Synthesis of oligosaccharide libraries from GBS capsular polysaccharides for structure-based selection of vaccine candidates

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Chapter 1

Synthetic glycoconjugate vaccines

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Introduction

Despite the enormous progress achieved by modern medicine, numerous diseases still have a profound impact on public health. Infectious pathologies are a global major concern both in industrialized and developing countries, and the rise of multidrug-resistant bacteria is a great concern. Vaccines are considered among the most cost-effective ways to prevent morbidity and mortality from infectious diseases. Over the last 50 years vaccines have greatly contributed to improve human health by controlling, and in some cases eradicating, many infectious diseases, both viral (for example, smallpox, measles and polio) and bacterial (for example, diphtheria and tetanus), that were the cause of many deaths and disability in the 20th century.

General principles of vaccination

The immune system is an important defense mechanism for our organism, being able to recognize infected cells (*non-self*) and trigger an immune response against them. The immune response can be divided into innate and adaptive immunity. The first fights intruding pathogens in a rapid and non-specific way and can be considered a first line of defense. Onset of adaptive immunity is delayed but directed specifically against the respective intruder. A multitude of pathogen-specific cells are created by clonal expansion of few precursor cells. Once the infection is resolved, some of these cells survive in the body to form immunological memory ready to fight the same pathogen faster when a second encounter occurs. Vaccination takes advantage of the adaptive immunity.
Indeed, the principle of vaccination is that exposure to a small sample of disease-causing microorganism (or to a part or portion of it) teaches the immune system to rapidly recognize the menace and to create memory that enables the body to fight efficiently the real pathogen during a later encounter. Vaccination induces an immune response in the host that leads to the production of immune effectors called antibodies, which are produced by B lymphocytes and are capable to recognize and specifically bind to a toxin or a pathogen (or to a portion representative of it)[1].
Two key players of the immune system are B and T lymphocytes, which both are provided with receptors to recognize specific antigens. When cell-antigen interactions occur, B-lymphocytes generate plasma cells and secrete specific antibodies against a particular antigen. B-lymphocytes are responsible for the humoral response, while T-lymphocytes produce T-cytotoxic and T-helper cells, involved in both humoral and cell-mediated immunity. Cooperation of B- and T-lymphocytes leads to the formation of memory B cells. When a second exposure to the same antigen takes place, memory B cells generate specific antibodies with particular combinatory sites capable of binding the structural-complementary antigens. They usually only recognize a small part of the molecule, called epitope. The activation of a B cell to create memory B cells requires the participation of macrophages or T-helper cells and this can only be stimulated by T-dependent (TD) antigens, like proteins. Following interaction with antigen-presenting cells (APC) like dendritic cells, macrophages and B-cells, protein antigens are internalized and processed into

small peptides which are then re-exposed and presented to T-lymphocytes in association with the MHC class II molecules. Interaction with T cells induces B cells to differentiate into plasma cells and memory B cells, thus initiating downstream adaptive immune responses. Unlike T-independent antigens, TD antigens are immunogenic early in infancy, the immune response induced can be boosted, is enhanced by adjuvants and is characterized by antibody class switch and production of antigen-specific IgG. The long polysaccharide chains found on bacterial capsule are T-independent antigens as they do not require T-cell activation for the induction of specific B-cell (antibody) response. Polysaccharide antigens directly activate polysaccharide-specific B cells which differentiate then into plasma cells to produce antibodies, but memory B cells are not formed. Moreover a pre-existing Memory B-cell pool can be depleted by immunization with unconjugated polysaccharide, with risk of hypo-responsiveness on subsequent immunizations[2].

Polysaccharide-based and glycoconjugate vaccines

Polysaccharides are important virulence factor especially for encapsulated bacteria that present these carbohydrate structure on their surface complex.

Around the 1930s the protective role of antibodies (Abs) induced by pneumococcal polysaccharides started to be investigated and in 1945 the first vaccine composed of purified polysaccharides from selected pneumococcal serotypes was tested in humans[3].

