Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 3598

www.rsc.org/obc

PERSPECTIVE

Carbohydrate chemistry in drug discovery

M. Carmen Galan,*[a] David Benito-Alifonso[a] and Gregory M. Watt[b]

Received 11th November 2010
DOI: 10.1039/c0ob01017k

The multitude of roles that carbohydrates and their glyco-conjugates play in biological processes has stimulated great interest in determining the nature of their interactions in both normal and diseased states. Manipulating such interactions will provide leads for drug discovery. Of the major classes of biomolecule, carbohydrates are the most structurally diverse. This hetereogeneity makes isolation of pure samples, and in sufficient amounts, from biological sources extremely difficult. Chemical synthesis offers the advantage of producing pure and structurally defined oligosaccharides for biological investigations. Although the complex nature of carbohydrates means that this is challenging, recent advances in the field have facilitated access to these molecules. The synthesis and isolation of oligosaccharides combined with progress in glycoarray technology have aided the identification of new carbohydrate-binding drug targets. This review aims to provide an overview of the latest advancements in carbohydrate chemistry and the role of these complex molecules in drug discovery, focusing particularly on synthetic methodologies, glycosaminoglycans, glycoprotein synthesis and vaccine development over the last few years.

1. Introduction

Carbohydrates are involved in many biological recognition processes such as protein folding, cell–cell communication, bacterial adhesion, viral infection, masking of immunological epitopes, fertilization, embryogenesis, neural development and cell proliferation and organization into specific tissues.[1–3] The nature of cell-surface carbohydrates can differ considerably between diseased and normal cells. Unique glycan markers of diseased cells can be exploited for early diagnosis, prevention (*via* vaccines) and treatment of illnesses (*via* drugs that target specifically the interaction of these glycans with their binding partner). Although carbohydrates are the most diverse and one of the most important classes of biomolecules in nature, there are relatively few carbohydrate-based drugs in the market.[4] This is mainly due to the high polarity of this class of drugs, which typically offers poor pharmacokinetic properties.[5] By altering their properties to

[a]*School of Chemistry, University of Bristol, Cantock's Close, Bristol, U.K BS8 1TS. E-mail: M.C.Galan@bristol.ac.uk*
[b]*Selcia Limited, Fyfield Rd, Ongar, Essex, U.K. CM5 0GS*

M. Carmen Galan

M. Carmen Galan received her Ph.D. in Organic Chemistry from the Complex Carbohydrate Research Center at The University of Georgia, USA, under the supervision of Prof. Geert-Jan Boons. She then moved to California to pursue post-doctoral research with Prof. Chi-Huey Wong at The Scripps Research Institute. After that, she continued her post-doctoral training at M.I.T with Prof. Sarah O'Connor. Carmen moved to the UK in October 2006 on a temporary lecturership at the Organic and Biological Chemistry Department in Bristol. Since 2008, she holds a Royal Society Dorothy Hodgkin Fellowship at the same department.

David Benito-Alifonso

David Benito-Alifonso received his Chemistry and Biochemistry degrees from the University Rovira i Virgili, and his Ph.D. in Organic Chemistry in 2008 from the same university working in the group of Prof. Sergio Castillon. After a short post-doctoral training with Dr David R. Carbery at the University of Bath in 2009 and then moved to the University of Bristol in 2010 as a Marie Curie Research Fellow working in the group of Dr M. Carmen Galan.

make them more 'drug-like', carbohydrate-based small molecules can be effective pharmaceuticals. The field of glycomimetic drugs has been reviewed recently by Ernst and Magnani.[5]

In order to understand glycan diversity and function, it is essential to have access to structurally defined oligosaccharides in sufficient purity and quantity. However, approaches to prepare diverse libraries of oligosaccharides in a rapid manner are greatly lacking and progress in glycobiology research has been hindered by having to rely on either isolated materials, target-oriented lengthy chemical syntheses or enzymatic approaches.[6]

In recent years, advancements in carbohydrate synthesis, analysis and glycoarray technology have facilitated the development of chemical approaches to "glycomics" that provide a better understanding of the biological processes involving complex carbohydrates. For instance, databases established by the Consortium for Functional Glycomics (CFG)[7] and the EUROCarbDB[8] are large research initiatives that provide resources and bioinformatic tools for the scientific community to use and are sources of reference and information on glycan-binding proteins, glycan structures, and glycosyltransferases. The focus of this review is the recent advances in carbohydrate chemistry and their application to drug discovery; however, due to length restrictions, these advances areas are not discussed in depth.

2. Synthetic oligosaccharide chemistry

Although the synthesis of many oligosaccharides can now be accomplished, albeit with considerable effort, the preparation of a complex structure can take from months to years due to the structural complexity of carbohydrates.[6,9]

In the last two decades, tremendous efforts have been devoted towards developing new strategies that can make oligosaccharide synthesis more accessible to main stream chemists. One-pot synthetic strategies have become a very attractive alternative to traditional sequential approaches since multiple glycosylation steps can be performed in a single reaction vessel (Fig. 1 A). Many of these one-pot convergent approaches are based on the selective activation of one glycosyl donor over another, a concept that was initially exemplified by Fraser-Reid's armed-disarmed methodology.[10] This idea was developed further thanks to the essential contributions of Ley's,[11] Wong's[12] and Huang's[13] groups into the field. The methodology has recently been exemplified with the modular assembly of a panel of heparin (HP)-like hexasaccharides (Fig. 2).[14] Matched donor and acceptor pairs were identified to allow stereospecific formation of the disaccharide building blocks, including those containing the challenging *cis*-1,4-linkages. From two advanced thioglycosyl disaccharide intermediates, all of the required disaccharide modules for library preparation were generated in a divergent manner. Preactivation-based, one-pot sequential glycosylations using the disaccharides led to the rapid construction of hexasaccharides in high yields.

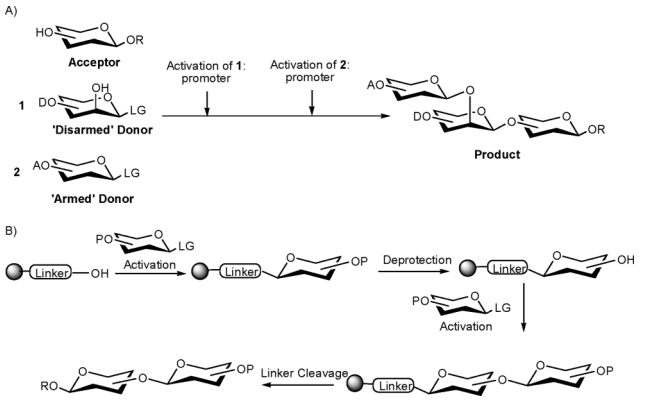

Fig. 1 General schematic representation for common oligosaccharide assembly strategies: A) Reactivity-based one-pot glycosylation synthesis. B) Supported phase oligosaccharide assembly on a polymer resin, fluorous tag, ionic-liquid-based tag or gold sticks. The glycan units are attached by means of a linker to the support and the cycle consists of activation and deprotection steps. Finally the linker is cleaved to procure the desired oligosaccharide. P (temporary protecting groups), LG (leaving group), R (hydrocarbon residue to be functionalized).

One of the main stumbling blocks for the automation of oligosaccharide synthesis is the requirement for purification after each step, which is normally accomplished by chromatographic methods. Polymer supported oligosaccharide syntheses were developed as a viable alternative[15–17] (Fig. 1 B). Solid support strategies have been typically associated with slow reaction rates, whereas soluble polymer supports suffer from low loading of saccharide and low solubility during the reaction and difficulties with product recovery. However, new advances in the area brought about by the use of new polymers, linkers and novel synthetic methodology has led to the synthesis of many complex oligosaccharides.[15] For instance, the first automated solid-phase synthesis of oligosaccharides containing the challenging β-mannosidic linkage has been reported.[18] Carboxybenzyl (CB) mannoside building blocks were used as effective β-mannosylation agents that resulted in excellent conversion and good to moderate selectivities. [(Triisopropylsilyl)oxy]-methyl ether (Tom), served as an orthogonal, minimally intrusive, and readily cleavable protecting group for the elongation of the C3 position of mannose (Fig. 3).

For all of the above polymer supported strategies to be useful, strict stereoselective control at each new forming glycoside bond has to be accomplished. In that respect, 1,2-*trans*-glycosides can

Gregory M. Watt

Gregory M. Watt received his Ph.D. in Organic Chemistry and Biochemistry from The University of Dundee, Scotland, under the supervision of Profs. John Brimacombe and Mike Ferguson. He then did post-doctoral research with Profs. Sabine Flitsch at the University of Edinburgh, Geert-Jan Boons at the Complex Carbohydrate Research Center, Georgia, USA, and Chi-Huey Wong at The Scripps Research Institute, California. Following a spell at Nature Publishing Group as an assistant editor Greg joined Prof. Tayaba Hasan's group at Harvard Medical School, Boston, as an instructor, before returning to the UK to join Selcia LTD., Essex, as a senior research scientist.

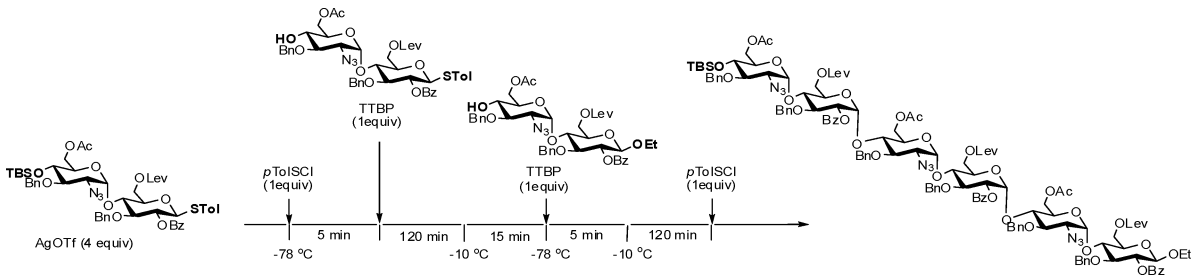

Fig. 2 Huang's one-pot modular synthesis of an HP-like hexasaccharide.

