254–3265 (2003)
36. Liu, Y.D., Bittman, R.: Synthesis of fluorescent lactosylceramide stereoisomers. Chem. Phys. Lipids **142**, 58–69 (2006)
37. Mikhalyov, I.I., Molotkovsky, J.G.: Synthesis and characteristics of fluorescent BODIPY-labeled gangliosides. Russ. J. Bioorg. Chem. **29**, 168–174 (2003)
38. Schwarzmann, G., Wendeler, M., Sandhoff, K.: Synthesis of novel NBD-GM1 and NBD-GM2 for the transfer activity of GM2-activator protein by a FRET-based assay system. Glycobiology **15**, 1302–1311 (2005)
39. Sarver, S.A., Keithley, R.B., Essaka, D.C., Tanaka, H., Yoshimura, Y., Palcic, M.M., Hindsgaul, O., Dovichi, N.J.: Preparation and electrophoretic separation of Bodipy-Fl-labeled glycosphingolipids. J. Chromatogr. A **1229**, 268–273 (2012)
40. Whitmore, C.D., Hindsgaul, O., Palcic, M.M., Schnaar, R.L., Dovichi, N.J.: Metabolic cytometry. Glycosphingolipid metabolism in single cells. Anal. Chem. **79**, 5139–5142 (2007)
41. Dada, O.O., Essaka, D.C., Hindsgaul, O., Palcic, M.M., Prendergast, J., Schnaar, R.L., Dovichi, N.J.: Nine orders of magnitude dynamic range: picomolar to millimolar concentration measurement in capillary electrophoresis with laser induced fluorescence detection employing cascaded avalanche photodiode photon counters. Anal. Chem. **83**, 2748–2753 (2011)
42. Essaka, D.C., Prendergast, J., Keithley, R.B., Palcic, M.M., Hindsgaul, O., Schnaar, R.L., Dovichi, N.J.: Metabolic cytometry: capillary electrophoresis with two-color fluorescence detection for the simultaneous study of two glycosphingolipid metabolic pathways in single primary neurons. Anal. Chem. **84**, 2799–2804 (2012)
43. Keithley, R.B., Rosenthal, A.S., Essaka, D.C., Tanaka, H., Yoshimura, Y., Palcic, M.M., Hindsgaul, O., Dovichi, N.J.: Capillary electrophoresis with three-color fluorescence detection for the analysis of glycosphingolipid metabolism. Analyst **138**, 164–170 (2013)
44. Daikoku, S., Ono, Y., Ohtake, A., Hasegawa, Y., Fukusaki, E., Suzuki, K., Ito, Y., Goto, S., Kanie, O.: Fluorescence-monitored zero dead-volume nanoLC-microESI-QIT-TOF MS for analysis of fluorescently tagged glycosphingolipids. Analyst **136**, 1046–1050 (2011)
45. Gross, H.J., Brossmer, R.: Enzymatic introduction of a fluorescent sialic-acid into oligosaccharide chains of glycoproteins. Eur. J. Biochem. **177**, 583–589 (1988)
46. Gross, H.J., Sticher, U., Brossmer, R.: A highly sensitive fluorometric assay for sialyltransferase activity using CMP-9-fluoresceinyl-NeuAc as donor. Anal. Biochem. **186**, 127–134 (1990)
47. Gross, H.J., Brossmer, R.: Characterization of human plasma sialyltransferase using a novel fluorometric assay. Clin. Chim. Acta **197**, 237–248 (1991)
48. Gross, H.J.: Fluorescent CMP-sialic acids as a tool to study the specificity of the CMP-sialic acid carrier and the glycoconjugate sialylation in permeabilized cells. Eur. J. Biochem. **203**, 269–275 (1992)
49. Wu, X.J., Tian, Y.P., Yu, M.Z., Lin, B.J., Han, J.H., Han, S.F.: A fluorescently labelled sialic acid for high performance intraoperative tumor detection. Biomater. Sci. **2**, 1120–1127 (2014)
50. Suzuki, K., Ohtake, A., Ito, Y., Kanie, O.: Synthesis of a fluorescently tagged sialic acid analogue useful for live-cell imaging. Chem. Commun. **48**, 9744–9746 (2012)