The research on vaccines development was subsequently slowed down by the introduction of antibiotics. However, with the emergence of drug resistant strains, the development of polysaccharide vaccines started again and several of them have been studied in large clinical studies. Polysaccharide vaccines against meningococcus serogroups ACWY, *Streptococcus pneumoniae* and *Haemophilus influenzae* type b were licensed between the seventies and the eighties[4, 5]. These bacteria possess polysaccharides which are either polymers formed by one monosaccharide unit (homopolymers) or more complex oligosaccharide repeats (heteropolymers), that can be charged or neutral.

Polysaccharides, although efficacious in adults, fail to evoke immunological memory or long-lived antibody production as they are T-independent antigen. Conjugate vaccines, obtained by covalent linkage of bacterial polysaccharides to carrier proteins, have allowed to overcome limitations of unconjugated polysaccharide vaccines.

The T cell help provided by protein epitopes present in glycoconjugates imparts the capacity to induce a long lasting and boostable IgG antibody production to the appended carbohydrates also in children below the age of two[6, 7]. Also, vaccination with glycoconjugates has been proven more effective than the one with polysaccharides in adult population[8, 9]. Finally, conjugate vaccines have been shown to reduce carriage or impact on transmission of meningococci, while there is some evidence that plain polysaccharide vaccines cannot[10].

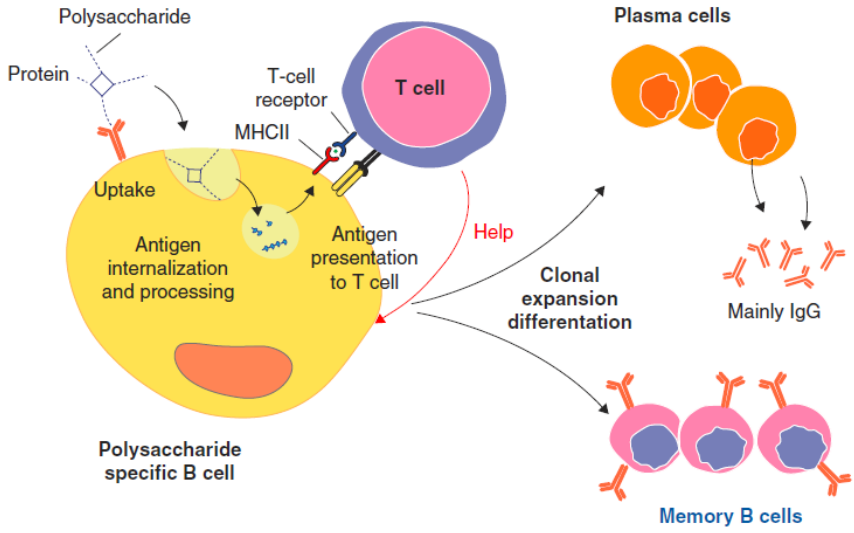

Figure 1. Mechanism of action of glycoconjugates vaccines[2]

Even though Avery and Goebel demonstrated already in 1929 that non-immunogenic sugar become able to induce antibodies in animal model after conjugation to a carrier protein, the first application of this concept to a vaccine for human use started in 1980 with the development of the first glycoconjugate vaccine against *Haemophilus influenzae* type b (Hib)[11]. Since then, glycoconjugates have been proven efficacious and cost effective to combat many life-threatening bacteria, such as *Streptococcus pneumoniae* (23 serotypes)[12], *Neisseria meningitidis* (A, C, W and Y)[13] and *Salmonella Typhi*[14-16].

A list of licensed glycoconjugate vaccines is reported in Table 1.