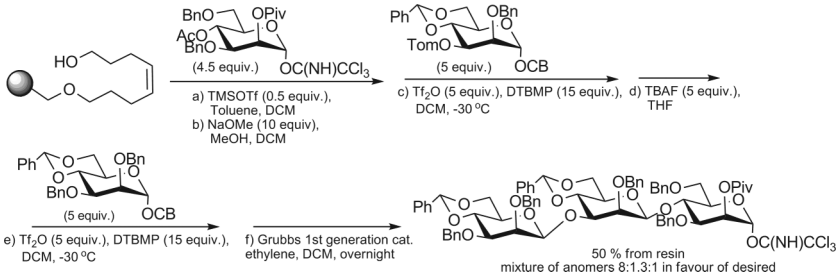

Fig. 3 Seeberger's automated synthesis of trimannoside on solid support.

be reliably introduced on solid-phase by taking advantage of neighbouring glycosylation of a 2-*O*-acyl functionality,[17] while Boons *et al.*[19] have recently reported the first solid-supported synthesis of an oligosaccharide having multiple 1,2-*cis*-glycosidic linkages by means of a chiral auxiliary. The strategy was applied to the synthesis of α-glucan pentasaccharide (Fig. 4). Complete anomeric control was achieved by using glycosyl donors having a participating (*S*)-(phenylthiomethyl)benzyl chiral auxiliary at C2. A branching point was installed by using 9-fluorenylmethyloxycarbonyl (Fmoc) and allyloxycarbonyl (Alloc) as a versatile set of orthogonal protecting groups. The synthetic strategy made it possible to attain partial on-resin deprotection of the completed oligosaccharide, thereby increasing the overall efficiency of the synthesis.

Another interesting recent development, the surface-tethered iterative carbohydrate synthesis (STICS), is a strategy based on the use of functionalized high surface area porous gold as an alternative solid support technology to perform cost efficient and simple synthesis of oligosaccharide chains.[20]

Fluorinated soluble support strategies (F-SPE) that show great promise have also been developed.[21–25] The methodology is of particular interest since protecting-group manipulations and glycosylations can be conducted under conditions typically used for solution-phase chemistry. The scope of the technology has recently been exemplified in the one-pot fluorous "catch and release" synthesis of linear and branched oligosaccharides, which featured the Lewis X trisaccharide antigen as one of their targets.[23] A fluorinated hydrazide linker-tag was reacted rapidly with ketone functionalized oligosaccharides post-glycosylation so that fluorous-tagged materials were selectively captured by filtration of the crude reaction mixture through a fluorous solid-phase extraction column and then released by elution with the appropriate solvent (Fig. 5). Beneficial features of the approach include rapid reaction rates and a substantially reduced volume of organic solvents employed for purification.

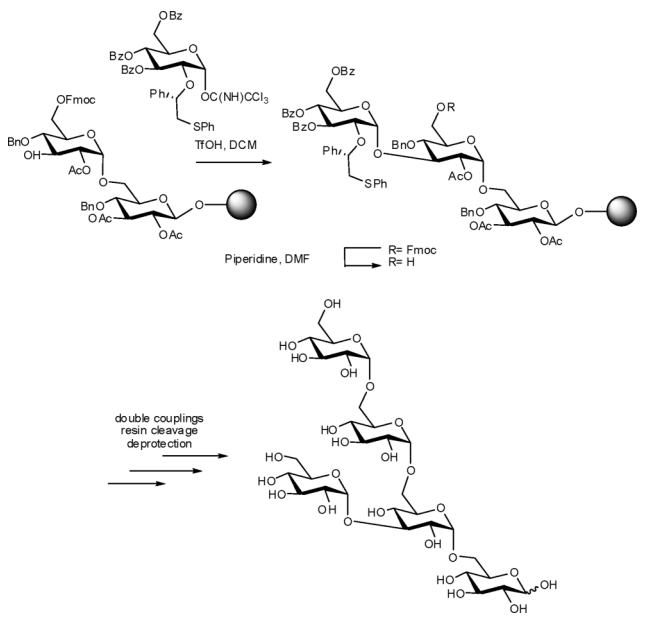

Fig. 4 Example of Boons' stereoselective solid-supported synthesis of α-glucan pentasaccharide.

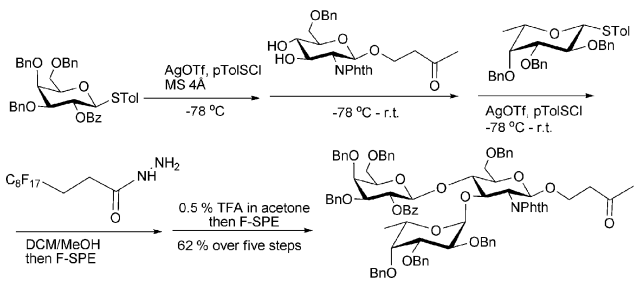

Fig. 5 Huang's synthesis of Lewis x trisaccharide *via* F-SPE.

In recent years, a new strategy based on the use of ionic liquids (ILs) as a soluble functional support has been developed and homolinear $\alpha(1\rightarrow6)$-linked di-, tri- tetra- and octa- saccharides have been successfully prepared using the approach.[26–29] Pathak *et al.*[28] have successfully applied this new IL supported technology to the synthesis of an activated oligomannan using a convergent assembly of a homolinear $\alpha(1\rightarrow6)$-linked octamannosyl thioglycoside starting from imidazolium cation-tagged mannosyl fluoride and thiomannoside using block couplings (Fig. 6).

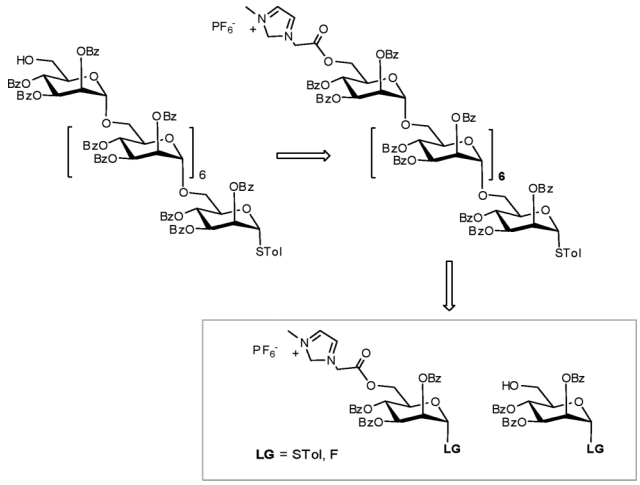

Fig. 6 Pathak's retrosynthetic analysis of IL-supported synthesis of $\alpha(1\rightarrow6)$-octamannoside using imidazolium cation-tagged donors.

One-pot approaches have also been applied to the preparation of orthogonally protected building blocks, which otherwise require lengthy and laborious protecting group manipulations, albeit there are fewer examples.[30,31] For instance, the groups of Beau[32,33] and Hung[34,35] have developed very elegant regio-selective one-pot protection strategies *via* Lewis acid catalyzed reactions on per-*O*-trimethylsilylated glucosides. Their route offers a tremendous advantage over sequential methods for the preparation of orthogonally protected glycosides ready to be used as building blocks in glycosylation reactions. This strategy has been recently highlighted in the regioselective protection of trehalose and maltose disaccharides using $FeCl_3{\cdot}6H_2O$ as an inexpensive and environmentally friendly catalyst starting from the corresponding per-*O*-silylated derivatives[33] (Fig. 7).

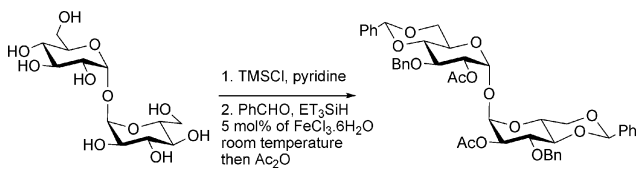

Fig. 7 Beau's one-pot regioselective protection of α,α-D-trehalose.

3. Synthetic glycosaminoglycans (GAGs)

GAGs are an important class of polysaccharide macromolecules that are heavily *O*-and *N*-sulfated and differ in the structures of the saccharide repeating units as well as the number of sulfo groups on the polysaccharide, with the exception of hyaluronic acid, which contains no sulfo groups.[36–38] Sulfated GAGs such as heparin, chondroitin sulfate (CS), and heparan sulfate (HS) are linear polysaccharides that are responsible for mediation of a wide range of biological actions through specific binding to a variety of proteins including those involved in blood coagulation, cell proliferation, differentiation and adhesion, and host–pathogen interactions.[36–38] Due to the diverse functions of HS and heparin, the possibility of exploiting its anticancer, anti-inflammatory and antiviral activities, in addition to its anticoagulant activity, has generated considerable interest (Fig. 8).

Pharmaceutical heparin, which is one of the oldest drugs currently still in widespread clinical use as an anticoagulant, is mainly isolated from porcine intestines; however the safety of heparin can be compromised by contamination from environmental factors. Batches of heparin, contaminated by over-sulfated chondroitin sulfate, were recently discovered in as many as 12 countries, and has led to over 80 deaths and nearly 1000 cases of allergic reactions in the US and Germany.[39] Therefore, the development of methods to prepare pure and structurally defined fragments of heparin and HS not only enables the characterization of the microstructure and the molecular level details of their interaction with peptides and proteins, but it is also crucial to develop improved and safer therapeutics that can be manufactured in properly regulated facilities.

The chemical synthesis of HS oligosaccharides with a defined sulfation pattern and length has proven effective in obtaining therapeutically active compounds that are smaller than hexasaccharides (Fig. 8 A).[40,41] For instance, a fully synthetic antithrombin III analogue has been marketed as a treatment for deep vein thrombosis and other synthetic heparin analogues have been developed as anticoagulants (Fig. 8 B).[42] In 2009, Boons and co-workers[43] reported a modular approach for the parallel combinatorial synthesis of a library of HS oligosaccharides which was employed to probe the structural features of HS for inhibiting the protease, BACE-1 (β-secretase). Moreover, the group of Baleux has devised a novel synthetic CD4–heparan sulfate glycoconjugate that inhibits simultaneously two highly conserved regions of HIV-1 envelope, gp120, which is key for viral invasion of host T-cells *via* the glycoprotein CD4 and co-receptors. The peptide binds to glycoprotein gp120, subsequently exposing a co-receptor binding domain allowing gp120 to further bind with the oligosaccharide moiety resulting in low-nanomolar antiviral activity (Fig. 8 C).[44]

The chemical synthesis of GAGs larger than octasaccharide is extremely difficult although encouraging steps have been made to overcome this problem. Hsieh-Wilson and coworkers,[45] have described the synthesis of novel chondroitin sulfate glycomimetics through ring-opening metathesis polymerization of sulfated monomers, a new approach that simplifies the synthesis of complex GAGs. The polymers obtained display biological activities comparable to those of natural chondroitin sulfate polysaccharides. Enzymatic synthesis offers a promising alternative for obtaining larger HS fragments with the desired biological functions.[46] Lindhart *et al.*[47] recently reported the first chemoenzymatic synthesis of a stable isotope-enriched heparin as a novel reagent for studying the interaction of heparin with proteins.[47] Even though enzymatic synthesis requires less steps than chemical approaches, there are still some limitations to overcome for these approaches to be viable for drug development. Some of the issues include: enzyme

