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Synthetic zwitterionic polysaccharides

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Zwitterionic polysaccharides (ZPSs) are a unique class of polysaccharides that are capable of eliciting a T-cell response after being processed by antigen presenting cells and presented on MHC II molecules. In addition, they have also been shown to be potent stimulators of the innate arm of the immune system. To unravel the molecular details of their remarkable immunological activity, various synthetic approaches to assemble fragments towards these polysaccharides have been reported. This review describes these efforts, illustrating the immense challenges presented by these inspiring structures.

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Zwitterionic polysaccharides: structure and activity

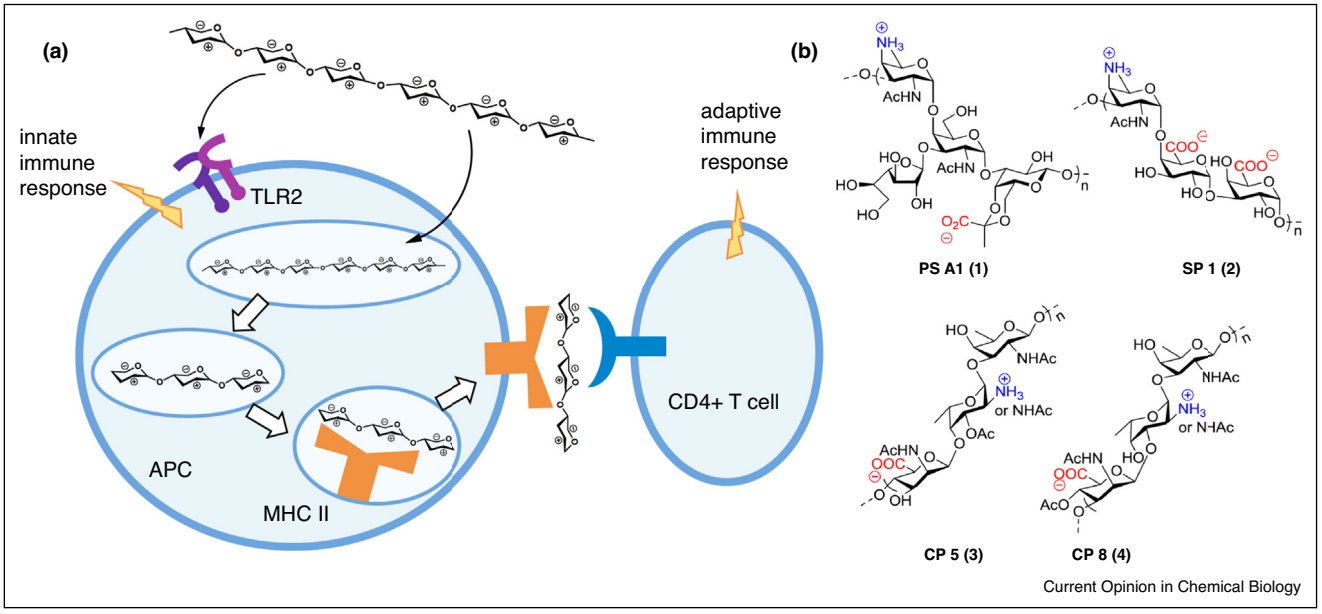
Bacteria are generally covered in polysaccharides featuring rare monosaccharide constituents and structural elements that differ from the mammalian glycan repertoire [1]. As such they represent excellent targets for the (human) innate and adaptive immune system to respond to [2]. However, bacterial polysaccharides behave poorly as stand-alone vaccine entities. Carbohydrates in general are poor immunogens and only trigger B-cell mediated IgM response, without switching to IgG production and memory development. Thus, in designing carbohydrate-based vaccines, bacterial oligo/polysaccharides are conjugated to a carrier protein to induce T-cell response to peptide epitopes embedded in the carrier protein [3–5]. This holds not true however for a unique class of carbohydrates that are characterized by the presence of both positively and negatively charged functionalities: the zwitterionic polysaccharides (ZPSs). These bacterial polysaccharides feature amino groups, protonated at physiological pH, and carboxylates or phosphates that are negatively charged at neutral pH. It is now well established that these unique structural features endow these saccharides with exceptional immunological properties [6••]. Zwitterionic polysaccharides are T-cell dependent antigens as they can be processed by antigen presenting cells, loaded onto MHC II molecules and presented to T-cells, and thus are able to elicit an immune response [7••]. As such, ZPSs behave like foreign proteins and this surprising activity has been show to arise from their unique structural elements [8,9]. Besides their activity in the adaptive part of the immune system, ZPS also interact with the innate arm of our immune system: various ZPS have been implied to interact with Toll like receptor (TLR) 2 [10••,11]. Figure 1 represents the structures of the most prominent ZPSs: PS A1 from *Bacteroides fragilis* (**1**) [7••], the *Streptococcus pneumonia* Sp1 saccharide (**2**) [7••] and the capsular polysaccharides of *Staphyococcus aureus* type 5 (**3**) and type 8 (**4**) [12]. Most mechanistic work on these ZPS has been conducted with ZPS **1** and **2**, isolated from the parent bacteria. Chemical modification of the isolated material (acetylation of the amines to remove the positive charges, reduction of the carboxylates, to remove the positive charges) has shown the prerequisite of the zwitterionic motif for activity [6••]. NMR studies combined with molecular dynamic calculations and supported by circular dichroism (CD) measurements revealed that ZPS **1** and **2** take up a helical structure, positioning their positive and negative charges at approximately equal distance [13,14,15•].