Table 1. Licensed glycoconjugate vaccines

Vaccine indication	Type of conjugate	Manufacturer
Haemophilus influenzae type b	PRP-TT	Sanofi-Pasteur
	PRP-OMPC	Merck
	PRP-CRM_{197}	Pfizer
	Hib-CRM_{197}	GSK
Haemophilus influenzae type b/Neisseria meningitidis group C	MenC/Hib-TT	GSK
Neisseria meningitidis serogroups $ACW_{135}Y$	MenA-TT	Serum Institute India
	MenC-CRM_{197}	Pfizer, GSK
	MenC-TT	Baxter
	MenACWY-DT	Sanofi-Pasteur
	MenACWY-CRM_{197}	GSK
	MenACWY-TT	Pfizer
Streptococcus pneumoniae serotypes	7 valent-CRM197 (4, 6B, 9V, 14, 18C, 19F, 23F)	Pfizer
	13 valent-CRM197 (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F)	Pfizer
	10 valent-DT/TT Protein D (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F)	GSK

Classical approaches for glycoconjugate vaccines preparation

As for other classes of vaccines, development of glycoconjugate vaccines is a long, complex and economically demanding process[17], that can last 10-15 years[18]. Increased complexity of future vaccines and need of reduced time to release have guided towards the development of faster approaches for glycoconjugate vaccines design.

Traditionally, polysaccharides are obtained by bacterial growths followed by harvesting and several purification steps, differing according to the nature of the carbohydrate (length, charge, necessity to preserve substitution patterns).

With long polysaccharides, chemical conjugation to the carrier protein is performed through random linkage along the saccharide chain, resulting in the formation of very high molecular weight cross-linked and rather undefined and heterogeneous structures[2, 19, 20]. Six proteins are used as carriers in licensed vaccines: Tetanus toxoid (TT), Diphtheria toxoid (DT), CRM_{197}, the Outer Membrane Protein Complex of meningococcus B (OMPC), Protein D from *H. influenzae* and recombinant Exotoxin A of *P. aeruginosa*[2, 21, 22].

Glycan-protein conjugation can take place using the carbohydrate functional moieties (e.g. hydroxyl, carboxyl groups) or alternatively derivatizing the polysaccharide with linkers to allow insertion of specific functionalities (e.g. thiols, bromide) and reduce the steric hindrance between protein and saccharide. One of the most employed transformations for conjugation entails the use of a $NaIO_4$ oxidation which generates aldehydes from *cis*-diols. These chemical groups can be directly linked to the ε-amine of the protein lysine residues by reductive amination, or further derivatized before linkage to the protein[23-26]. CDAP chemistry (1-Cyano-4-dimethylaminopyridinium tetrafluoroborate) is also randomly applied to commonly use hydroxyl groups of polysaccharides for condensation with the amines of the protein[27]. Recently, pneumococcal polysaccharides, pre-activated with sodium periodate, have been conjugated to CRM_{197} carrier protein by an in vacuo glycation method, and capped using sodium borohydride to prepare a 13-valent vaccine which was shown to give an immune response comparable to Prevnar for 10 out of 13 serotypes[28]. Dry-glycation (or in vacuo glycation) is a water free conjugation method consisting of colyophilization of the protein with the activated sugar, and then subjecting the lyophilized mixture to a vacuum at high temperature.

On a manufacture standpoint, use of shorter oligosaccharides facilitates consistency of production, sterile filtration, purification of the resulting conjugate, particularly from unreacted polysaccharide, and its characterization. Oligosaccharides can be linked to the protein through the end reducing sugar directly, or via a spacer. Production of well-defined and uniform lower molecular weight polysaccharide populations is typically achieved by chemical or mechanical polysaccharide fragmentation followed by size fractionation[29-31]. Depending on the polysaccharide structure, $NaIO_4$ oxidation results in simultaneous fragmentation and generation of terminal aldehyde groups available for conjugation (e.g. *H. influenzae* type b CPS). Alternatively, the polysaccharides can be fragmented by treatment with hydrogen peroxide[32] or via acid hydrolysis, and sized by chromatography or ultrafiltration techniques to isolate populations more defined in length for conjugation[31, 33]. Individual bacterial strains may also express a wide range of saccharide sizes, as it is usually the case of *O*-polysaccharides, that, in some instances, may not include the desired target size for vaccine development. Also, in this case, isolation of the sugar with desired length is required, impacting on overall yield and complexity of the process.

