Bacterial glycomimetics: synthesis and applications

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Conclusions and future prospects

This work has described synthetic strategies towards well-defined structures resembling capsular polysaccharide (CPS) fragments, CPS mimics, teichoic acid (TA) fragments as well as a third-generation ring-closing tandem metathesis (RCM) linker to better exploit the potential of automated synthesis. The synthesis of diheteroglycan (DHG) fragments of the Gram-positive *Enterococcus faecalis* has been described and preliminary biological properties shown. CPS-mimics of *Neisseria meningitidis* A, a Gram-negative bacterium and one of the major causes of bacterial meningitis, could provide an alternative to the inherent instability of current glycoconjugate vaccines. TA fragments presented in this thesis, typical for Gram-positive bacteria, have been equipped with labile D-alanine appendages to further investigate their biological relevance in interactions with the host immune system. A tandem-RCM linker has been developed and tested on a carbohydrate automated synthesizer, providing a proof of concept of its use.

Chapter 1 has summarized approaches towards glycoconjugate vaccine production, from the antigen production to its conjugation to the carrier protein, with a particular focus on semi-synthetic conjugate production, showing principal strategies for the production of the antigen part and on the choice of the protein.

Chapter 2 has described the efficient synthesis of "carba-analogues" of the *Neisseria meningitidis* type A CPS, with the implementation of acetyl decorations on the C-3-OH of the carba-mannoside, which has been shown to be fundamental for inducing a strong immune response. Generated fragments, through iterative phosphoramidite couplings, up to 10 repeating units have been successfully synthesized and the carba-3OAc octamer has been used in competitive SPR experiment and compared with a native CPS fragment with an average degree of polymerization of 15 and the native CPS, showing strong inhibition of the binding between anti-MenA mAb and MenA CPS immobilized on the chip, albeit to a lesser extent as the native structures. These results should still be considered an improvement from previous work done, as the IC_{50} value of the carba-3OAc octamer vastly improved compared to its non-acetylated counterpart described in a recent paper by Codée, Lay and Adamo.[1] In addition, the carba-3OAc octamer has been conjugated to CRM_{197} as carrier protein to investigate its ability to elicit an immune response specific against MenA bacteria in mice. In order to get further insight on the

role of acetyl appendages a new set of carba analogues with different acetylation patterns could be synthesized and this is shown in Chapter 3 this thesis.

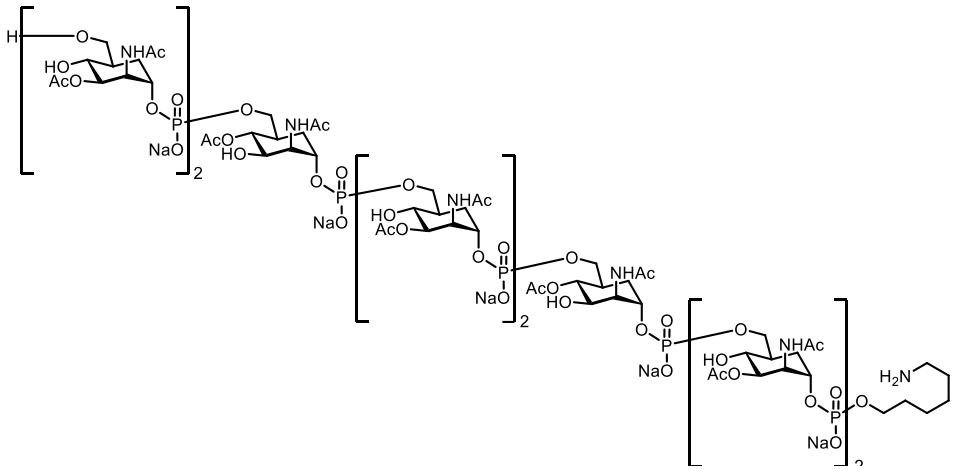


Figure 1 3-4OAc carba octamer structure

Chapter 3 describes a novel strategy to obtain carba-MenA analogues building blocks, acetylated on the C-4-OH and their implementation in the synthetic strategy to obtain fragments up to 8 repeating units with a well-defined acetylation pattern (Figure 1), inspired by recent results described by Adamo and co-workers.[2] The IC_{50} value of these structures was evaluated by performing competitive SPR experiment. Immunization experiments will be needed to further substantiate these results. A lot of small, but progressive steps have been undertaken on this ambitious project: from the idea of replacing the endocyclic oxygen of the mannoside with a carbon atom to stabilize the overall structure in water, the first assembly of a small trimeric structure to the generation of libraries of structures differentiated by lenght, acetyl presence and acetylation pattern.[3, 4]