51. Turner, D.I., Chakraborty, N., d'Alarcao, M.: A fluorescent inositol phosphate glycan stimulates lipogenesis in rat adipocytes by extracellular activation alone. Bioorg. Med. Chem. Lett. **15**, 2023–2025 (2005)
52. Hagihara, S., Miyazaki, A., Matsuo, I., Tatami, A., Suzuki, T., Ito, Y.: Fluorescently labeled inhibitor for profiling cytoplasmic peptide: *N*-glycanase. Glycobiology **17**, 1070–1076 (2007)
53. Hekmat, O., Florizone, C., Kim, Y.-W., Eltis, L.D., Warren, R.A.J., Withers, S.G.: Specificity fingerprinting of retaining β-1, 4-glycanases in the *Cellulomonas fimi* secretome using two fluorescent mechanism-based probes. Chembiochem **8**, 2125–2132 (2007)
54. Suzuki, K., Tobe, A., Adachi, S., Daikoku, S., Hasegawa, Y., Shioiri, Y., Kobayashi, M., Kanie, O.: *N*-Hexyl-4-aminobutyl glycosides for investigating structures and biological functions of carbohydrates. Org. Biomol. Chem. **7**, 4726–4733 (2009)

55. Uppal, T., Bhupathiraju, N.V.S.D.K., Vicente, M.G.H.: Synthesis and cellular properties of near-IR BODIPY-PEG and carbohydrate conjugates. Tetrahedron **69**, 4687–4693 (2013)
56. Papalia, T., Siracusano, G., Colao, I., Barattucci, A., Aversa, M.C., Serroni, S., Zappalà, G., Campagna, S., Sciortino, M.T., Puntoriero, F., Bonaccorsi, P.: Cell internalization of BODIPY-based fluorescent dyes bearing carbohydrate residues. Dyes Pigments **110**, 67–71 (2014)
57. van Tilbeurgh, H., Loontiens, F.G., Debruyne, C.K., Claeyssens, M.: Fluorogenic and chromogenic glycosides as substrates and ligands of carbohydrases. Methods Enzymol. **160**, 45–59 (1988)
58. Öckerman, P.A.: Identity of β-glucosidase β-xylosidase and one of β-galactosidase activities in human liver when assayed with 4-methylumbelliferyl-β-D-glycosides studies in cases of Gauchers disease. Biochim. Biophys. Acta **165**, 59–62 (1968)
59. van Tilbeurgh, H., Claeyssens, M., de Bruyne, C.K.: The use of 4-methylumbelliferyl and other chromophoric glycosides in the study of cellulolytic enzymes. FEBS Lett. **149**, 152–156 (1982)
60. Szweda, R., Spohr, U., Lemieux, R.U., Schindler, D., Bishop, D.F., Desnick, R.J.: Synthesis of 4-methylumbelliferyl glycosides for the detection of α-D-galactopyranosaminidases and β-D-galactopyranosaminidases. Can. J. Chem. **67**, 1388–1391 (1989)
61. Lee, J.C., Chang, S.W., Liao, C.C., Chi, F.C., Chen, C.S., Wen, Y.S., Wang, C.C., Kulkarni, S.S., Puranik, R., Liu, Y.H., Hung, S.C.: From D-glucose to biologically potent L-hexose derivatives: synthesis of α-L-iduronidase fluorogenic detector and the disaccharide moieties of bleomycin A_2 and heparan sulfate. Chem. Eur. J. **10**, 399–415 (2004)
62. Yasukochi, T., Fukase, K., Suda, Y., Takagaki, K., Endo, M., Kusumoto, S.: Enzymatic synthesis of 4-methylumbelliferyl glycosides of trisaccharide and core tetrasaccharide, Gal(β1-3)Gal(β1-4)Xyl and GlcA(β1-3)Gal(β1-3)Gal(β1-4)Xyl, corresponding to the linkage region of proteoglycans. Bull. Chem. Soc. Jpn. **70**, 2719–2725 (1997)
63. Wang, L.X., Keyhani, N.O., Roseman, S., Lee, Y.C.: 4-Methylumbelliferyl glycosides of *N*-acetyl 4-thiochitooligosaccharides as fluorogenic substrates for chitodextrinase from *Vibrio furnissii*. Glycobiology **7**, 855–860 (1997)
64. Honda, Y., Tanimori, S., Kirihata, M., Kaneko, S., Tokuyasu, K., Hashimoto, M., Watanabe, T.: Chemo- and enzymatic synthesis of partially and fully *N*-deacetylated 4-methylumbelliferyl chitobiosides: fluorogenic substrates for chitinase. Bioorg. Med. Chem. Lett. **10**, 827–829 (2000)