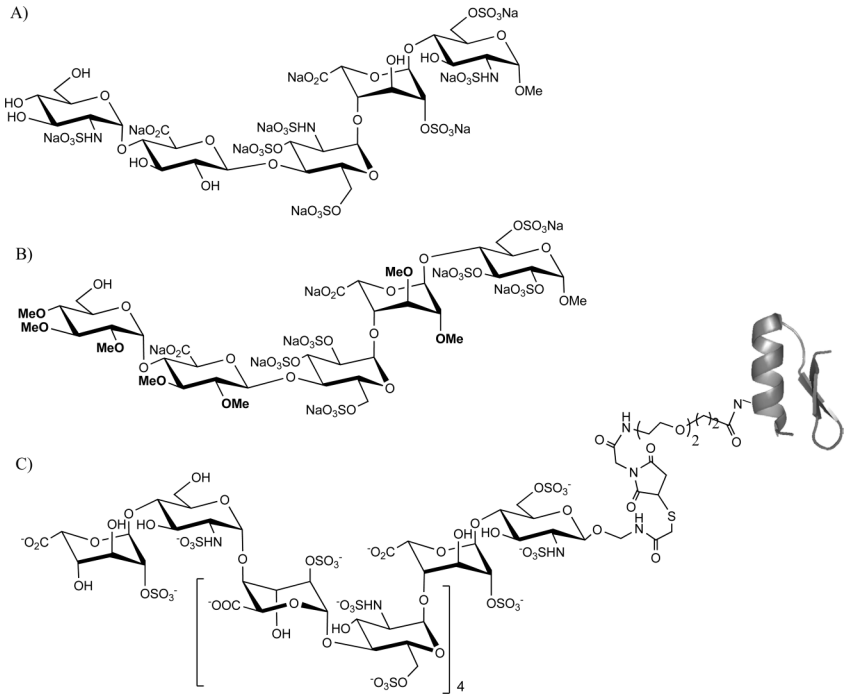

Fig. 8 GAGs. A) Structure of the anticoagulant drug Arixtra; B) Structure of anticoagulant candidate drug Idraparinux; C) mCD4-HS12 chimera.

accessibility, control of product structural variability, scalability limitations and synthesis of unnatural derivatives.

4. *O*-GlcNAc

The *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modification is an abundant post-translational modification found in all eukaryotic organisms. *O*-GlcNAc moiety is attached to the hydroxyl side chain of a serine or threonine residue.[48] The incorporation and removal of *O*-GlcNAc is a dynamic process which is found in nuclear and cytosolic proteins. The modification involves the incorporation of a single glucosamine unit and the carbohydrate is not further elongated into more elaborate structures. That feature, along with the fact that this is a temporary modification, is what makes this protein modification so peculiar and more analogous to phosphorylation/dephosphorylation signaling processes than to normal protein glycosylation. In fact, *O*-GlcNAc shares many common traits with *O*-phosphate. In a similar way as kinases/phosphatases regulate phosphorylation/dephosphorylation process, *O*-GlcNAc is attached to proteins by an *O*-GlcNAc transferase, UDP-GlcNAc:polypeptide transferase (OGT),[49] and removed by a β-*N*-acetylglucosaminidase (*O*-GlcNAcase).[50] Because OGT associates with a phosphatase[51] forming a dephosphorylating-glycosylating complex, and both modifications often occur on the same residues,[52] it was believed that both processes might have a reciprocal relationship, but recent evidence suggests that the relationship within the tandem *O*-GlcNAc glycosylation/phosphorylation is more complex.[53–55]

O-GlcNAc glycosylation processes are involved in cellular events such as transcription,[56] apoptosis,[57,58] glucose homeostasis,[59] and signal transduction.[54] Important advances have been made in the understanding of the role of *O*-GlcNAc glycosylation. Although the development of new immunologic[60–62] and chemical tools[63–67] have provided rapid and sensitive methods for detecting this particular protein modification, there is still a need for more efficient tools to study the role of *O*-GlcNAc in cells.

In this context, Hsieh-Wilson and co-workers have made a big contribution to the field, developing methodologies for the identification and quantification of *O*-GlcNAc modified proteins.[63,64] In their latest contribution,[68] the authors describe a strategy based on the tagging of a protein *via* enzymatic modification of the *O*-GlcNac residues with a UDP-ketogalactose analog using a methodology previously developed within the group[64] and posterior derivatization with an aminooxy-functionalized polyethylene glycol moiety (Fig. 9). The proteins with the tag incorporated are then easily visualized on SDS-PAGE and posterior immunoblotting. This methodology enables rapid quantification of *in vivo* glycosylation levels of endogenous proteins with little processing and avoids the use of expensive detection methods.

A new insight into the biological role of *O*-GlcNAc has been reported by Boons and co-workers.[55] Using fully synthetic three-component immunogens, the authors obtained a large set of *O*-GlcNAc-specific monoclonal antibodies able to identify more than 200 modified proteins including a large number of new glycoproteins.

One of the main problems associated with the study of *O*-GlcNAc protein modification is the low presence on protein substrates (usually less than stoichiometric amounts), and the difficulty in detecting this modification by mass spectrometry. To circumvent this issue, Hart and co-workers[69] have developed a methodology for the enrichment and characterization of *O*-GlcNAc sites from complex samples. The strategy involves the tagging of *O*-GlcNAc modified peptides with a photocleavable biotinylation reagent, followed by enrichment by affinity

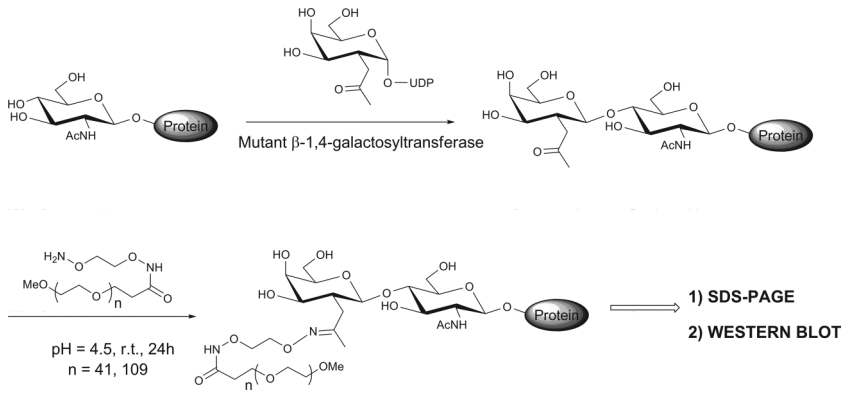

Fig. 9 Hsieh-Wilson's mass-tagging strategy for *O*-GlcNAc stoichiometry and dynamics quantification.

chromatography. The peptides are then released from the solid support bearing an ionic label which facilitates the characterization by electron transfer dissociation (ETD) mass spectrometry. Using this strategy, eight *O*-GlcNAc sites were mapped from a tau-enriched sample from rat brain. Sites of GlcNAcylation were characterized on important neuronal proteins such as tau, synucleins, and methyl CpG-binding protein 2.

5. Synthetic glycoconjugates

Carbohydrates linked to other biomolecule classes are termed 'glycoconjugates'. They include ubiquitous and biologically important biomolecule sub-classes, such as glycoproteins, peptidoglycans and glycolipids. Many naturally occurring glycoconjugates are important drugs, for example antibiotics vancomycin and erythromycin, which are both glycosylated macrocycles, and glycoprotein-based drugs including Erythropoetin and therapeutic antibodies such as Avastin. Synthetic glyco-conjugation gives chemists the potential to develop new pharmaceutically active compounds with improved and broad spectra of activity.

Glycoproteins

The post-translational glycosylation pattern of natural glycoproteins is highly diverse, with the glycan not only ensuring the activity and stability of proteins but also adding additional functionalities, such as recognition by carbohydrate-binding proteins. The multiple glycoforms of glycoproteins makes the study of their biological function extremely difficult and their production as drugs more complex, with significant bioengineering required to provide higher proportions of effective glycoproteins. The incorporation of pure, chemically synthesized glycans at specific sites in proteins to produce homogeneous glycoproteins, could potentially afford active glycoproteins, without the complication of multiple glycoforms.[70,71] Glycoprotein synthesis has been recently reviewed.[72,73] There are two major strategies to prepare glycoproteins (Fig. 10). The main difference between these methods is at what stage the glyco-peptide bond is formed in the synthetic process. One approach involves linking the glycan to a specific point on a completed protein (Fig. 10 A). In the other approach the glycopeptide bond is first formed by coupling the glycan to a single amino acid or small peptide and the rest of the protein is synthesized afterwards (Fig. 10 B). The advantage of this approach is that a natural glycopeptide link can be formed. The latter approach has been greatly enhanced by the discovery of native chemical ligation (NCL)[74] and expressed protein ligation (EPL).[75] The coupling of two unprotected peptides, one with an N-terminal cysteine and the other with a C-terminal thioester, occurs *via* an $S \rightarrow N$ acyl shift, forming a longer peptide or protein with native linkages. EPL of short glycopeptide chains with larger recombinant peptide fragments has been used to prepare complex glycoproteins,[76,77] including an active analogue of erythropoietin with two complex-type glycans.[78] A recent elaborate synthesis of active RNase C[79,80] involved EPL of a complex-type glycosylated peptide with a cysteine-rich recombinant N-terminal fragment. Carboxyethylthio-capping of the cysteine thiol groups was necessary to stabilise the cysteine-rich peptide during ligation. MacMillan *et al.* [81,82] have recently discovered conditions for reversing NCL; peptides are cleaved at low pH in the presence of a thio-alcohol in an $N \rightarrow S$ acyl shift affording thioesters, which in-turn are used for NCL with glycopeptides. Peptides can be also ligated without the need of an N-terminal cysteine, instead peptide coupling is aided by a thiol-bearing sugar attached to the N-terminal peptide.[83] Although this approach is limited by both the nature of the attached glycan and also by the peptide sequence to be formed.[84,85]

One of the most exciting developments in selective protein modification in recent years has came from the Davis group,[86] who introduced chemically dual orthagonal post-translational modifications to an essentially inactive bacterially produced protein that not only activated the production of protein to levels of those produced naturally, but also allowed the study of post-translational modification in a biological system. More recently, the same group have expanded their protein chemical glycosylation techniques to include an interesting cross-metathesis approach that utilizes magnesium chloride as a weak ligand to block unwanted protein interactions with the ruthenium catalyst[87] and an efficient Suzuki–Miyaura cross-coupling.[88]

6. Carbohydrate vaccines

Carbohydrate based vaccines have been used to induce immunity since the 1980s when the first polysaccharide vaccine was commercially launched by Merck and Co. Since then, many developments in this ever expanding area have taken place. In this section, we aim to present a summary of the most recent applications of