Because of the appealing biological activities and their unique structures, the ZPSs have been the subject of several synthetic endeavors [16••,17•,18••,19–27]. Given the structural features of the target molecules (*cis*-glycosidic linkages, the presence of multiple functional groups, i.e. amines, acetamides, carboxylates and the rare monosaccharide constituents), these total synthesis campaigns have not been without a challenge. In this review, we will present an overview of the accomplished syntheses to date and the — limited — biological data that has been gathered with the resulting zwitterionic oligosaccharides.

Zwitterionic polysaccharides: synthesis

Several synthetic routes towards fragments of the ZPS depicted in Figure 1b have been disclosed. Because the synthesis strategies towards capsular polysaccharides of *S. aureus* are not compatible with the incorporation of free, positively charged amine functionalities (next to the

Figure 1



Zwitterionic polysaccharides. **(a)** ZPS can stimulate the innate and adaptive arm of the immune system through interaction with TLR2 and by binding to MHC II, respectively. **(b)** Examples of naturally occurring ZPS from *Bacteroides fragilis* (PS A1, **1**), *Streptococcus pneumonia* (SP 1, **2**), *S. aureus* (type 5, **3** and type 8, **4**).

acetamides) in the generated fragments [23–27] these syntheses will not be reviewed here. The overview of the successful syntheses of Sp1 and PS A1 fragments clearly illustrate the challenges associated with the complex structures of these molecules. One of the bottlenecks in the assembly of these structures is represented by the requirement of sufficient amounts of a suitably functionalized trideoxydiaminogalactose (TDDAG) building block. A recent review [28] details the variant approaches taken to generate such building blocks.

The Sp1 polysaccharide is built up from trimer repeats composed of α-D-2-*N*-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose and α-D-galacturonic acid residues (see Figure 1b). The presence of the rare TDDAG and galacturonic acid (GalA) residues and the fact that they are all interconnected through *cis*-glycosidic linkages present a huge synthetic challenge. Bundle and co-workers were the first to complete the assembly of a fragment of this ZPS [16••]. They reported the synthesis of a monomer and dimer of the repeating trisaccharide as depicted in Figure 2. The TDDAG motif was generated from a rather advanced disaccharide synthon (**7**–**8**). After coupling with galactose donor **10**, the trisaccharide **12** was obtained. Of this intermediate the primary alcohol functions were unmasked to set the stage for the double oxidation step. The two carboxylates were installed using a TEMPO/NaOCl oxidation procedure, after which global deprotection of the trisaccharide was accomplished by a catalytic hydrogenation event. The assembly of the hexasaccharide, encompassing two repeating units, required the generation of a new trisaccharide. Again, a disaccharide synthon (**9**) was generated, this time bearing an anomeric *tert*-butyldimethyl (TBS) group as a temporary protecting, to allow for the generation of a trisaccharide donor (**14**). The crucial condensation of the trisaccharide donor **14** and acceptor **13** required careful tuning of the reaction conditions and hexasaccharide **15** could be obtained in 85% yield. Deacetylation of **15** then provided the tetraol, ready for the crucial oxidation step. Complete oxidation of the tetraol **17** proved more difficult than the corresponding oxidation of diol **16**. It is commonly observed in the assembly of uronic acid containing oligosaccharides that it is significantly more challenging to perform multiple oxidations on a large substrate than to oxidize smaller fragments. Using Huangs oxidation procedure that entails a biphasic TEMPO oxidation, followed by a Pinnick oxidation of the formed aldehydes, the tetra uronic acid was obtained. After benzylation of the carboxylates, the hexasaccharide **19** could be purified and it was obtained in 52% yield starting from tetra-acetate **15**. A single hydrogenation event then delivered the target hexasaccharide **21**. The authors report that the tri-saccharide and hexasaccharide **20** and **21**, respectively, were evaluated for their T-cell activating capacity but that no activity was found. However, no details for these experiments have been disclosed. It has been postulated that ZPSs take up helical shapes and that this 3-dimensional structure may be relevant for the unique MHC-II binding capacity of the ZPSs. The NMR spectra of the