65. Eneyskaya, E.V., Ivanen, D.R., Shabalin, K.A., Kulminskaya, A.A., Backinowsky, L.V., Brumer III, H., Neustroev, K.N.: Chemo-enzymatic synthesis of 4-methylumbelliferyl β-(1->4)-D-xylooligosides: new substrates for β-D-xylanase assays. Org. Biomol. Chem. **3**, 146–151 (2005)
66. Mazzaferro, L.S., Piñuel, L., Erra-Balsells, R., Giudicessi, S.L., Breccia, J.D.: Transglycosylation specificity of *Acremonium sp* α-rhamnosyl-β-glucosidase and its application to the synthesis of the new fluorogenic substrate 4-methylumbelliferyl-rutinoside. Carbohydr. Res. **347**, 69–75 (2012)
67. Deng, L.H., Tsybina, P., Gregg, K.J., Mosi, R., Zandberg, W.F., Boraston, A.B..., Vocadlo, D.J.: Synthesis of 4-methylumbelliferyl α-D-mannopyranosyl-(1->6)-β-D-mannopyranoside and development of a coupled fluorescent assay for GH125 exo-α-1,6-mannosidases. Bioorg. Med. Chem. **21**, 4839–4845 (2013)
68. Zbiral, E., Schreiner, E., Salunkhe, M.M., Schulz, G., Kleineidam, R.G., Schauer, R.: Synthesis of the 4-methylumbelliferyl 2α-glycosides of 7-epi-*N*-acetylneuraminic, 8-epi-*N*-acetylneuraminic, and 7,8-bis(epi)-*N*-acetylneuraminic acids, as well as of 7-deoxy-*N*-acetylneuraminic, 8-deoxy-*N*-acetylneuraminic, 9-deoxy-*N*-acetylneuraminic, and 4,7-dideoxy-*N*-acetylneuraminic acids and their behavior towards sialidase from *Vibrio cholerae*. Liebigs Ann. Chem. 519–526 (1989)
69. Kleineidam, R.G., Furuhata, K., Ogura, H., Schauer, R.: 4-Methylumbelliferyl-α-glycosides of partially *O*-acetylated *N*-acetylneuraminic acids as substrates of bacterial and viral sialidases. Biol. Chem. Hoppe Seyler **371**, 715–719 (1990)
70. Zamora, C.Y., d'Alarcao, M., Kumar, K.: Fluorogenic sialic acid glycosides for quantification of sialidase activity upon unnatural substrates. Bioorg. Med. Chem. Lett. **23**, 3406–3410 (2013)
71. Engstler, M., Talhouk, J.W., Smith, R.E., Schauer, R.: Chemical synthesis of 4-trifluoromethylumbelliferyl-α-D-*N*-acetylneuraminic acid glycoside and its use for the fluorometric detection of poorly expressed natural and recombinant sialidases. Anal. Biochem. **250**, 176–180 (1997)
72. Ge, Y., Antoulinakis, E.G., Gee, K.R., Johnson, I.: An ultrasensitive, continuous assay for xylanase using the fluorogenic substrate 6,8-difluoro-4-methylumbelliferyl β-D-xylobloside. Anal. Biochem. **362**, 63–68 (2007)
73. Wu, M., Nerinckx, W., Piens, K., Ishida, T., Hansson, H., Sandgren, M., Ståhlberg, J.: Rational design, synthesis, evaluation and enzyme-substrate structures of improved fluorogenic substrates for family 6 glycoside hydrolases. FEBS J. **280**, 184–198 (2013)
74. Wiederschain, G.Y., Kozlova, I.K., Ilyina, G.S., Mikhaylova, M.A., Beyer, E.M.: The use of glycosides of 6-acylamino and 8-acylamino-4-methylumbel-liferone in studies of the specificity and properties of human lysosomal glycolipid hydrolases. Carbohydr. Res. **224**, 255–272 (1992)
75. Miller, S.P.F., French, S.A., Kaneski, C.R.: Synthesis and characterization of a novel lysosomotropic enzyme substrate that fluoresces at intracellular pH. J. Org. Chem. **56**, 30–34 (1991)
76. Renaudet, O., Dumy, P.: Oxime-based synthesis of new chromogenic and fluorogenic oligosaccharides. Eur. J. Org. Chem. 5383–5386 (2008)