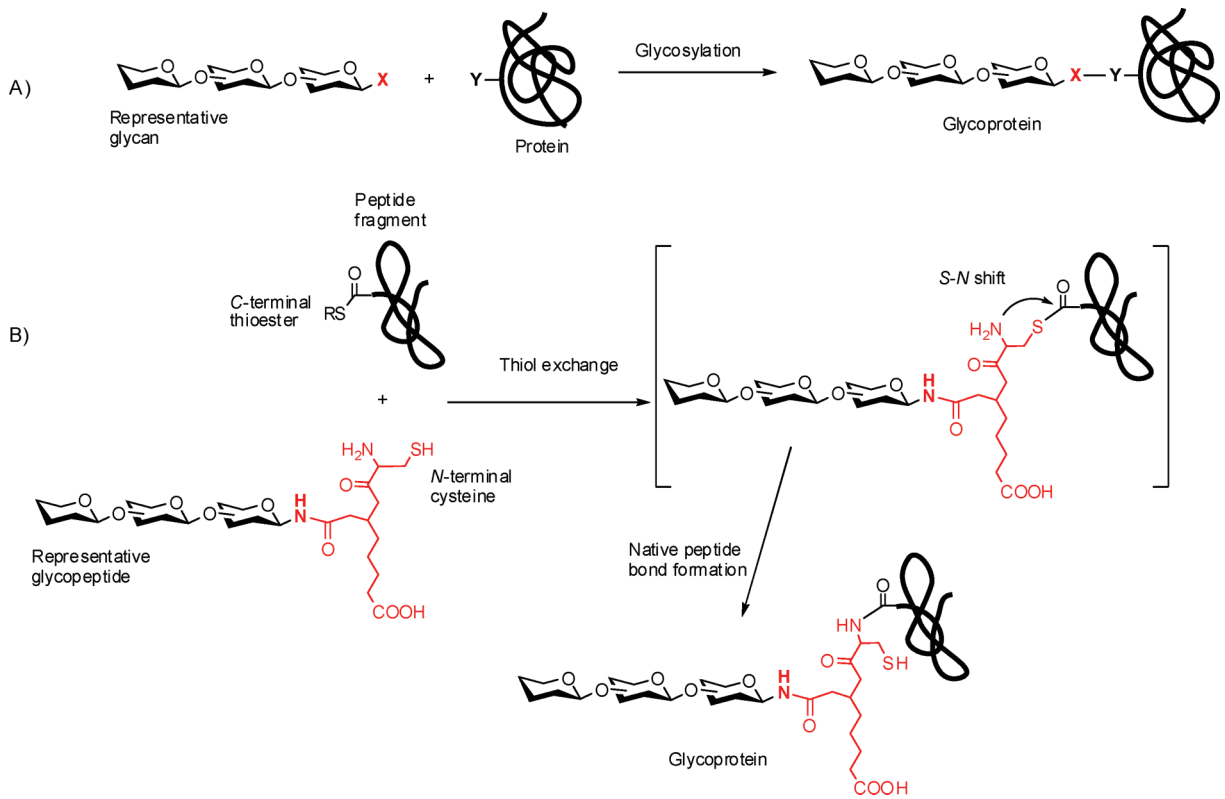

Fig. 10 Strategies towards glycoprotein synthesis: A) Site-specific glycosylation of proteins (Y = reactive functionality at specific point on protein; X = activated group for coupling with Y); B) NCL of pre-formed glycopeptide with a peptide block containing C-terminal thioester.

carbohydrates in vaccine development, focusing on the chemical synthesis and the effectiveness of the vaccine candidates. For more in detail analysis of the main issues involved in the development of carbohydrate vaccines, challenges and future prospects refer to the excellent review written by Astronomo and Burton.[89]

The use of carbohydrates in vaccines has been limited because of their poor immunogenic properties. Unlike protein antigens which mobilize $CD4^+$ T cells and trigger the generation of highly specific and long-lived antibodies, carbohydrate antigens activate independent T-cell humoral responses which create short-lived antibodies mobilizing immunoglobulin G.[90] Nonetheless, synthetic carbohydrate-based vaccines present the advantage of a well defined chemical structure and the complete absence of impurities, two conditions difficult to accomplish with vaccines derived from biological sources.

Different approaches have been successfully developed to overcome this lack of immune response. The use of adjuvants, covalent attachment of carbohydrates to immune-stimulants (conjugate vaccines) or integration into complex multi-component vaccines have helped in the progress of carbohydrate vaccine development leading to a renewed interest in the field. (Fig. 11)

6.1 Antiviral vaccines

Unlike other exogenous pathogens (bacteria and fungi), viruses are unable to replicate by themselves and use host replication machinery. The consequence is that viruses present in their outer shell carbohydrate structures of the host organism, which makes them a difficult target.[91] Nonetheless, in recent years several achievements have been made in the progress of antiviral vaccines development, especially in the field of anti-HIV vaccines.

The discovery of a broadly neutralizing antibody against HIV1 2G12 that was able to recognize conserved clustered oligomannose glycans ($Man_9(GlcNAc)_2$) present on the gp120 receptor-binding glycoprotein of the virus, has given scientists hope that the "glycan shield" defense of the virus can be breached, and that these structures, under the right circumstances can act as potential targets for vaccine development.[92] Indeed, most of the recent strategies developed towards obtaining an anti-HIV vaccine involved the use of oligomannose structures as antigenic entities.[93–97]

Wang *et al.*[97] developed a very efficient strategy for the synthesis of highly functionalized oligomannose dendrons, attaching oligosaccharides of different lengths to an alkynyl dendrimeric scaffold *via* the copper(I) catalysed alkyne-azide 1,3-dipolar cycloaddition reaction (Fig. 11 A). Although the structures obtained were not suitable for vaccine purposes, glycan microarray assays showed that the second generation glycodendrons carrying linear chains of mannose nonasaccharides gave the best affinities, suggesting that a multivalent display of carbohydrates might be a practical solution for inducing 2G12-like antibodies and blocking viral infection.

A similar approach has been recently used by Kabanova *et al.*[93] The authors describe the development of polyamidoamine-based dendrons displaying oligomannose clusters of HIV-related antigens Man_4, Man_6, and Man_8. Although IgG antibodies were generated, the antisera failed to recognize gp120 proteins.

Following the same multivalent presentation principle, Astronomo *et al.*[95] developed neoglycoconjugates displaying variable

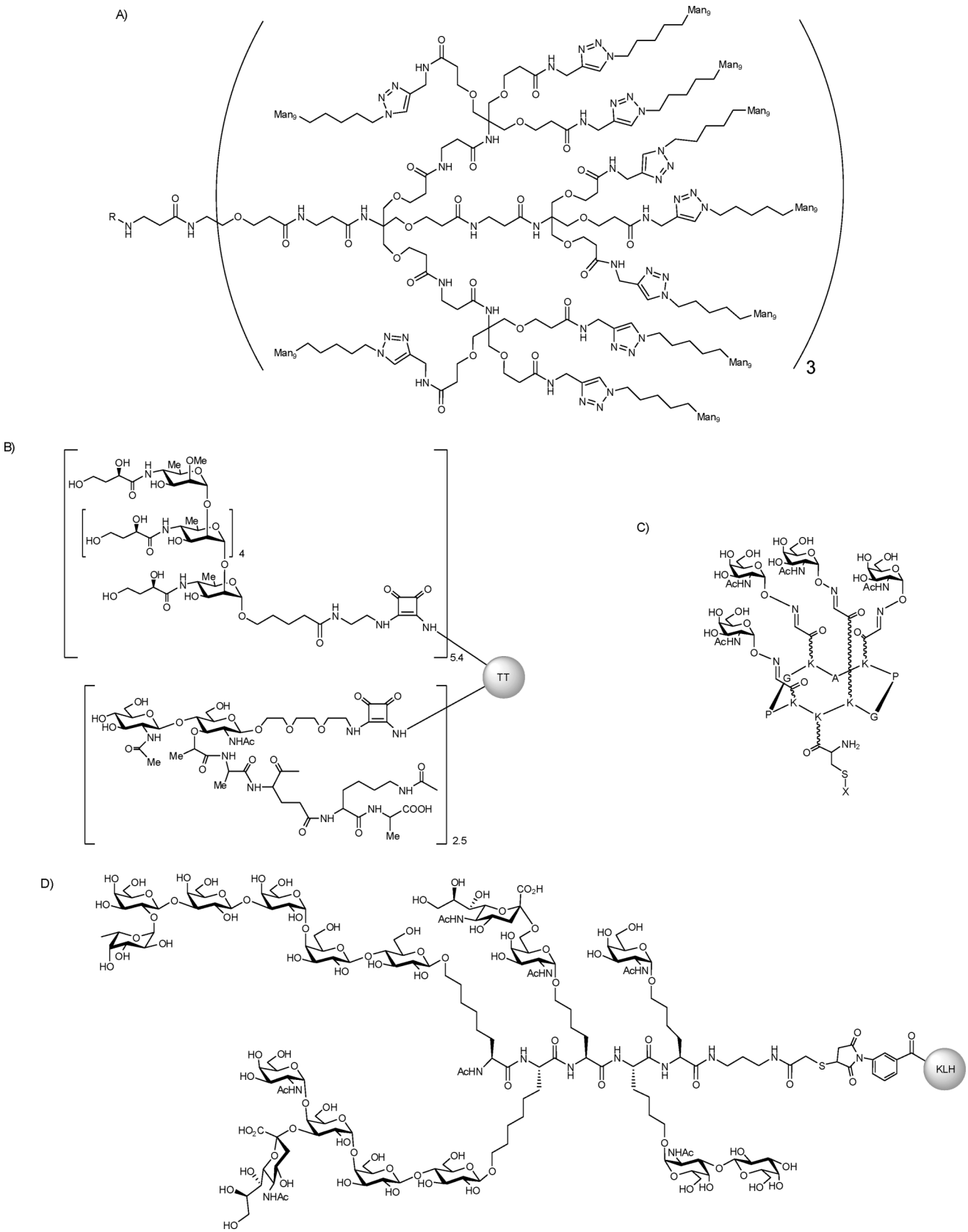

Fig. 11 Antigen presentation strategies employed in the development of carbohydrate-based vaccines in order to circumvent the low immunogenicity of carbohydrates. A) Wang's 2nd generation antiviral oligomannose dendrimer, Man = Mannose; B) Bongat's squarate antibacterial vaccine incorporating carbohydrate antigen and glycolipid for enhanced immunogenicity. TT: Tetanus toxin fragment; C) Renaudet *et al.* anticancer RAFT-based vaccine candidate; D) Zhu's unimolecular pentavalent anticancer vaccine candidate. KLH: Keyhole Limpet Hemocyanin.

copy numbers of synthetic Man_4 directly conjugated to bovine serum albumin (BSA). Although BSA-$(Man_4)_{14}$ was immunogenic, inducing the formation of 2G12 antibodies in rabbits, those antibodies did not bind to gp120. The authors suggest that the heterogeneity of the BSA surface and therefore the irregular distribution of the glycans might be the main cause for the lack of efficacy of their glycoconjugate as a viable vaccine.