Chapter 4 has described the synthesis of two sets of libraries of *Enterococcus faecalis* diheteroglycan (DHG) structures, based on [Glc*p*-Gal*f*] or [Gal*f*-Glc*p*] dimer repeating units, resembling the DHG-backbone, but lacking the lactic acid and acetyl substituents (Figure 2).

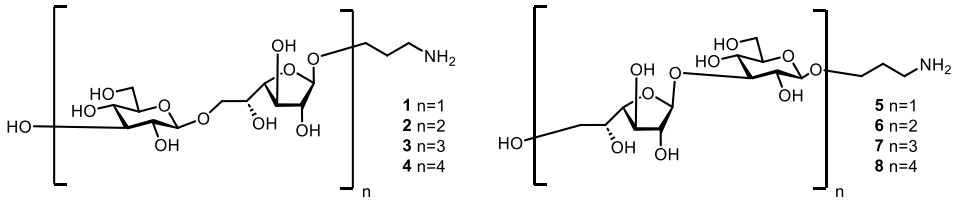
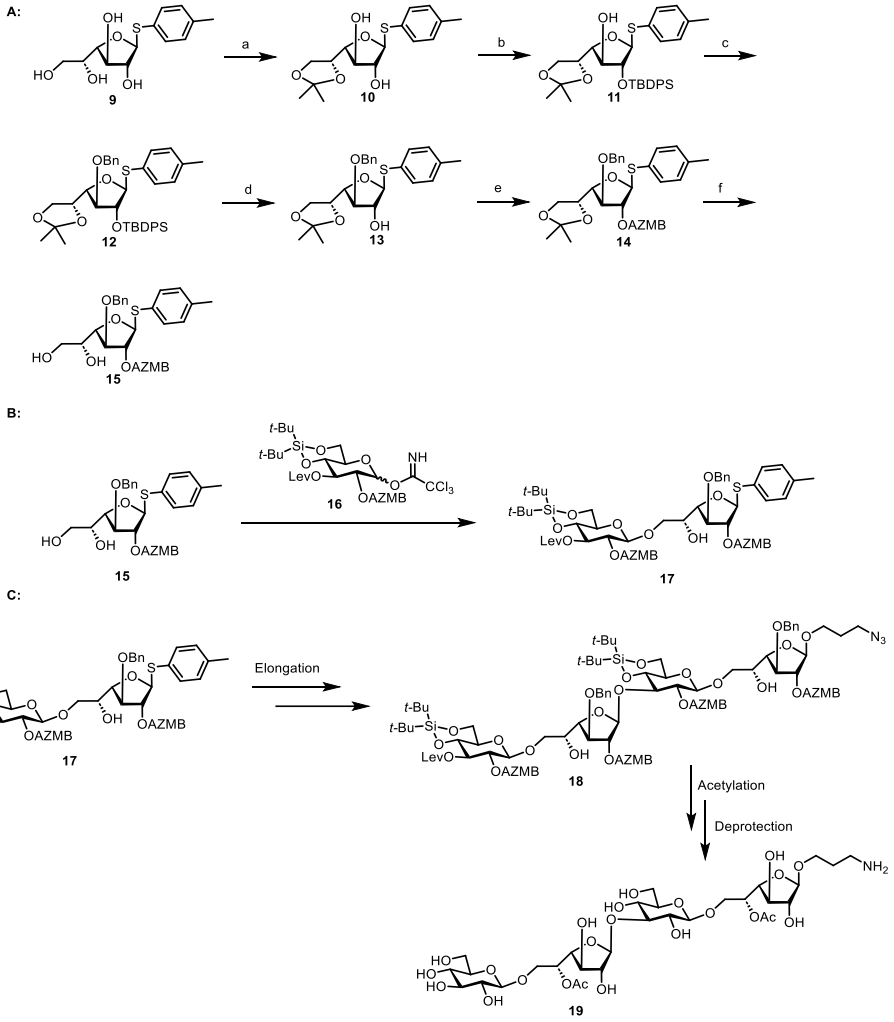