77. Ibatullin, F.M., Banasiak, A., Baumann, M.J., Greffe, L., Takahashi, J., Mellerowicz, E.J., Brumer, H.: A real-time fluorogenic assay for the visualization of glycoside hydrolase activity in planta. Plant Physiol. **151**, 1741–1750 (2009)
78. Yadav, A.K., Shen, D.L., Shan, X., He, X., Kermode, A.R., Vocadlo, D.J.: Fluorescence-quenched substrates for live cell imaging of human glucocerebrosidase activity. J. Am. Chem. Soc. **137**, 1181–1189 (2015)
79. Park, S., Lee, M.R., Shin, I.: Carbohydrate microarrays as powerful tools in studies of carbohydrate-mediated biological processes. Chem. Commun. 4389–4399 (2008)
80. Kiessling, L.L., Splain, R.A.: Chemical approaches to glycobiology. Annu. Rev. Biochem. **79**, 619–653 (2010)
81. Chevolot, Y.: Carbohydrate microarrays: methods and protocols. Methods in Molecular Biology, vol. 808. Humana Press, New York (2012)
82. Lee, M., Shin, I.: Facile preparation of carbohydrate microarrays by site-specific, covalent immobilization of unmodified carbohydrates on hydrazide-coated glass slides. Org. Lett. **7**, 4269–4272 (2005)
83. Park, S., Sung, J.W., Shin, I.: Fluorescent glycan derivatives: their use for natural glycan microarrays. ACS Chem. Biol. **4**, 699–701 (2009)
84. Xia, B.Y., Kawar, Z.S., Ju, T.Z., Alvarez, R.A., Sachdev, G.P., Cummings, R.D.: Versatile fluorescent derivatization of glycans for glycomic analysis. Nat. Methods **2**, 845–850 (2005)
85. Song, X.Z., Xia, B.Y., Lasanajak, Y., Smith, D.F., Cummings, R.D.: Quantifiable fluorescent glycan microarrays. Glycoconj. J. **25**, 15–25 (2008)
86. Song, X.Z., Xia, B.Y., Stowell, S.R., Lasanajak, Y., Smith, D.F., Cummings, R.D.: Novel fluorescent glycan microarray strategy reveals ligands for galectins. Chem. Biol. **16**, 36–47 (2009)
87. Song, X.Z., Lasanajak, Y., Rivera-Marrero, C., Luyai, A., Willard, M., Smith, D., Cummings, R.: Generation of a natural glycan

microarray using 9-fluorenylmethyl chloroformate (FmocCl) as a cleavable fluorescent tag. Anal. Biochem. **395**, 151–160 (2009)
88. Gamblin, D.P., Scanlan, E.M., Davis, B.G.: Glycoprotein synthesis: an update. Chem. Rev. **109**, 131–163 (2009)
89. Bohorov, O., Andersson-Sand, H., Hoffmann, J., Blixt, O.: Arraying glycomics: a novel bi-functional spacer for one-step microscale derivatization of free reducing glycans. Glycobiology **16**, 21C–27C (2006)
90. Niikura, K., Kamitani, R., Kurogochi, M., Uematsu, R., Shinohara, Y., Nakagawa, H., Deguchi, K., Monde, K., Kondo, H., Nishimura, S.I.: Versatile glycoblotting nanoparticles for high-throughput protein glycomics. Chem. Eur. J. **11**, 3825–3834 (2005)
91. Vila-Perelló, M., Gallego, R.G., Andreu, D.: A simple approach to well-defined sugar-coated surfaces for interaction studies. Chembiochem **6**, 1831–1838 (2005)
92. Zhou, X.C., Zhou, J.H.: Oligosaccharide microarrays fabricated on aminooxyacetyl functionalized glass surface for characterization of carbohydrate-protein interaction. Biosens. Bioelectron. **21**, 1451–1458 (2006)
93. Tarentino, A.L., Plummer, T.H.: Enzymatic deglycosylation of asparagine-linked glycans - purification, properties, and specificity of oligosaccharide-cleaving enzymes from *Flavobacterium meningosepticum*. Methods Enzymol. **230**, 44–57 (1994)