Alternative strategies for the presentation of the antigen in an effective way involved the use of a semi-rigid cyclic peptide as scaffold.[96] Danishefsky and co-workers designed a 2G12 mimotope consisting in up to 3 copies of the epitope $Man_9GlcNAc_2$ attached to a 14 amino acid peptide. The synthetic structures were able to bind 2G12 in *in vitro* assays, but immunization results in animal models showed no functional immune response, suggesting that the glycoconjugates do not mimic the carbohydrate epitope of an infectious HIV-1 virion, probably due to the poor rigidity of the glycans.

Abdel-Motal *et al.* [98] exploited the high expression of anti-α-gal epitope antibodies to develop glycoconjugates formed by a fusion protein (gp120/p24, surface and matrix proteins of HIV1) and α-gal epitope. The constructs elicit an effective immune response facilitated by the presence of the α-gal epitope, suggesting that the use of this particular glycan, which is not present in humans, could be a useful strategy to target vaccines with low immunogenic antigens.

A different approach has been recently developed by the joint efforts of the Davis, Burton, Wilson amd Finn groups.[94] The observation that 2G12 binds D-fructose with higher affinity than D-mannose [99] led the authors to develop a series of analogues of the epitope Man_4 modified at the terminal sugar unit, where unnatural sugars based on D-fructose were incorporated. The immunogenicity of the corresponding glycoconjugates was tested as well, but unfortunately no HIV-reactive antibodies were elicited.

6.2 Antibacterial vaccines

Antibiotic resistance has become one of the main concerns in bacterial disease treatment. Vaccination is one of the most effective methods in the prevention and control of bacterial infections. The effectiveness of carbohydrate-based antibacterial vaccines is evidenced by the presence on the market of a variety of antibacterial vaccines *e.g.* anti-meningococcal, -pneumococcal or anti-*Haemophilus influenzae* type B bacteria. Commercial vaccines are composed of a polysaccharide extracted from an organism which is linked to a carrier protein, but to date, no fully synthetic oligosaccharide containing vaccines are in clinical use. Nevertheless, significant progress has been made in the last years.[100] Different studies have been published [101–104] concerning the synthesis of immunogenic structures and conjugation methods. Along with these synthetic papers, several vaccine candidates appeared in the last two years.[105–107] P. Simerska and H.L. Istvan Toth *et al.*[106] developed a self-adjuvanting vaccine conjugate targeting *Streptococcus pyogenes*. In this work, a glucoside derivative was used as a chiral scaffold derivatized with antigenic peptides and a lipid moiety which acts as an adjuvant. To date no immunological tests have been reported, and although the authors point out that the lipoaminoacid structure makes this glycoconjugate a feasible candidate for intranasal or oral administration, the immunogenicity of the vaccine has not yet been demonstrated. Bongat *et al.*[107] prepared divalent conjugates from different synthetic oligosaccharides attached as squarate derivatives to a recombinant tetanus toxin carrier. In these conjugates one of the ligands is a synthetic carbohydrate antigen and the other is a synthetic glycolipid to enhance the immunogenicity of the construct. Immunological implications of these conjugates are also to be demonstrated (Fig. 11 B).

6.3 Antifungal and antiparasitic pathogens vaccines

In the context of their ongoing research into conjugate vaccines, the Bundle research group has developed conjugated glycopeptide and glycopolymer conjugated vaccines candidates for the treatment of candidiasis and other fungal infections. Using a non immunogenic heterobifunctional linker based on water-soluble triethylene glycol developed in his group,[108] The group prepared a vaccine against candidiasis incorporating a small carbohydrate epitope (β-mannan trisaccharide) and a short peptide derived from pathogen cell wall proteins (Fig. 12).[109] This glycoconjugate was sufficient to induce protective responses against both the carbohydrate and peptide carrier components, avoiding the use of any other carrier such as bacterial toxoids. Similarly, the same carbohydrate epitope was activated for co-polymerization with acrylamide, and derivatized for conjugation to chicken serum albumin.[110] The constructs presented the oligosaccharide units in a clustered form, inducing a robust immune response and eliciting antibodies that bound cell wall antigen of *C. Albicans* in mouse models.

The field of antiparasitic vaccines has experienced less scientific impetus since the appearance of the first synthetic carbohydrate vaccine against malaria by Verez-Bencomo and co-workers.[111]

6.4 Anticancer vaccines

Carbohydrate-based anticancer vaccines have gained much scientific interest over the last few years.[112–127] The glycosylation pattern and the density of cell-surface sugars are determined genetically. Cancer cells express aberrant surface carbohydrate structures, either in their sequence (truncated glycosylation), or in their increased expression. These glycans are known as tumor-associated carbohydrate antigens (TACAs) and represent attractive targets for vaccine development. The use of TACAs in vaccine development presents added problems in comparison to other carbohydrate-based vaccines, in addition to the low immunogenicity of carbohydrate epitopes, TACAs are self antigens and can be tolerated by the immune system. Nonetheless, different approaches have been made in order to circumvent this. Most strategies try to reproduce the presentation of glycans on the cell surface, whereby different scaffolds have been used to this end. In this sense, P. Dumy and co-workers [114,117] developed a self-adjuvanting multivalent glycopeptide cancer vaccine prototype attaching four copies of the Tn-antigen onto a cyclic decapeptide that also bore a $CD4^+$ T cell epitope peptide, a $CD8^+$ T cell epitope peptide and a palmitic acid moiety as adjuvant. This construct induced strong antitumor B and T cell protective immunity when tested in mouse model systems (Fig. 11 C).

Unimolecular pentavalent vaccine candidates developed by Danishefsky and co-workers gave such promising results that some of them are ready to enter phase I clinical trials.[125] In these constructs, the scaffold is a linear peptide which incorporates a cluster of up to 5 different carbohydrate epitopes conjugated

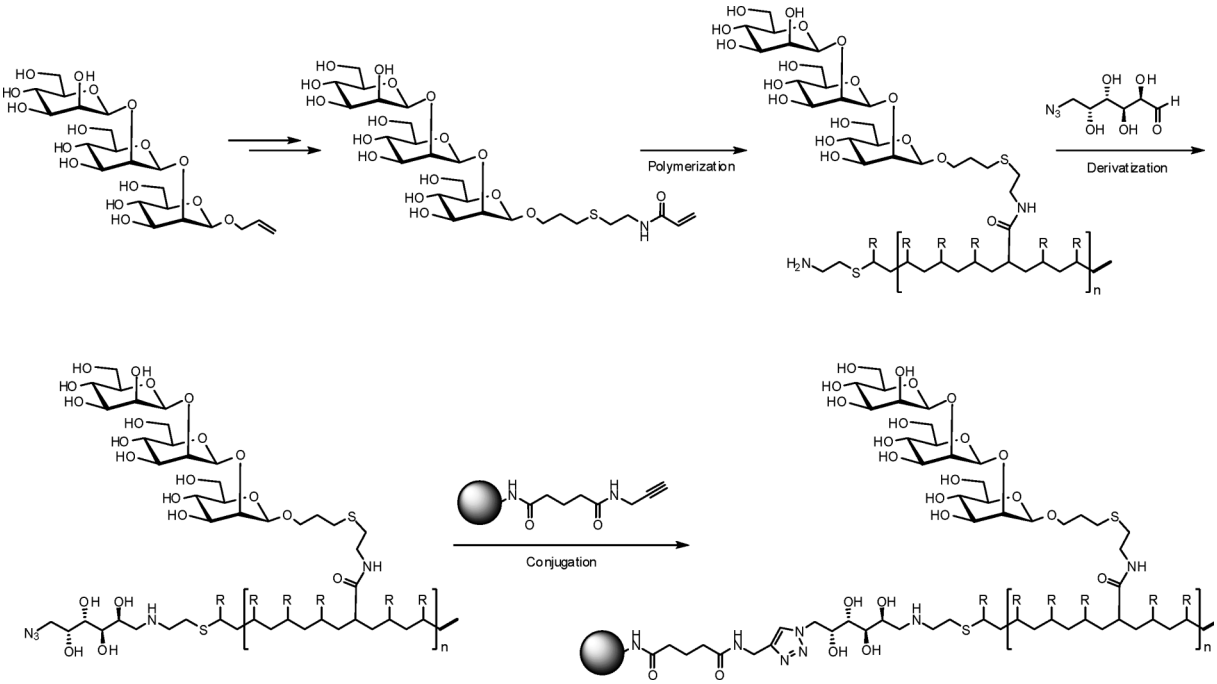

Fig. 12 Bundle's glycopolymer conjugate with *Candida albicans* β-mannan trisaccharide.

to KLH (Keyhole Limpet Hemocyanin) carrier protein. This approach demonstrates the effectiveness of clustering in generating an immune response. With the main objective of obtaining a well defined and regular scaffold for adequate antigen display, other approaches involved the use of cowpea mosaic virus capsid[113] or calixarenes[112] as carbohydrate presenting platforms (Fig. 11 D).

Although multivalent constructs can successfully elicit immune responses, monovalent vaccine candidates have also shown good results in producing antibodies against TACAs.[116,118,124] In this sense, Boons and co-workers[118] developed a vaccine candidate composed of a glycopeptide (B-epitope), a T-cell epitope and a TLR-2 ligand (Toll-Like Receptor ligand) to facilitate the uptake of the glyco-lipopeptide by antigen-presenting cells and B-lymphocyte. This construct was able to elicit strong IgG antibody responses that recognized cancer cells. Ragupathi *et al.* [124] were also successful in developing a monovalent vaccine targeting sialyl Lewis[a] (sLe[a]), eliciting IgG and IgM antibodies which reacted strongly with sLe[a] positive cancer cells.