Synthetic and enzymatic approaches for glycoconjugate vaccines

Although isolation of polysaccharides from bacterial growth is the primary approach used for manufacturing licensed vaccines, approaches avoiding large scale pathogen fermentation have emerged over the last decades[34]. Specifically, organic synthesis has shown it can meet the demand of bacterial related oligosaccharides, with the advantages of a more defined structure, ease of characterization, lack of bacterial contaminants, higher batch-to-batch reproducibility, and more robust correlation of the elicited immune response with the oligosaccharide chemical structure[35]. Feasibility of large scale production of synthetic carbohydrates for vaccine manufacturing has been shown for the *H. influenzae* type b Quimi-Hib vaccine[36], which was produced by a polymerization approach based on H-phosphonate chemistry. Recently, a synthetic oligosaccharide based vaccine to combat *S. flexneri* serotype 2a has been tested in Phase 1 clinical trials[37]. Despite these examples, chemical synthesis of oligosaccharides remains challenging and time consuming, especially compared to the synthesis of other biopolymers such as peptides and oligonucleotides. The difficulty of oligosaccharides assembly is due to the presence of multiple hydroxy groups on the sugar monomers that must be discriminated during the synthesis; moreover, the union of two saccharides through formation of a new glycosidic linkage generates a new stereocenter[38]. A variety of methods, both in solution and in solid phase, have been pursued to accelerate glycans assembly and provide faster access to oligosaccharide well-defined structures[39].

One-pot multi-step strategies (Figure 2A) to assemble complex oligosaccharides integrate several glycosylation steps into one synthetic operation to furnish target oligosaccharides in a short period of time and without the need for protecting group manipulation and intermediate isolation[40, 41]. Databases based on the relative reactivities values (RRVs) of a variety of building blocks have been developed to aid the generation of the target oligosaccharides using reactivity guided synthesis design[42].

Automated synthetic approaches have now become available (Figure 2B). Starting from an adapted peptide synthesizer, via multiple home-built systems, different versions of the Glyconeer automated synthesizer (currently 2.1) have been developed to assemble synthetic glycans of increasing length and complexity by repeating cycles of glycosylation, capping and deprotection steps on a polystyrene Merrifield resin[43-45]. This system has been shown applicable for multimilligram synthesis of diverse glycans, varying from a homopolymeric mannans up to a 30-mer[46] to complex structures, such as alginates forming the *P. aeruginosa* exopolysaccharide featuring the chemically challenging 1,2-*cis*-mannosidic bond[47], as well as biologically relevant oligosaccharides bearing various *cis*-galactosidic and *cis*-glucosidic linkages[45].

Automation is also feasible via a HPLC system[48] (Figure 2C) or a combination of automated solution phase synthesis and use of fluorous tags[49].

At the moment, fragments obtained by automated glycan assembly (AGA) or other fast assembling procedures are prepared in a quantity sufficient for structural characterization through

methods such as Saturation Transfer Difference Nuclear Magnetic Resonance (STD-NMR), Surface Plasmon Resonance (SPR), or X-ray crystallography which individually or in combination allow selection of structures to be further investigated in animal models as vaccine candidates, but we are still far from the scale required for process development. Among the techniques to study interactions of carbohydrates with protective antibodies, glycan microarray enables fast screening of synthesized glycans against animal or human antibodies requiring minimal quantities of analytes[34]. The combined approach of AGA and glycoarray has led to identification of crucial epitopes from polysaccharide antigens and to the selection of synthetic vaccine candidates. For this reason, it has been shown as a valid and rapid alternative to surface polysaccharide isolation for many glycan antigens, including capsular polysaccharide from carbapenem resistant *K. pneumoniae*[50], *Clostridium difficile* PS-I[51], and *S. aureus* teichoic acids[52]. AGA has been also applied to *S. pneumoniae* vaccine development, in particular to the identification of optimal oligosaccharide epitopes from different serogroups of *S. pneumoniae* which are not included in the currently marketed anti-pneumococcal vaccines[53-55]. The synthetic approach has been recently shown able to provide antigens for the formulation of a pentavalent glycoconjugate vaccine comprising *S. pneumoniae* serogroups 2, 3, 5, 8 and 14. Such synthetic formulation elicited antibodies in rabbits with strong opsonophagocytic activity. Furthermore, coformulation of the anti-pneumococcal licensed vaccines Prevnar-13 and Synflorix with synthetic glycoconjugates from serotypes currently not covered by vaccination proved effective and led to improved 15- and 13-valent vaccines, respectively[56].