Figure 2 Synthetic DHG structures

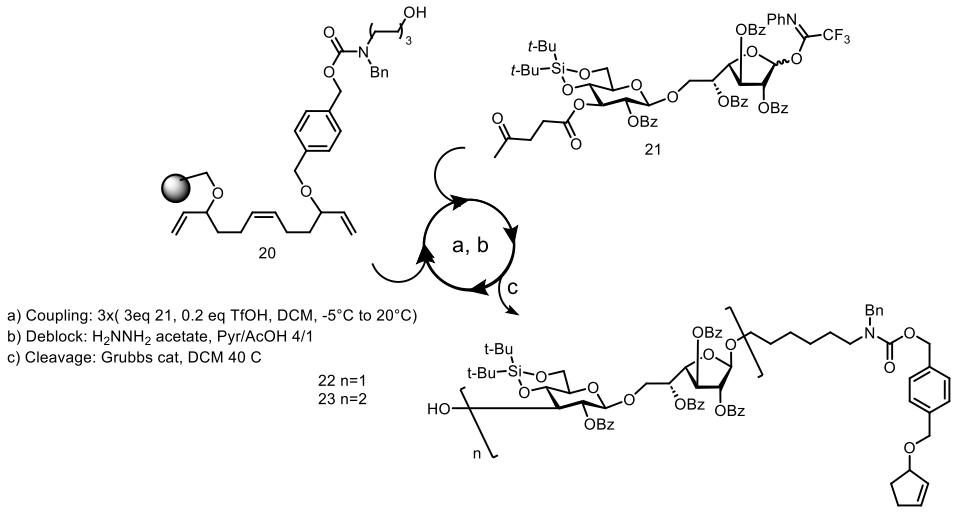
Serum, raised against native DHG, was shown to recognize the synthetic structures but to a significantly smaller extent than the native polysaccharide. BSA-conjugates have been generated using octasaccharides **4** and **8** and immunization experiments has been carried out on rabbits. The resulting sera have been evaluated in OPA, OPIA and *in vivo* experiments, showing observable killing activity towards the target bacterial strains. In



Scheme 1 ***A:*** *synthesis of building block* ***15*** *a) Acetone, PTSA, reflux, b) TBDPSCl, Imidazole, DMF, c) BnBr, NaH, DMF, d) TBAF, THF, e) AZMBCl, pyridine, f) MeOH, AcOH.* ***B:*** *Key glycosylation reaction TMSOTf, MS3Å, DCM, -20°C,* ***C:*** *Elongation and deprotection approach.*

addition, a solid phase approach has been developed to generate DHG structures showing the possibility to streamline the synthesis. As a further development the incorporation of the naturally occurring substituents on the synthetic structures would be necessary to determine whether their presence can improve the immune response.

To establish structure-immunogenicity relationships, two octasaccharides could be generated, one bearing the acetyl esters, while the other features the lactic acids. In a later stage, structures can be generated bearing both functional groups. This strategy would ensure a deeper understanding on the role of each substituent and establish whether they have a synergic effect in eliciting an immune response. For the installation of the acetyl moiety a proposed route is depicted in Scheme 1; Galf building block **15** can be obtained starting from **9** by selectively protecting the C-5 and C-6-OH with an



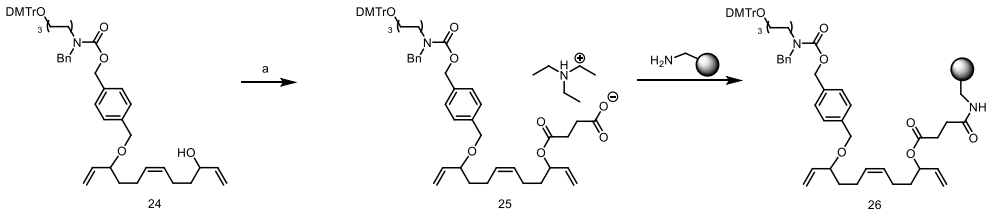
Scheme 2 Automated synthesis using RCM linker loaded on Merrifield resin