Kunz and co-workers also obtained strong immune responses with synthetic multicomponent constructs incorporating the MUC1 glycopeptide with either sialyl-Tn saccharide side chain[128] or TF antigen[129] coupled to a tetanus toxoid carrier protein (Fig. 13). A different synthetic approach has been used by R. J. Payne and co-workers.[130] The authors describe the synthesis of self-adjuvating multicomponent vaccine candidates combining MUC1 glycopeptides in combination with a T-cell helper peptide and the lipopeptide immunoadjuvant Pam3CysSer using an efficient and convergent method for the assembling of the

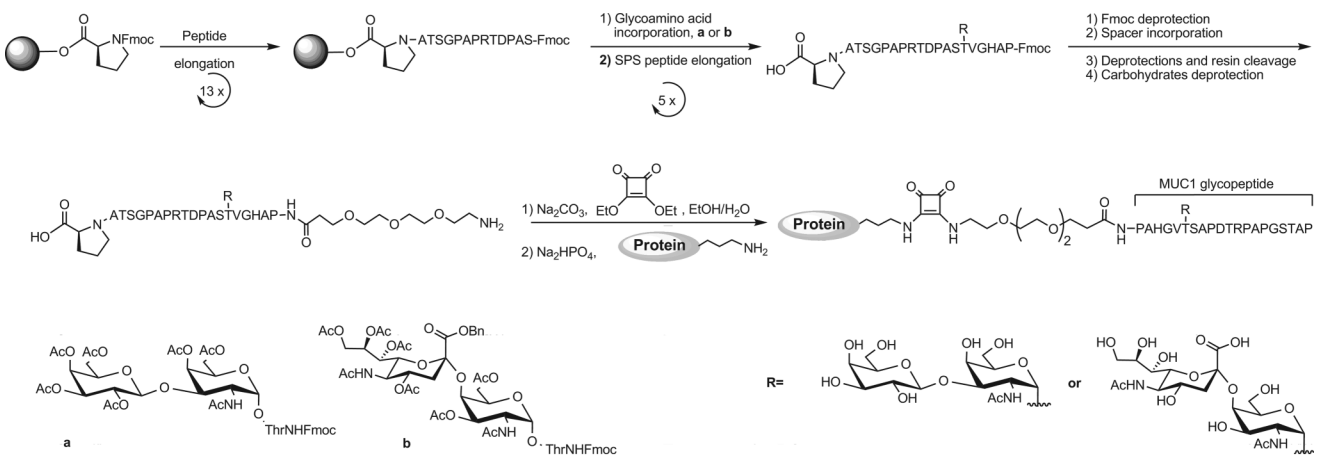

Fig. 13 Kunz's synthesis of MUC1 multicomponent vaccine construct.

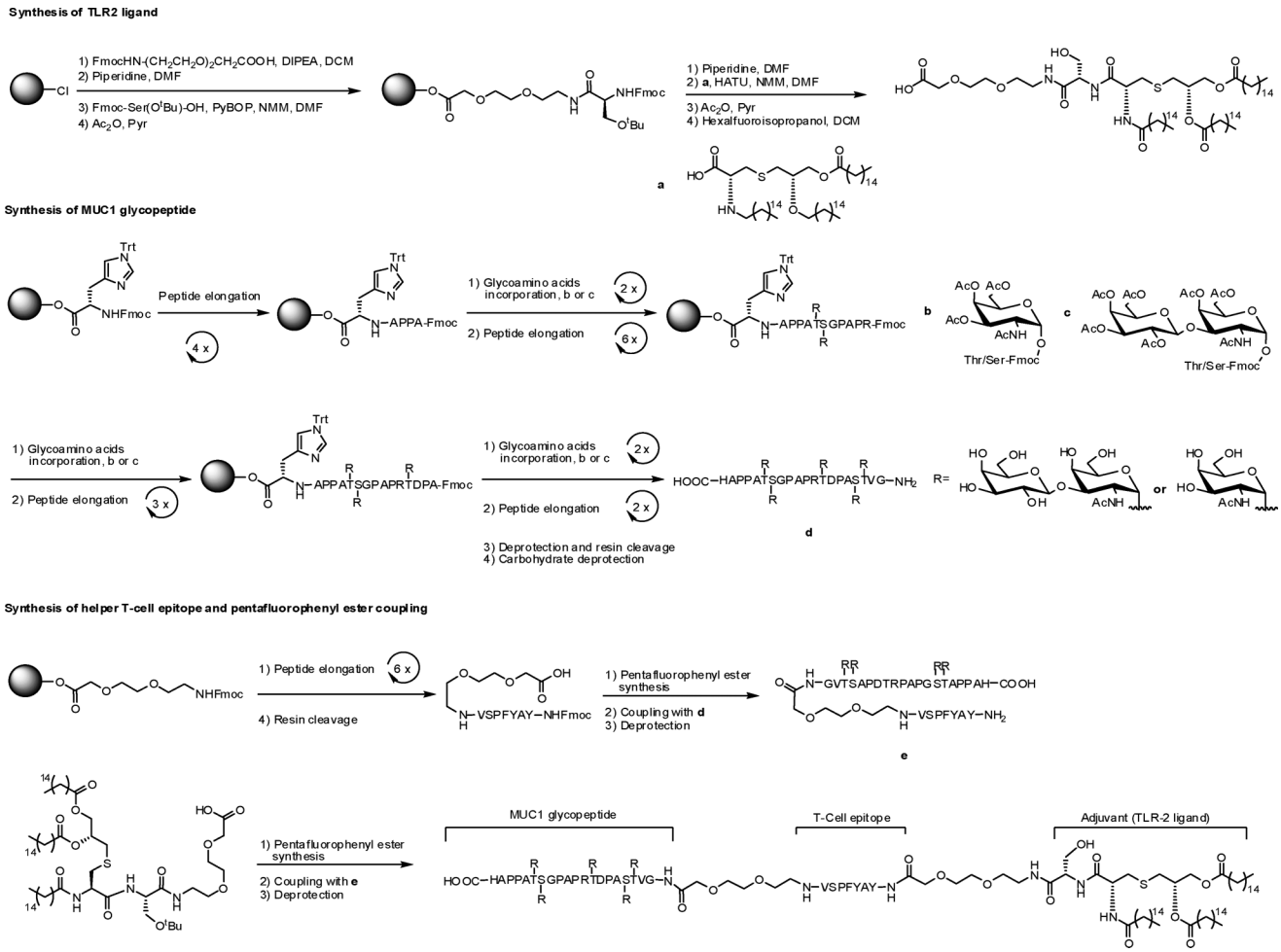

Fig. 14 Payne's fragment condensation strategy for the synthesis of MUC1 self-adjuvating multicomponent vaccine construct.

different building blocks *via* pentafluorophenyl ester condensation (Fig. 14).[130,131]

In some cases monovalent constructs gave better results than the divalent ones as shown by Bay *et al.*,[116] where conjugation of bacterially produced GM2 ([GalNAcβ-4(NeuAcα-3)Galβ-4Glc] ganglioside) to a $CD4^+$ T cell epitope led to synthetic glycopeptides capable of inducing tumor cell-specific antibodies after immunization in the case of the monovalent conjugate, whereas the divalent construct was unable to elicit any immune response at all.

Similarly to anti-HIV vaccines candidates incorporating α-Gal epitopes,[98] alternative approaches for enhancing the immunogenicity of the vaccine candidates involve the use of the naturally occurring anti-rhamnose antibodies present in the human serum to develop monovalent three-component vaccine. Incorporation of L-rhamnose (Tn - helper T cell epitope - Rhamnose) improves the antigenicity of the conjugates through antibody-mediated antigen uptake.[132]

Other strategies to improve antigenicity involve the design of conjugates incorporating chemical modifications on the carbohydrate epitopes.[129,133,134] The majority of constructs incorporating modified STn epitope have been shown to be immunogenic. In particular, the sera obtained from mice treated with modified carbohydrate synthetic vaccines developed by Zhang, Ye and coworkers strongly reacted with STn-positive tumor cells.[133]

6. Conclusions

The biological significance of glycan structures and their conjugates calls for access to adequate samples of pure materials to explore the interactions with their specific binding partners. Although carbohydrate synthesis has been improved greatly over the last 30 years, a fully automated system that can rapidly generate a complex of oligosaccharide structures as easily as other biomolecules, such as peptide or nucleotide sequences, remains elusive. Until this is realized, a thorough exploration of glycan interactions with potential binding partners cannot be achieved rapidly. The relative weakness of glycan interactions has been essentially solved by the development of glycan chips containing arrays of multivalent oligosaccharides. Such glycan-chips have been used for diagnosis of certain diseases, allowing potentially life-saving early therapeutic intervention. By exploiting differences in the cell-surface glycosylation pattern of normal and diseased states, scientists have explored the development of vaccines to a variety of ailments including cancer.

In areas utilizing traditional carbohydrate drugs such as anticoagulant heparin, there have been significant advances, which include the synthesis of therapeutic heparin fragments that are safer than the parent drug. As drugs, carbohydrates themselves are generally poor candidates as they have low tissue permeability and

short serum half-lives. These properties, however, can be improved though by the design of glycomimetics, where recent progress in X-ray crystallography and NMR spectroscopy has provided greater understanding of structural aspects of carbohydrate interactions, thereby realizing the potential to unlock access to new therapeutic sources.