Modern synthetic techniques are, therefore, becoming powerful tools to accelerate identification of carbohydrate antigens without the need for polysaccharide purification from a biological matrix.

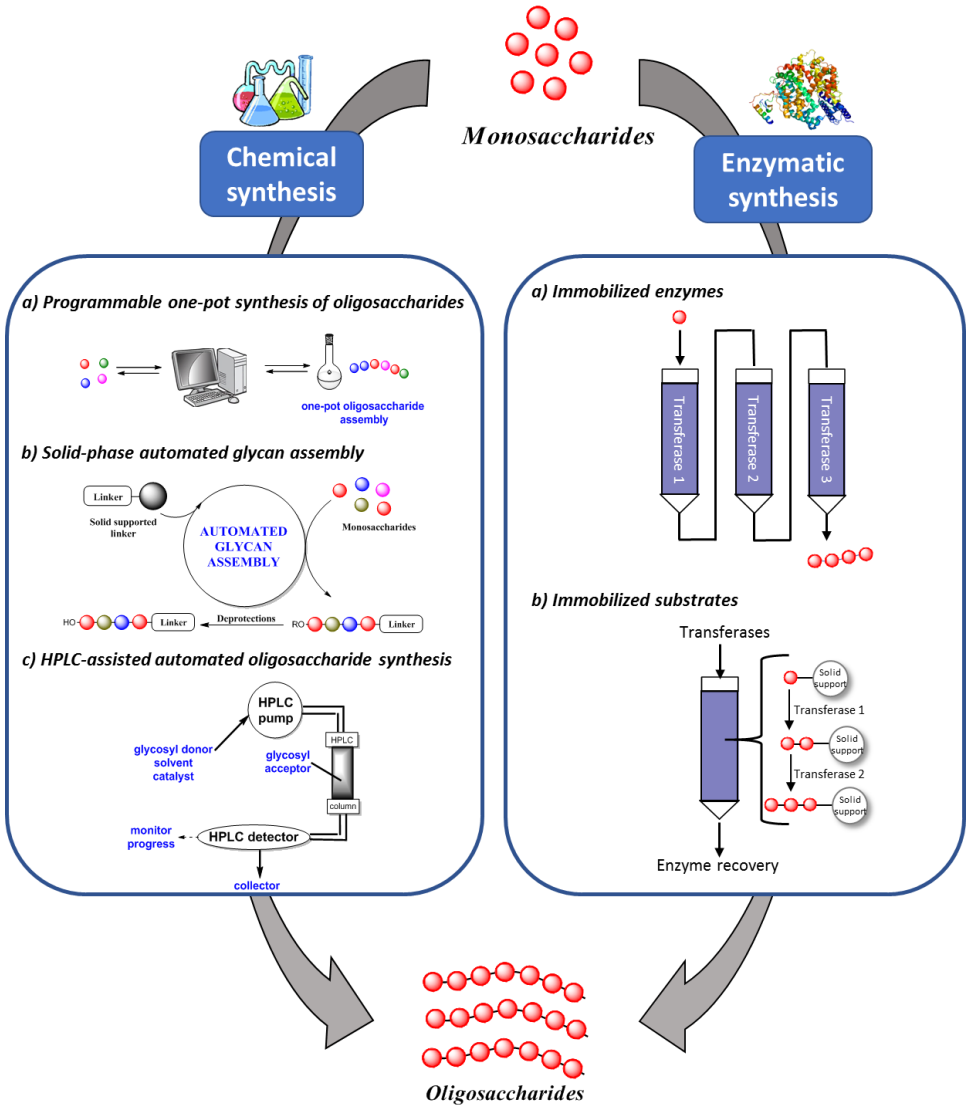

Figure 2. Automated chemo-enzymatic approaches to oligosaccharide antigens preparation

Enzyme-catalyzed oligosaccharide assembly is an interesting alternative to chemical synthesis of complex bacterial glycans since enzymatic glycosylation by glycosyltransferases allows stereo- and regioselectivity without the need to undergo tedious protecting group manipulation. Enzymatic reactions occur in aqueous solutions and only require to control a few reaction conditions such as temperature, system pH, and in certain cases metal catalysis. Also, there are no toxic byproducts produced in this glycosylation process, making the enzymatic synthesis of oligosaccharides an environmentally friendly approach[57]. These advantages make enzyme-based carbohydrate production attractive for industrialization.