94. Patel, T.P., Parekh, R.B.: Release of oligosaccharides from glycoproteins by hydrazinolysis. Methods Enzymol. **230**, 57–66 (1994)
95. Galanina, O., Feofanov, A., Tuzikov, A.B..., Rapoport, E., Crocker, P.R., Grichine, A., Egret-Charlier, M., Vigny, P., Le Pendu, J., Bovin, N.V.: Fluorescent carbohydrate probes for cell lectins. Spectrochim. Acta A **57**, 2285–2296 (2001)
96. Erdogan, B., Wilson, J.N., Bunz, U.H.F.: Synthesis and mesoseopic order of a sugar-coated poly(*p*-phenyleneethynylene). Macromolecules **35**, 7863–7864 (2002)
97. Lavigne, J.J., Broughton, D.L., Wilson, J.N., Erdogan, B., Bunz, U.H.F.: "Surfactochromic" conjugated polymers: surfactant effects on sugar-substituted PPEs. Macromolecules **36**, 7409–7412 (2003)
98. Disney, M.D., Zheng, J., Swager, T.M., Seeberger, P.H.: Detection of bacteria with carbohydrate-functionalized fluorescent polymers. J. Am. Chem. Soc. **126**, 13343–13346 (2004)
99. Kelly, T.L., Lam, M.C.W., Wolf, M.O.: Carbohydrate-labeled fluorescent microparticles and their binding to lectins. Bioconjug. Chem. **17**, 575–578 (2006)
100. Chen, Q., Xu, Y.H., Du, Y.G., Han, B.H.: Triphenylamine-based fluorescent conjugated glycopolymers: synthesis, characterization and interactions with lectins. Polymer **50**, 2830–2835 (2009)
101. Shi, J.B., Cai, L.P., Pu, K.Y., Liu, B.: Synthesis and characterization of water-soluble conjugated glycopolymer for fluorescent sensing of concanavalin A. Chem. Asian. J. **5**, 301–308 (2010)
102. Kim, I.B., Wilson, J.N., Bunz, U.H.F.: Mannose-substituted PPEs detect lectins: a model for Ricin sensing. Chem. Commun. 1273–1275 (2005)
103. Xue, C.H., Jog, S.P., Murthy, P., Liu, H.Y.: Synthesis of highly water-soluble fluorescent conjugated glycopoly(*p*-phenylene)s for lectin and *Escherichia coli*. Biomacromolecules **7**, 2470–2474 (2006)
104. Xue, C.H., Donuru, V.R.R., Liu, H.Y.: Facile, versatile prepolymerization and postpolymerization functionalization approaches for well-defined fluorescent conjugated fluorene-based glycopolymers. Macromolecules **39**, 5747–5752 (2006)
105. Phillips, R.L., Kim, I.B., Carson, B.E., Tidbeck, B., Bai, Y., Lowary, T.L., Tollbert, L.M., Bunz, U.H.F.: Sugar-substituted poly(*p*-phenyleneethynylene)s: sensitivity enhancement toward lectins and bacteria. Macromolecules **41**, 7316–7320 (2008)
106. Xue, C., Velayudham, S., Johnson, S., Saha, R., Smith, A., Brewer, W., Murthy, P., Bagley, S.T., Liu, H.: Highly water-soluble, fluorescent, conjugated fluorene-based glycopolymers with poly(ethylene glycol)-tethered spacers for sensitive detection of *Escherichia coli*. Chem. Eur. J. **15**, 2289–2295 (2009)
107. Phillips, R.L., Kim, I.B., Tolbert, L.M., Bunz, U.H.F.: Fluorescence self-quenching of a mannosylated poly(*p*-phenyleneethynylene) induced by concanavalin A. J. Am. Chem. Soc. **130**, 6952–6954 (2008)
108. Yang, W., Pan, C.Y., Luo, M.D., Zhang, H.B.: Fluorescent mannose-functionalized hyperbranched poly(amido amine)s: synthesis and interaction with *E. coli*. Biomacromolecules **11**, 1840–1846 (2010)
109. Ruff, Y., Buhler, E., Candau, S.J., Kesselman, E., Talmon, Y., Lehn, J.M.: Glycodynamers: dynamic polymers bearing oligosaccharides residues - generation, structure, physicochemical, component exchange, and lectin binding properties. J. Am. Chem. Soc. **132**, 2573–2584 (2010)