isopropylidene group. Subsequent treatment with *tert*-butyl(chloro)diphenylsilane (TBDPSCl) and imidazole followed by installation of a benzyl ether on the C3-OH will lead to fully protected Galf **12**. At this stage the silyl group could be removed to introduce a 2-(azidomethyl)benzoyl ester (AZMB) to ensure participation during the glycosylation with the glucose building block and orthogonality with the acetyl group. The isopropylidene can then be removed under acidic conditions to form building block **15**.[5] The key step of this synthesis would be the glycosylation step between **15** and **16** which would exploit the higher reactivity of the primary alcohol of the Galf residue to obtain disaccharide **17**, which can be acetylated at the C-5'-OH. Next the structures can undergo the same elongation process described in Chapter 4. Having obtained the desired length, the generated fragments can be deprotected by removing the silyl groups and reductive removal of the remaining benzyl ethers the azidomethylbenzoates and concomitant transformation of the azide in the spacer to the corresponding primary amine.

Chapter 5 described the design and synthesis of a novel tandem ring closing metathesis (RCM) linker system and the first application on an automated carbohydrate synthesizer for the formation of a DHG di- and tetrasaccharide (Scheme 2). This successful application represents a proof of concept that needs to be further explored with different substrates

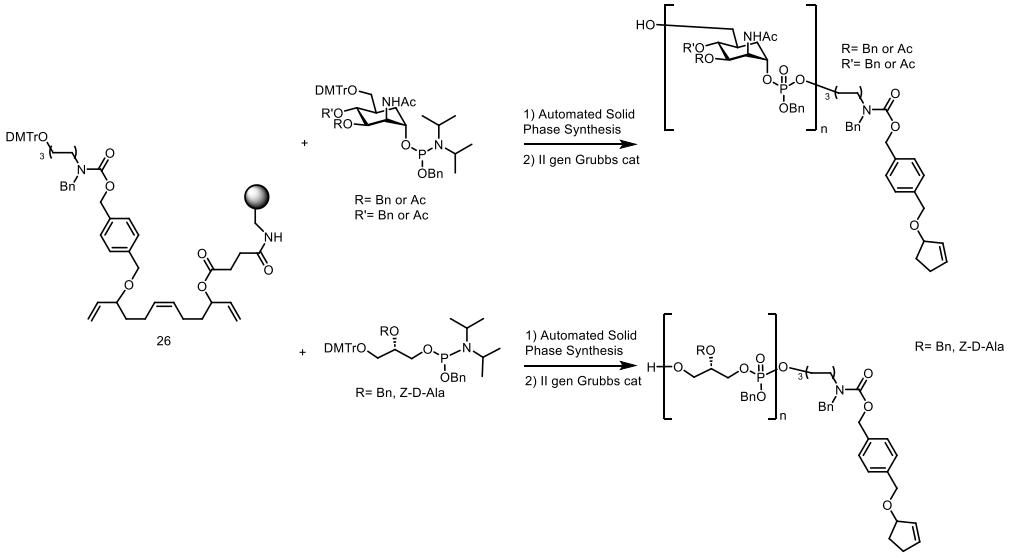
in order to establish its consistency. In addition, the newly developed RCM linker could be installed on a controlled pore glass (CPG) resin as depicted in Scheme 3.

The installation on the RCM linker on CPG as solid support would open the possibility to expand the applicability of this system also to oligonucleotide automated synthesizers.



Scheme 3 Loading of RCM linker on CPG resin a) Succinic anhydride, TEA, DCM, b) DIC, ACN, CPG Resin.

For instance, the carba-MenA building blocks described in chapters 2 and 3 could be used together with **26** on a DNA synthesizer to streamline the synthesis of a library of compounds differing in the degree and pattern of *O*-acetylation pattern (Scheme 4). Lastly, the neutral conditions used for the cleavage of the linker from the solid support can be exploited by using building blocks containing acid or base labile decorations such as the D-Ala-equipped TA-building blocks.



Scheme 4 Proposed applications for RCM linker on Oligonucleotide synthesizer

Chapter 6 described the synthesis of well-defined polyglycerol TAs decorated with a D-Ala residue on predetermined positions along the chain (Figure 3). The generated fragments can be tested to explore their immunogenicity and the role of the D-Ala

substituent in interaction with (circulating) antibodies and in the generation of an antibody response.