A first automated carbohydrate synthesizer employing enzymatic synthesis has recently been reported[58] (Figure 2).

Recent progress made with the cloning and functional expression of capsule polymerases and pioneering studies that demonstrate the suitability of recombinant enzymes for the *in vitro* production of capsular polysaccharides (CPS) have opened new perspectives for the economic and safe production of conjugate vaccines[59, 60]. For example, Gerardy-Schahn's and coworkers have developed enzyme-based approaches focusing on the synthesis of meningococcal capsular polysaccharides. In 2016, they described a scalable *in vitro* synthesis of CPS serogroup X from a chemically pure precursor using recombinant *Neisseria meningitidis* X capsule polymerase[61].

As for serogroup X, a soluble version of the polymerase has been developed also for *in vitro* synthesis of *N. meningitidis* serogroup A polysaccharide[59].

A chemoenzymatic method using recombinant sialyl-transferases to develop well-defined glycoconjugates has been reported for the *N. meningitidis* serogroup C by Vann's group[62, 63].

The biosynthesis of the other two relevant *N. meningitidis* polysaccharides, serogroup W and Y, involves different catalytic strategies for sialic acid and hexose transfer, and enzymes responsible for these processes have been identified[64, 65]. Therefore, fully-chemoenzymatic manufacturing of polysaccharides needed for MenACWY vaccine is potentially feasible.

The combination of chemical and enzymatic methods can also be exploited for fast and efficient production of oligosaccharides as vaccine components. An example of this combined strategy is the use of a sialyltransferase isolated from rat liver to achieve a double α-sialylation at the position 3 of galactose in a near quantitative yield for the assembly of dimeric repeating units of the type III Group B *Streptococcus* polysaccharide[66]. Production of the pentasaccharide hapten for a vaccine candidate against endemic shigellosis has also been shown feasible by combined chemical and enzymatic steps[67].

Currently, two main approaches are under investigation for the automated enzyme-mediated bacterial oligosaccharide synthesis, using immobilizing enzymes or substrates (Figure 2) on an appropriate support or resin[68]. Chemical and enzymatic steps have been combined for the preparation of *N. meningitidis* serogroup X fragments[69, 70] by using immobilized enzyme. Starting from a fully synthetic trisaccharide acceptor and a truncated immobilized construct of *N. meningitidis* X capsular polymerase (CsxA), an on-column method was developed to provide rapidly conjugation-ready oligosaccharides with a size optimal for immunogenicity[70]. Enzyme-immobilization is a mature technology but it has the limitation that only immobilized enzymes can be removed at the end of the sugar elongation process, while the oligosaccharide product is still mixed with other reagents[57]. To overcome this, a different strategy has been recently developed where glycosyltransferase-catalyzed reactions are performed to generate oligosaccharides equipped with sulfonate tags for purification by a selective catch and release from adequate resins[58]. Although this method has been applied so far only to mammalian glycans, it has the potential to be extended to microbial oligosaccharides.