110. Ruff, Y., Lehn, J.M.: Glycodynamers: fluorescent dynamic analogues of polysaccharides. Angew. Chem. Int. Ed. **47**, 3556–3559 (2008)
111. Kikkeri, R., Garcia-Rubio, I., Seeberger, P.H.: Ru(II)-carbohydrate dendrimers as photoinduced electron transfer lectin biosensors. Chem. Commun. 235–237 (2009)
112. Tian, X., Pai, J., Baek, K.H., Ko, S.K., Shin, I.: Fluorophore-labeled, peptide-based glycoclusters: synthesis, binding properties for lectins, and detection of carbohydrate-binding proteins in cells. Chem. Asian. J. **6**, 2107–2113 (2011)
113. Kikkeri, R., Hossain, L.H., Seeberger, P.H.: Supramolecular one-pot approach to fluorescent glycodendrimers. Chem. Commun. 2127–2129 (2008)
114. Chen, Q.S., Wei, W.L., Lin, J.M.: Homogeneous detection of concanavalin A using pyrene-conjugated maltose assembled graphene based on fluorescence resonance energy transfer. Biosens. Bioelectron. **26**, 4497–4502 (2011)
115. Grünstein, D., Maglinao, M., Kikkeri, R., Collot, M., Barylyuk, K., Lepenies, B., Kamena, F., Zenobi, R., Seeberger, P.H.: Hexameric supramolecular scaffold orients carbohydrates to sense bacteria. J. Am. Chem. Soc. **133**, 13957–13966 (2011)
116. Zhou, J., Butchosa, N., Jayawardena, H.S.N., Zhou, Q., Yan, M.D., Ramström, O.: Glycan-functionalized fluorescent chitin nanocrystals for biorecognition applications. Bioconjug. Chem. **25**, 640–643 (2014)
117. Sanji, T., Shiraishi, K., Nakamura, M., Tanaka, M.: Fluorescence turn-on sensing of lectins with mannose-substituted tetraphenylethenes based on aggregation-induced emission. Chem. Asian. J. **5**, 817–824 (2010)
118. Das, S., Mandal, S., Mukhopadhyay, B., Zade, S.S.: Synthesis of carbohydrate-functionalized thiophene-capped cyclopenta[c]thiophene for concanavalin A recognition. Tetrahedron Lett. **53**, 1464–1467 (2012)
119. Wang, K.R., Wang, Y.Q., An, H.W., Zhang, J.C., Li, X.L.: A Triazatruxene-based glycocluster as a fluorescent sensor for concanavalin A. Chem. Eur. J. **19**, 2903–2909 (2013)
120. Wang, J.X., Chen, Q., Bian, N., Yang, F., Sun, J., Qi, A.D., Yan, C.G., Han, B.H.: Sugar-bearing tetraphenylethylene: novel fluorescent probe for studies of carbohydrate-protein interaction based on aggregation-induced emission. Org. Biomol. Chem. **9**, 2219–2226 (2011)
121. Chen, X., Ramström, O., Yan, M.D.: Glyconanomaterials: emerging applications in biomedical research. Nano Res. **7**, 1381–1403 (2014)
122. Marin, M.J., Schofield, C.L., Field, R.A., Russell, D.A.: Glyconanoparticles for colorimetric bioassays. Analyst **140**, 59–70 (2015)
123. Robinson, A., Fang, J.M., Chou, P.T., Liao, K.W., Chu, R.M., Lee, S.J.: Probing lectin and sperm with carbohydrate-modified quantum dots. Chembiochem **6**, 1899–1905 (2005)

124. Babu, P., Sinha, S., Surolia, A.: Sugar-quantum dot conjugates for a selective and sensitive detection of lectins. Bioconjug. Chem. **18**, 146–151 (2007)
125. Bavireddi, H., Kikkeri, R.: Glyco-β-cyclodextrin capped quantum dots: synthesis, cytotoxicity and optical detection of carbohydrate-protein interactions. Analyst **137**, 5123–5127 (2012)
126. Mukhopadhyay, B., Martins, M.B., Karamanska, R., Russell, D.A., Field, R.A.: Bacterial detection using carbohydrate-functionalised CdS quantum dots: a model study exploiting *E. coli* recognition of mannosides. Tetrahedron Lett. **50**, 886–889 (2009)