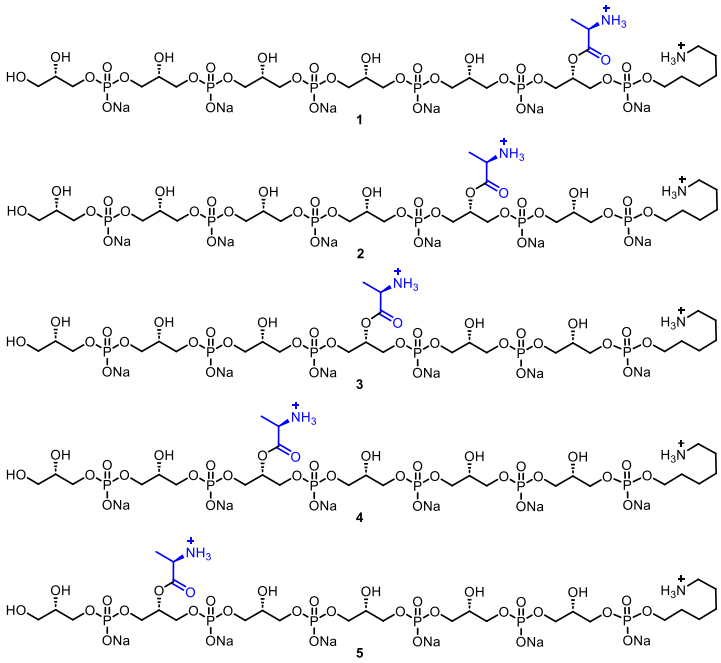
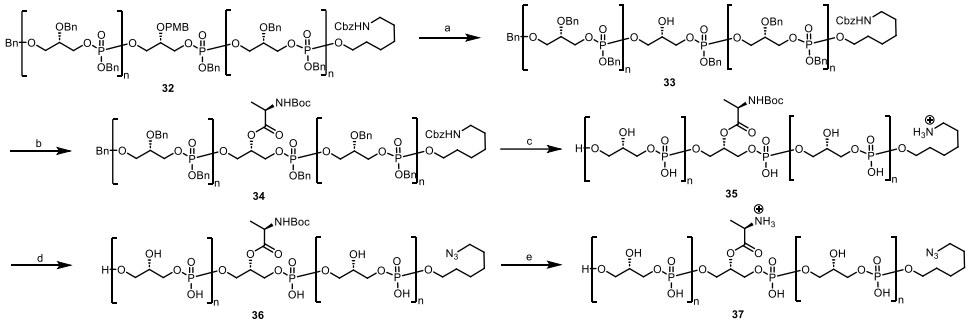


Figure 3 Generated synthetic D-Ala TAs

In addition, it would be relevant to determine a strategy for the conjugation of these structures, for instance to a carrier protein, that would preserve the labile ester of the D-Ala residue. To do so, it would be fundamental to find a way to differentiate the amine of the linker with the one of the ester. In Scheme 5 a synthetic approach towards this end is proposed: the first part of the synthesis resembles the already well-established route described in chapter 6, while it differentiates after the removal of the PMB moiety. Instead of a Cbz protected D-Ala, a *N-tert*-butyloxycarbonyl (Boc)-protected D-Ala could

be installed, followed by a hydrogenolysis reaction to remove all other protecting groups to provide compound **35** (Scheme 5).



*Scheme 3 Proposed synthetic approach towards **37**: a) DDQ, DCM/MeOH; b) Boc-D-Ala, PyBOP, Me-Im, ACN; c) Pd black, H_2, H_2O/1,2-Dioxane d) FSO_2N_3, MTBE/DMF/H_2O; e) HCl/ HFIP, H_2O*

At this point the novel metal-free diazotransfer method could be used to transform the primary amine on the linker into an azide to obtain **36** which could be then treated with HCl/HFIP to remove the Boc group producing final compound **37**.[6, 7] Fragments generated in this way could be easily conjugated via click chemistry for further structure activity relationship studies.

In conclusion the work in this thesis has described synthetic methods for the development of well-defined mimics of natural occuring antigens and their biological evaluation to pave the way to generate future vaccine modalities.

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