Automated chemical and enzymatic synthesis appear highly complementary and the combination of both will greatly expand the chemical space that can be rapidly accessed in the coming years. While in the absence of structural information, long oligosaccharides have to be employed to fully cover all of the relevant glycotopes, an understanding of the structural basis for the immune recognition of carbohydrate epitopes and the elucidation of minimal saccharide antigens responsible for an effective immune response is relevant to support the rational design of modern glycoconjugate vaccines with short oligosaccharides obtained by synthetic, chemoenzymatic, or bioengineering methods[71, 72]. An interesting concept that could simplify the use of synthetic carbohydrates is the identification of glycotopes expressed across multiple microorganisms. This is the case of Poly-N-acetyl glucosamine (PNAG), a polysaccharide found in a large number of bacterial, fungal and eukaryotic pathogens[73]. Natural antibodies to PNAG are poorly effective at mediating *in vitro* microbial killing or *in vivo* protection. However, synthetic deacetylated glucosamine 5-mer or 9-mer have been shown to elicit, after conjugation to carrier proteins, broad-based immunity (among others, *Mycobacterium tuberculosis*, *Burkholderia cenocepacia*, *A. baumannii*, *N. meningitidis* serogroup B, *Streptococcus pyogenes*)[74]. The safety profile and potential interactions with microbiota and off-target toxicity in humans, however, are aspects that still need to be clarified for this broadly active conjugate.
A synthetic lipoteichoic acid fragment from *Enterococci faecalis* and *faecium* has also been shown to induce functional and cross-reactive antibodies against *S. aureus*, suggesting that this class of molecules can induce broadly protective response across different Gram-positive bacteria[75].

Perspectives

Glycoconjugate vaccines have been proven cost-effective tools to prevent dramatic infectious diseases such as pneumonia and meningitis. Historically they have been developed to overcome limitations of plain polysaccharides and confer protection and boostable response in children. Lifecyle management of existing vaccines requires incessant monitoring of possible serotype replacement and mutable epidemiology which can demand increased vaccine coverage. In addition, the use of an increased number of glycoconjugate vaccines with a relatively small number of carrier proteins (primarily TT, DT and CRM_{197}) and the complexity of the immunization schedules have highlighted in certain cases reduced immunogenicity against the polysaccharide hapten due to preexisting immunity towards the protein (so called "carrier epitope suppression")[76]. Technologies enabling the use of alternative carriers in conjunction with simplified vaccine formulations are, therefore, requested for the future vaccines.
Several additional factors encourage the development of novel and faster methods for glycoconjugates design and production. A large part of the world composed is constituted by low and middle-income countries, where infectious diseases account for around half of all deaths with around 90% of this mortality being attributed to diarrheal and respiratory diseases, AIDS, tuberculosis and malaria[77]. Methodologies for fast and affordable vaccine production combined

with sustainable models for capacity building and technology transfer are necessary to render vaccination accessible to these populations. The success of the MenAfriVac vaccine and program for extension to a multivalent ACWXY conjugate is generating a model for development and implementation of vaccination in low income countries[78].

Also, the need of novel therapeutic approaches to tackle antimicrobial resistant pathogens has been clearly underscored by recent analyses from WHO and CDC[79, 80]. In addition, fungal infections are a concern for immunocompromised patients, such those with immune systems impaired by cancer chemotherapy, or hospitalized individuals[81]. Finally, increased life expectation in the developed countries and increased numbers of travelers are also expanding the request for vaccination of adult and elder population in the developed countries[82].

Among the emerging approaches for carbohydrate production, chemical and enzymatic strategies, and their automation, hold the potential to enable the pathogen-free production of carbohydrates and rationally selected glycoepitopes for vaccine design. These methodologies are now giving access to a large variety of complex glycans including those deriving from pathogen for which vaccines are not available (e.g. *S. flexneri* type 2a O-antigen, *K. pneumoniae* CPS, *S. aureus* PNAG). Advancement of mass spectrometry approaches for sugar fingerprint analysis coupled with automated chemical or enzymatic synthesis is expected to further progress the field in the near future[83]. Proof of feasibility for a multivalent pneumococcal conjugate vaccine based on synthetic oligosaccharides has been recently shown. Nevertheless, these methods at the present still require rather extensive case-by-case optimization of the synthetic strategy and large-scale production might constitute a challenge for multivalent vaccines. Therefore, effort is expected to focus in adapting these novel approaches for manufacturing.

As an emerging technology, combination of nanoparticle scaffolds with chemical and enzymatic methodologies for carbohydrate synthesis appears very appealing in the design of totally synthetic constructs. Whereas this approach is at a very early stage of development, it could represent the future frontier of pathogen contaminant-free, highly characterized, totally synthetic carbohydrate-based vaccines. Its advancement will depend on how quickly accessible the synthetic carbohydrate production will be, as discussed above, and how consistent the manufacturing for large scale production will be.