127. Huang, C.C., Chen, C.T., Shiang, Y.C., Lin, Z.H., Chang, H.T.: Synthesis of fluorescent carbohydrate-protected Au nanodots for detection of concanavalin A and *Escherichia coli*. Anal. Chem. **81**, 875–882 (2009)
128. Pfaff, A., Schallon, A., Ruhland, T.M., Majewski, A.P., Schmalz, H., Freitag, R., Müller, A.H.E.: Magnetic and fluorescent glycopolymer hybrid nanoparticles for intranuclear optical imaging. Biomacromolecules **12**, 3805–3811 (2011)
129. Kim, B.S., Yang, W.Y., Ryu, J.H., Yoo, Y.S., Lee, M.: Carbohydrate-coated nanocapsules from amphiphilic rod-coil molecule: binding to bacterial type 1 pili. Chem. Commun. 2035–2037 (2005)
130. Ryu, J.H., Lee, E., Lim, Y.B., Lee, M.: Carbohydrate-coated supramolecular structures: transformation of nanofibers into spherical micelles triggered by guest encapsulation. J. Am. Chem. Soc. **129**, 4808–4814 (2007)
131. Bonaccorsi, P., Aversa, M.C., Barattucci, A., Papalia, T., Puntoriero, F., Campagna, S.: Artificial light-harvesting antenna systems grafted on a carbohydrate platform. Chem. Commun. **48**, 10550–10552 (2012)
132. Maisonneuve, S., Métivier, R., Yu, P., Nakatani, K., Xie, J.: Multichromophoric sugar for fluorescence photoswitching. Beilstein J. Org. Chem. **10**, 1471–1481 (2014)
133. Ågren, J.K.M., Billing, J.F., Grundberg, H.E., Nilsson, U.J.: Synthesis of a chiral and fluorescent sugar-based macrocycle by 1,3-dipolar cycloaddition. Synthesis 3141–3145 (2006)
134. Xie, J., Menand, M., Maisonneuve, S., Métivier, R.: Synthesis of bispyrenyl sugar-aza-crown ethers as new fluorescent molecular sensors for Cu(II). J. Org. Chem. **72**, 5980–5985 (2007)
135. Hsieh, Y.C., Chir, J.L., Wu, H.H., Guo, C.Q., Wu, A.T.: Synthesis of a sugar-aza-crown ether-based cavitand as a selective fluorescent chemosensor for Cu^{2+} ion. Tetrahedron Lett. **51**, 109–111 (2010)
136. Hsieh, Y.C., Chir, J.L., Yang, S.T., Chen, S.J., Hu, C.H., Wu, A.T.: A sugar-aza-crown ether-based fluorescent sensor for Cu^{2+} and Hg^{2+} ions. Carbohydr. Res. **346**, 978–981 (2011)
137. Hsieh, Y.C., Chir, J.L., Wu, H.H., Chang, P.S., Wu, A.T.: A sugar-aza-crown ether-based fluorescent sensor for Hg^{2+} and Cu^{2+}. Carbohydr. Res. **344**, 2236–2239 (2009)
138. Yu, Y.H., Bogliotti, N., Tang, J., Xie, J.: Synthesis and properties of carbohydrate-based BODIPY-functionalised fluorescent macrocycles. Eur. J. Org. Chem. 7749–7760 (2013)
139. Thakur, A., Mandal, D., Deb, P., Mondal, B., Ghosh, S.: Synthesis of triazole linked fluorescent amino acid and carbohydrate bioconjugates: a highly sensitive and skeleton selective multiresponsive chemosensor for Cu(II) and Pb(II)/Hg(II) ions. RSC Adv. **4**, 1918–1928 (2014)
140. Li, K.B., Zhang, H.L., Zhu, B., He, X.P., Xie, J., Chen, G.R.: A per-acetyl glycosyl rhodamine as a novel fluorescent ratiometric probe for mercury (II). Dyes Pigments **102**, 273–277 (2014)
141. Yang, L.J., Yalagala, R.S., Hutton, S., Lough, A., Yan, H.B.: Reactions of BODIPY fluorophore with cupric nitrate. Synlett **25**, 2661–2664 (2014)
142. Fei, Y.Y., Sun, Y.S., Li, Y.H., Lau, K., Yu, H., Chokhawala, H.A., Huang, S.S., Landry, J.P., Chen, X., Zhu, X.D.: Fluorescent labeling agents change binding profiles of glycan-binding proteins. Mol. Biosyst. **7**, 3343–3352 (2011)