Notes and references

1 A. Varki and J. B. Lowe, in *Essentials of glycobiology*, ed. A. Varki, Cold Spring Harbor Laboratory Press2009, vol. Part I, Chapter 6, pp. 80–81.
2 Y. van Kooyk and G. A. Rabinovich, *Nat. Immunol.*, 2008, **9**, 593–601.
3 R. D. Cummings, *Mol. BioSyst.*, 2009, **5**, 1087–1104.
4 J. C. McAuliffe and O. Hindsgaul in *Molecular and Cellular Glycobiology*, M. Fukuda and O. Hindsgaul, ed., Oxford University Press, Oxford, 2000.
5 B. Ernst and J. L. Magnani, *Nat. Rev. Drug Discovery*, 2009, **8**, 661–677.
6 T. J. Boltje, T. Buskas and G.-J. Boons, *Nat. Chem.*, 2009, **1**, 611–622.
7 CFG, http://www.functionalglycomics.org.
8 EurocarbDB, http://www.eurocarbdb.org/.
9 X. M. Zhu and R. R. Schmidt, *Angew. Chem., Int. Ed.*, 2009, **48**, 1900–1934.
10 D. R. Mootoo, P. Konradsson, U. Udodong and B. Fraser-reid, *J. Am. Chem. Soc.*, 1988, **110**, 5583–5584.
11 N. L. Douglas, S. V. Ley, U. Lucking and S. L. Warriner, *J. Chem. Soc., Perkin Trans. 1*, 1998, 51–66.
12 Z. Y. Zhang, I. R. Ollmann, X. S. Ye, R. Wischnat, T. Baasov and C. H. Wong, *J. Am. Chem. Soc.*, 1999, **121**, 734–753.
13 X. Huang, L. Huang, H. Wang and X.-S. Ye, *Angew. Chem., Int. Ed.*, 2004, **43**, 5221–5224.
14 Z. Wang, Y. M. Xu, B. Yang, G. Tiruchinapally, B. Sun, R. P. Liu, S. Dulaney, J. A. Liu and X. F. Huang, *Chem.–Eur. J.*, 2010, **16**, 8365–8375.
15 H. M. I. Osborn and T. H. Khan, *Tetrahedron*, 1999, **55**, 1807–1850.
16 P. H. Seeberger and W. C. Haase, *Chem. Rev.*, 2000, **100**, 4349–4393.
17 P. H. Seeberger, *Chem. Soc. Rev.*, 2008, **37**, 19–28.
18 J. D. C. Codee, L. Krock, B. Castagner and P. H. Seeberger, *Chem.–Eur. J.*, 2008, **14**, 3987–3994.
19 T. J. Boltje, J.-H. Kim, J. Park and G.-J. Boons, *Nat. Chem.*, 2010, **2**, 552–557.
20 P. Pornsuriyasak, S. C. Ranade, A. X. Li, M. C. Parlato, C. R. Sims, O. V. Shulga, K. J. Stine and A. V. Demchenko, *Chem. Commun.*, 2009, 1834–1836.
21 I. T. Horvath and J. Rabai, *Science*, 1994, **266**, 72–75.
22 F. Zhang, W. Zhang, Y. Zhang, D. P. Curran and G. Liu, *J. Org. Chem.*, 2009, **74**, 2594–2597.
23 B. Yang, Y. Q. Jing and X. F. Huang, *Eur. J. Org. Chem.*, 2010, 1290–1298.
24 F. A. Jaipuri and N. L. Pohl, *Org. Biomol. Chem.*, 2008, **6**, 2686–2691.
25 G.-S. Chen and N. L. Pohl, *Org. Lett.*, 2008, **10**, 785–788.
26 X. He and T. H. Chan, *Synthesis*, 2006, 1645–1651.
27 A. K. Pathak, C. K. Yerneni, Z. Young and V. Pathak, *Org. Lett.*, 2007, **10**, 145–148.
28 C. K. Yerneni, V. Pathak and A. K. Pathak, *J. Org. Chem.*, 2009, **74**, 6307–6310.
29 J. Y. Huang, M. Lei and Y. G. Wang, *Tetrahedron Lett.*, 2006, **47**, 3047–3050.
30 M. Filice, J. M. Guisan and J. M. Palomo, *Curr. Org. Chem.*, 2010, **14**, 516–532.
31 R. A. Jones, R. Davison, A. T. Tran, N. Smith and M. C. Galan, *Carbohydr. Res.*, 2010, **345**, 1842–1845.
32 A. Francais, D. Urban and J. M. Beau, *Angew. Chem., Int. Ed.*, 2007, **46**, 8662–8665.
33 Y. Bourdreux, A. Lemetais, D. Urban and J.-M. Beau, *Chem. Commun.*, 2011, **47**, 2146–2148.
34 C. C. Wang, J. C. Lee, S. Y. Luo, S. S. Kulkarni, Y. W. Huang, C. C. Lee, K. L. Chang and S. C. Hung, *Nature*, 2007, **446**, 896–899.
35 K.-L. Chang, M. M. L. Zulueta, X.-A. Lu, Y.-Q. Zhong and S.-C. Hung, *J. Org. Chem.*, 2010, **75**, 7424–7427.
36 J. R. Bishop, M. Schuksz and J. D. Esko, *Nature*, 2007, **446**, 1030–1037.
37 N. S. Gandhi and R. L. Mancera, *Chem. Biol. Drug Des.*, 2008, **72**, 455–482.
38 X. X. Xu and Y. Dai, *J. Cell. Mol. Med.*, 2010, **14**, 175–180.
39 H. Y. Liu, Z. Q. Zhang and R. J. Linhardt, *Nat. Prod. Rep.*, 2009, **26**, 313–321.
40 K. D. Johnstone, T. Karoli, L. G. Liu, K. Dredge, E. Copeman, C. P. Li, K. Davis, E. Hammond, I. Bytheway, E. Kostewicz, F. C. K. Chiu, D. M. Shackleford, S. A. Charman, W. N. Charman, J. Harenberg, T. J. Gonda and V. Ferro, *J. Med. Chem.*, 2010, **53**, 1686–1699.
41 M. de Kort, R. C. Buijsman and C. A. A. van Boeckel, *Drug Discovery Today*, 2005, **10**, 769–779.
42 M. Petitou and C. A. A. van Boeckel, *Angew. Chem., Int. Ed.*, 2004, **43**, 3118–3133.
43 S. Arungundram, K. Al-Mafraji, J. Asong, F. E. Leach, I. J. Amster, A. Venot, J. E. Turnbull and G. J. Boons, *J. Am. Chem. Soc.*, 2009, **131**, 17394–17405.
44 F. Baleux, L. Loureiro-Morais, Y. Hersant, P. Clayette, F. Arenzana-Seisdedos, D. Bonnaffe and H. Lortat-Jacob, *Nat. Chem. Biol.*, 2009, **5**, 743–748.
45 M. Rawat, C. I. Gama, J. B. Matson and L. C. Hsieh-Wilson, *J. Am. Chem. Soc.*, 2008, **130**, 2959–2961.
46 S. Peterson, A. Frick and J. Liu, *Nat. Prod. Rep.*, 2009, **26**, 610–627.
47 Z. Q. Zhang, S. A. McCallum, J. Xie, L. Nieto, F. Corzana, J. Jimenez-Barbero, M. Chen, J. Liu and R. J. Lindhardt, *J. Am. Chem. Soc.*, 2008, **130**, 12998–13007.
48 C. Torres and G. Hart, *J. Biol. Chem.*, 1984, **259**, 3308–3317.
49 R. S. Haltiwanger, M. A. Blomberg and G. W. Hart, *J. Biol. Chem.*, 1992, **267**, 9005–9013.
50 L. Wells, Y. Gao, J. A. Mahoney, K. Vosseller, C. Chen, A. Rosen and G. W. Hart, *J. Biol. Chem.*, 2002, **277**, 1755–1761.
51 L. Wells, L. K. Kreppel, F. I. Comer, B. E. Wadzinski and G. W. Hart, *J. Biol. Chem.*, 2004, **279**, 38466–38470.
52 G. W. Hart, K. D. Greis, L. Y. Dong, M. A. Blomberg, T. Y. Chou, M. S. Jiang, E. P. Roquemore, D. M. Snow, L. K. Kreppel, R. N. Cole, F. I. Comer, C. S. Arnold and B. K. Haynes, *Adv. Exp. Med. Biol.*, 1955, **376**, 115–123.
53 J. J. Kohler, *Nat. Chem. Biol.*, 2010, **6**, 634–635.
54 C. Slawson and G. W. Hart, *Curr. Opin. Struct. Biol.*, 2003, **13**, 631–636.
55 C. F. Teo, S. Ingale, M. A. Wolfert, G. A. Elsayed, L. G. Nöt, J. C. Chatham, L. Wells and G.-J. Boons, *Nat. Chem. Biol.*, 2010, **6**, 338–343.
56 D. C. Love and J. A. Hanover, *Sci. STKE*, 2005, **2005**, re13-.
57 L. Wells and G. W. Hart, *FEBS Lett.*, 2003, **546**, 154–158.
58 L. Wells, S. A. Whelan and G. W. Hart, *Biochem. Biophys. Res. Commun.*, 2003, **302**, 435–441.
59 N. E. Zachara and G. W. Hart, *Biochim. Biophys. Acta, Gen. Subj.*, 2004, **1673**, 13–28.
60 M. K. Park, M. D'Onofrio, M. C. Willingham and J. A. Hanover, *Proc. Natl. Acad. Sci. U. S. A.*, 1987, **84**, 6462–6466.
61 C. M. Snow, A. Senior and L. Gerace, *J. Cell Biol.*, 1987, **104**, 1143–1156.
62 Y. Sakaidani, K. Furukawa and T. Okajima, in *Methods in Enzymology*, ed. F. Minoru, Academic Press, 2010, vol. Volume 480, pp. 355–373.
63 P. M. Clark, J. F. Dweck, D. E. Mason, C. R. Hart, S. B. Buck, E. C. Peters, B. J. Agnew and L. C. Hsieh-Wilson, *J. Am. Chem. Soc.*, 2008, **130**, 11576–11577.
64 N. Khidekel, S. B. Ficarro, E. C. Peters and L. C. Hsieh-Wilson, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 13132–13137.
65 B. J. Gross, B. C. Kraybill and S. Walker, *J. Am. Chem. Soc.*, 2005, **127**, 14588–14589.
66 D. J. Vocadlo, H. C. Hang, E.-J. Kim, J. A. Hanover and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 9116–9121.
67 C. Gurcel, A.-S. Vercoutter-Edouart, C. Fonbonne, M. Mortuaire, A. Salvador, J.-C. Michalski and J. Lemoine, *Anal. Bioanal. Chem.*, 2008, **390**, 2089–2097.
68 J. E. Rexach, C. J. Rogers, S. H. Yu, J. F. Tao, Y. E. Sun and L. C. Hsieh-Wilson, *Nat. Chem. Biol.*, 2010, **6**, 645–651.
69 Z. Wang, N. D. Udeshi, M. O'Malley, J. Shabanowitz, D. F. Hunt and G. W. Hart, *Mol. Cell. Proteomics*, 2010, **9**, 153–160.
70 D. Macmillan, R. M. Bill, K. A. Sage, D. Fern and S. L. Flitsch, *Chem. Biol.*, 2001, **8**, 133–145.
71 G. M. Watt, J. Lund, M. Levens, V. S. K. Kolli, R. Jefferis and G. J. Boons, *Chem. Biol.*, 2003, **10**, 807–814.