In parallel with these novel technologies to obtain glycoconjugate vaccines, advancements of techniques for deciphering surface polysaccharide structure[84] and carbohydrate-protein interactions[85], as well as progresses in the field of analytics and immunoassays, including novel tools and high-throughput methodologies for rapid analysis of sera specificity[86] and functional activity[87, 88], are contributing to make antigen identification and structure guided vaccine design faster.

Outline of the thesis

This Chapter has provided an overview of glycoconjugate vaccines, generally describing their mechanism of action compared to polysaccharide-based vaccines. Classical approaches to produce glycoconjugate vaccines were described, with a focus on chemical and enzymatic methods to provide fast access to synthetic oligosaccharide antigens.
In **Chapter 2** Group B streptococcus (GBS) epidemiology is outlined, underlining the correlation between different serotypes and clinical symptoms to highlight the need for a multivalent vaccine. The synthesis of GBS repeating units from different serogroups is also reported, showing feasibility of a GBS conjugate vaccine based on synthetic oligosaccharides. In **Chapter 3** a regioselective glycosylation approach to the disaccharide synthon GlcNAcβ(1→3)Galβ is introduced, with the aim to obtain branched oligosaccharides using fewer protecting groups and therefore reducing the number of steps to the final target compounds. In particular, the impact of the protecting groups on the glycosyl acceptor and donor as well as the leaving group on the glycosyl donor were evaluated and a combination of building blocks to achieve a fully regioselective glycosylation was identified. Disaccharide synthons bearing temporary protecting groups at C-3 and C-4 were synthesized to be used as intermediates for the synthesis of GBS type Ia and Ib related glycans. In **Chapter 4** the appropriate disaccharide synthon prepared through regioselective glycosylation was used as starting material for elongation to obtain a panel of GBS Ia related oligosaccharides, ranging from a linear tetrasaccharide up to a heptasaccharide to be used for epitope mapping of relevant glyco epitopes of the corresponding polysaccharide. Each fragment was prepared with an aminopropyl linker at the terminal sugar for future conjugation to carrier proteins and immunological studies. The regioselective reaction to the GlcNAcβ(1→3)Galβ disaccharide also has a central role in **Chapter 5**, where the first synthesis of GBS type Ib repeating units, represented by two frameshifts, a linear and a branched structure, is described. These structures differ from GBS type Ia glycans only for the connection of the side chain Neu5Acα(2→3)-Galβ to the glucosamine, which is a β(1→3) linkage instead of β(1→4) as in type Ia. To overcome low reactivity of the glucosamine 3-OH, the bulky, electron withdrawing NPhth protecting group on the glucosamine was replaced by the Troc group and a more flexible silylidene ring was inserted to block glucosamine 4- and 6-hydroxyls. The synthetic repeating units of GBS Ia and Ib were used to validate a structural model aiming to elucidate the conformation of the corresponding polysaccharides. In **Chapter 6**, structural studies on the panel of Ia and Ib related fragments synthesized are reported. In particular, the synthetic fragments were used as inhibitors in competitive Surface Plasmon Resonance experiments of the binding of PSIa and Ib to the specific mAbs. To investigate the interactions between the glycans and the mAb better, STD-NMR experiments were undertaken. Finally, the fragments were conjugated to carrier proteins for immunogenicity evaluation *in vivo*. **Chapter 7** presents the synthesis of a hexasaccharide from GBS type III CPS corresponding to the minimal epitope recognized by a protective mAb according to the published crystal structure of the derived Fab with a

semisynthetic DP2 fragment. This oligosaccharide was first used to map the relevant interactions between the sugar and the mAb, and then conjugated to CRM_{197} and tested in vivo. Immunological results showed that the hexasaccharide was able to elicit an IgG titer comparable to the CRM_{197}-PSIII conjugate, directly related to a high glycosylation degree. **Chapter 8** summarizes the findings of this thesis and outlines future plans and strategies.

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