72 D. P. Gamblin, E. M. Scanlan and B. G. Davis, *Chem. Rev.*, 2009, **109**, 131–163.
73 B. G. Davis, *Pure Appl. Chem.*, 2009, **81**, 285–298.
74 P. E. Dawson, T. W. Muir, I. Clarklewis and S. B. H. Kent, *Science*, 1994, **266**, 776–779.
75 T. W. Muir, D. Sondhi and P. A. Cole, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 6705–6710.
76 D. Macmillan and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2004, **43**, 1355–1359.
77 N. Yamamoto, Y. Tanabe, R. Okamoto, P. E. Dawson and Y. Kajihara, *J. Am. Chem. Soc.*, 2007, **130**, 501–510.
78 K. Hirano, D. Macmillan, K. Tezuka, T. Tsuji and Y. Kajihara, *Angew. Chem., Int. Ed.*, 2009, **48**, 9557–9560.
79 C. Piontek, D. Varón Silva, C. Heinlein, C. Pöhner, S. Mezzato, P. Ring, A. Martin, F. X. Schmid and C. Unverzagt, *Angew. Chem., Int. Ed.*, 2009, **48**, 1941–1945.
80 C. Piontek, P. Ring, O. Harjes, C. Heinlein, S. Mezzato, N. Lombana, C. Pöhner, M. Püttner, D. Varón Silva, A. Martin, F. X. Schmid and C. Unverzagt, *Angew. Chem., Int. Ed.*, 2009, **48**, 1936–1940.
81 J. P. Richardson, C.-H. Chan, J. Blanc, M. Saadi and D. Macmillan, *Org. Biomol. Chem.*, 2010, **8**, 1351–1360.
82 J. Masania, J. Li, S. J. Smerdon and D. Macmillan, *Org. Biomol. Chem.*, 2010, **8**, 5113–5119.
83 A. Brik, Y. Y. Yang, S. Ficht and C. H. Wong, *J. Am. Chem. Soc.*, 2006, **128**, 5626–5627.
84 C. S. Bennett, S. M. Dean, R. J. Payne, S. Ficht, A. Brik and C. H. Wong, *J. Am. Chem. Soc.*, 2008, **130**, 11945–11952.
85 R. J. Payne, S. Ficht, S. Tang, A. Brik, Y. Y. Yang, D. A. Case and C. H. Wong, *J. Am. Chem. Soc.*, 2007, **129**, 13527–13536.
86 S. I. van Kasteren, H. B. Kramer, H. H. Jensen, S. J. Campbell, J. Kirkpatrick, N. J. Oldham, D. C. Anthony and B. G. Davis, *Nature*, 2007, **446**, 1105–1109.
87 Y. A. Lin, J. M. Chalker, N. Floyd, G. J. L. Bernardes and B. G. Davis, *J. Am. Chem. Soc.*, 2008, **130**, 9642.
88 J. M. Chalker, C. S. C. Wood and B. G. Davis, *J. Am. Chem. Soc.*, 2009, **131**, 16346–16347.
89 R. D. Astronomo and D. R. Burton, *Nat. Rev. Drug Discovery*, 2010, **9**, 308–324.
90 J. J. Mond, A. Lees and C. M. Snapper, *Annu. Rev. Immunol.*, 1995, **13**, 655–692.
91 J. Lue, M. Hsu, D. Yang, P. Marx, Z. Chen and C. Cheng-Mayer, *J. Virol.*, 2002, **76**, 10299–10306.
92 R. D. Astronomo, E. Kaltgrad, A. K. Udit, S. K. Wang, K. J. Doores, C. Y. Huang, R. Pantophlet, J. C. Paulson, C. H. Wong, M. G. Finn and D. R. Burton, *Chem. Biol.*, 2010, **17**, 357–370.
93 A. Kabanova, R. Adamo, D. Proietti, F. Berti, M. Tontini, R. Rappuoli and P. Costantino, *Glycoconjugate J.*, 2010, **27**, 501–513.
94 K. J. Doores, Z. Fulton, V. Hong, M. K. Patel, C. N. Scanlan, M. R. Wormald, M. G. Finn, D. R. Burton, I. A. Wilson and B. G. Davis, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 17107–17112.
95 R. D. Astronomo, H.-K. Lee, C. N. Scanlan, R. Pantophlet, C.-Y. Huang, I. A. Wilson, O. Blixt, R. A. Dwek, C.-H. Wong and D. R. Burton, *J. Virol.*, 2008, **82**, 6359–6368.
96 J. G. Joyce, I. J. Krauss, H. C. Song, D. W. Opalka, K. M. Grimm, D. D. Nahas, M. T. Esser, R. Hrin, M. Feng, V. Y. Dudkin, M. Chastain, J. W. Shiver and S. J. Danishefsky, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 15684–15689.
97 S.-K. Wang, P.-H. Liang, R. D. Astronomo, T.-L. Hsu, S.-L. Hsieh, D. R. Burton and C.-H. Wong, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 3690–3695.
98 U. M. Abdel-Motal, S. Wang, A. Awad, S. Lu, K. Wigglesworth and U. Galili, *Vaccine*, 2010, **28**, 1758–1765.
99 D. A. Calarese, C. N. Scanlan, M. B. Zwick, S. Deechongkit, Y. Mimura, R. Kunert, P. Zhu, M. R. Wormald, R. L. Stanfield, K. H. Roux, J. W. Kelly, P. M. Rudd, R. A. Dwek, H. Katinger, D. R. Burton and I. A. Wilson, *Science*, 2003, **300**, 2065–2071.
100 V. Pozsgay, *Curr. Top. Med. Chem.*, 2008, **8**, 126–140.
101 C. Mukherjee and A. Misra, *Glycoconjugate J.*, 2008, **25**, 111–119.
102 D. Safari, H. A. T. Dekker, J. A. F. Joosten, D. Michalik, A. C. de Souza, R. Adamo, M. Lahmann, A. Sundgren, S. Oscarson, J. P. Kamerling and H. Snippe, *Infect. Immun.*, 2008, **76**, 4615–4623.
103 C. Leung, A. Chibba, R. F. Gómez-Biagi and M. Nitz, *Carbohydr. Res.*, 2009, **344**, 570–575.
104 J. D. M. Olsson and S. Oscarson, *Tetrahedron: Asymmetry*, 2009, **20**, 875–882.
105 A. Phalipon, M. Tanguy, C. Grandjean, C. Guerreiro, F. Belot, D. Cohen, P. J. Sansonetti and L. A. Mulard, *J. Immunol.*, 2009, **182**, 2241–2247.
106 P. Simerska, H. Lu and I. Toth, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 821–824.
107 A. Bongat, R. Saksena, R. Adamo, Y. Fujimoto, Z. Shiokawa, D. Peterson, K. Fukase, W. Vann and P. Kováč, *Glycoconjugate J.*, 2010, **27**, 69–77.
108 S. Dziadek, S. Jacques and David R. Bundle, *Chem.–Eur. J.*, 2008, **14**, 5908–5917.
109 H. Xin, S. Dziadek, D. R. Bundle and J. E. Cutler, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 13526–13531.
110 T. Lipinski, P. I. Kitov, A. Szpacenko, E. Paszkiewicz and D. R. Bundle, *Bioconjugate Chem.*, 2010, DOI: 10.1021/bc100397b.
111 V. Verez-Bencomo, V. Fernandez-Santana, E. Hardy, M. E. Toledo, M. C. Rodriguez, L. Heynngnezz, A. Rodriguez, A. Baly, L. Herrera, M. Izquierdo, A. Villar, Y. Valdes, K. Cosme, M. L. Deler, M. Montane, E. Garcia, A. Ramos, A. Aguilar, E. Medina, G. Torano, I. Sosa, I. Hernandez, R. Martinez, A. Muzachio, A. Carmenates, L. Costa, F. Cardoso, C. Campa, M. Diaz and R. Roy, *Science*, 2004, **305**, 522–525.
112 C. Geraci, G. M. L. Consoli, E. Galante, E. Bousquet, M. Pappalardo and A. Spadaro, *Bioconjugate Chem.*, 2008, **19**, 751–758.
113 A. Miermont, H. Barnhill, E. Strable, X. Lu, K. A. Wall, Q. Wang, M. G. Finn and X. Huang, *Chem.–Eur. J.*, 2008, **14**, 4939–4947.
114 O. Renaudet, L. BenMohamed, G. Dasgupta, I. Bettahi and P. Dumy, *Chem Med Chem*, 2008, **3**, 737–741.
115 Q. Wang, S. A. Ekanayaka, J. Wu, J. Zhang and Z. Guo, *Bioconjugate Chem.*, 2008, **19**, 2060–2067.
116 S. Bay, S. Fort, L. Birikaki, C. Ganneau, E. Samain, Y.-M. Coïc, F. Bonhomme, E. Dériaud, C. Leclerc and R. Lo-Man, *Chem Med. Chem*, 2009, **4**, 582–587.
117 I. Bettahi, G. Dasgupta, O. Renaudet, A. Chentoufi, X. Zhang, D. Carpenter, S. Yoon, P. Dumy and L. BenMohamed, *Cancer Immunol. Immunother.*, 2009, **58**, 187–200.
118 S. Ingale, M. A. Wolfert, T. Buskas and G.-J. Boons, *Chem Bio Chem*, 2009, **10**, 455–463.
119 I. Jeon, K. Iyer and S. J. Danishefsky, *J. Org. Chem.*, 2009, **74**, 8452–8455.
120 E. Khatuntseva, Y. Tsvetkov, R. Stepanenko, R. Vlasenko, R. Petrov and N. Nifantiev, *Russ. Chem. Bull.*, 2009, **58**, 450–456.
121 D. Lee and S. J. Danishefsky, *Tetrahedron Lett.*, 2009, **50**, 2167–2170.
122 P. Nagorny, W. H. Kim, Q. Wan, D. Lee and S. J. Danishefsky, *J. Org. Chem.*, 2009, **74**, 5157–5162.
123 T. E. Newsom-Davis, D. Wang, L. Steinman, P. F.-T. Chen, L.-X. Wang, A. K. Simon and G. R. Screaton, *Cancer Res.*, 2009, **69**, 2018–2025.
124 G. Ragupathi, P. Damani, G. Srivastava, O. Srivastava, S. Sucheck, Y. Ichikawa and P. Livingston, *Cancer Immunol. Immunother.*, 2009, **58**, 1397–1405.
125 J. Zhu, Q. Wan, D. Lee, G. Yang, M. K. Spassova, O. Ouerfelli, G. Ragupathi, P. Damani, P. O. Livingston and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2009, **131**, 9298–9303.
126 C. L. Brooks, A. Schietinger, S. N. Borisova, P. Kufer, M. Okon, T. Hirama, C. R. MacKenzie, L.-X. Wang, H. Schreiber and S. V. Evans, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 10056–10061.
127 O. Renaudet, G. Dasgupta, I. Bettahi, A. Shi, A. B. Nesburn, P. Dumy and L. BenMohamed, *PLoS One*, 2010, **5**, e11216.
128 A. Kaiser, N. Gaidzik, U. Westerlind, D. Kowalczyk, A. Hobel, E. Schmitt and H. Kunz, *Angew. Chem., Int. Ed.*, 2009, **48**, 7551–7555.
129 A. Hoffmann-Röder, A. Kaiser, S. Wagner, N. Gaidzik, D. Kowalczyk, U. Westerlind, B. Gerlitzki, E. Schmitt and H. Kunz, *Angew. Chem., Int. Ed.*, 2010, **49**, 8498–8503.
130 B. L. Wilkinson, S. Day, L. R. Malins, V. Apostolopoulos and R. J. Payne, *Angew. Chem., Int. Ed.*, 2011, **50**, 1635–1639.
131 B. L. Wilkinson, L. R. Malins, C. K. Y. Chun and R. J. Payne, *Chem. Commun.*, 2010, **46**, 6249–6251.
132 S. Sarkar, S. A. Lombardo, D. N. Herner, R. S. Talan, K. A. Wall and S. J. Sucheck, *J. Am. Chem. Soc.*, 2010, **132**, 17236–17246.
133 F. Yang, X.-J. Zheng, C.-X. Huo, Y. Wang, Y. Zhang and X.-S. Ye, ACS Chemical Biology, 201010.1021/cb100287q.
134 S. Sahabuddin, T.-C. Chang, C.-C. Lin, F.-D. Jan, H.-Y. Hsiao, K.-T. Huang, J.-H. Chen, J.-C. Horng, J.-a. A. Ho and C.-C. Lin, *Tetrahedron*, 2010, **66**, 7510–7519.

