# Total synthesis of alginate and zwitterionic SP1 oligosaccharides

Zhang, Q.

# Summary and Future prospects

The work in this thesis has been focused on two subjects. The first is the assembly of alginate oligosaccharides and the generation of building blocks for the enzymatic synthesis of alginate, and the second is the total synthesis of large fragments of the zwitterionic SP1 polysaccharide. With these fragments, details about alginate biosynthesis can be obtained through binding studies with biosynthesis enzymes, conjugate vaccines can be generated and binding studies with major histocompatibility complex II molecules can be studied. This Chapter summarizes the work described in this Thesis and provides an outlook for future research.

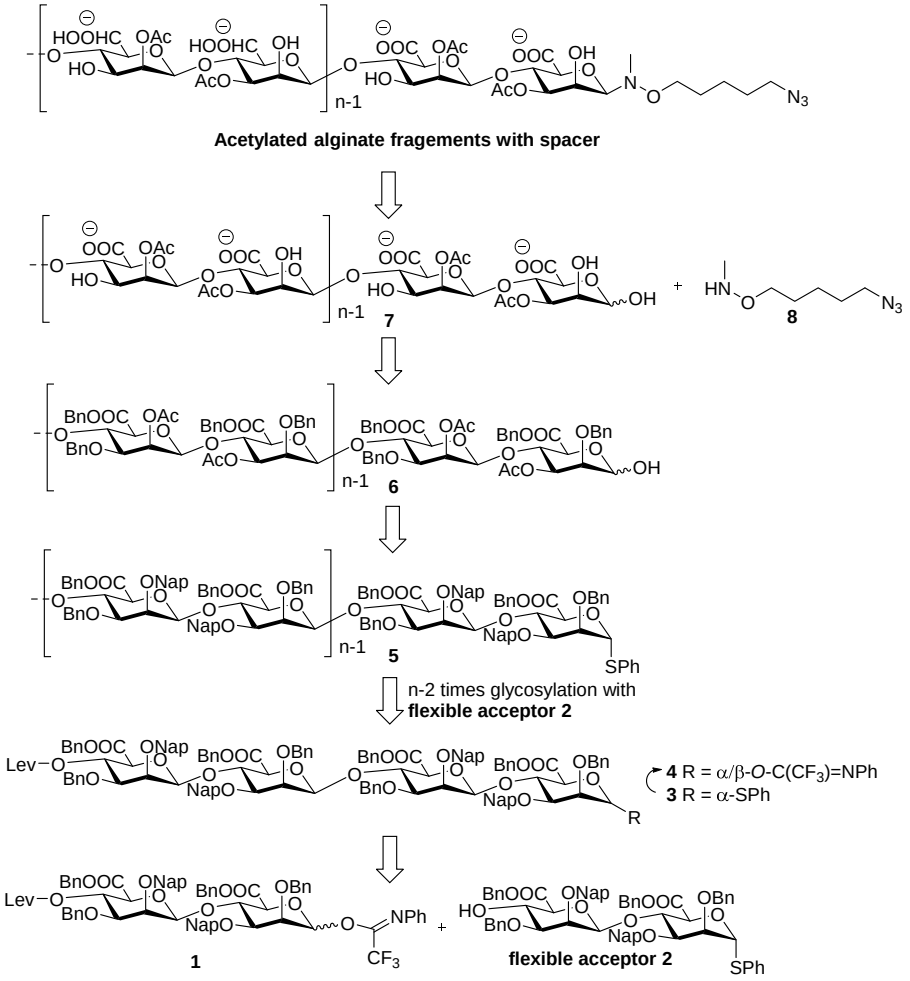
## 8.1 Synthesis of alginate fragments and GDP-mannuronic acid building blocks

**Chapter 1** provides a concise overview of the synthesis of alginate oligosaccharides reported to date. So far, only alginate oligomers have been generated composed of a single type of building block, *i.e.* $\beta$-D-mannuronic acids or $\alpha$-L-guluronic acids. **Chapter 3** and **Chapter 4** describe the synthesis of alginate fragments containing both $\beta$-D-mannuronic and $\alpha$-L-guluronic acids. In **Chapter 3**, both monomeric and dimeric guluronic acid and gulose acceptors were synthesized and coupled with monomeric and dimeric donors to examine their reactivity in glycosylation reactions. It was found that the gulosyl C-4 hydroxyl is a relatively poor nucleophile. Quite surprisingly, the functional group at the C5, neighboring the C4-hydroxyl, had little influence on the reactivity of the nucleophile, with the guluronic acid acceptors performing similar to the gulose acceptors. A striking effect was found of the flexibility of the acceptor disaccharides. A $\beta$-*O*-mannuronic acid on the reducing end, equipped with a rigid spacer, led to a very poor nucleophile, while a flexible $\alpha$-configured 1-thio mannuronic acid reducing end residue provided a much more reactive acceptor. The conformational flexibility of $\alpha$-*S*-tolyl mannuronic acid reducing ends was reflected in the $^{1}$H NMR and $^{13}$C NMR spectra of the disaccharides, where the signals of the mannuronic acid ring appeared as broad and poorly resolved resonances at room temperature. At low temperature (–60 °C), two resonance sets became apparent that coalesced with increasing temperature. In **Chapter 4**, the fully stereoselective assembly of alginate fragments containing $\beta$-D-mannuronic and $\alpha$-L-guluronic acids has been reported for the first time. A set of alginate fragments, comprised of GM, GMG,

GMGM, GMGMG, GMGMGM, GMGMGMG and GMGGMG sequences was assembled. During the assembly of the oligomers the conformational flexibility of the GM acceptors was revealed as an all-important factor determining the efficiency of the coupling reactions. **Chapter 5** describes the assembly of a triad of guanosine diphosphate mannuronic acids, comprising GDP-ManA and its C-4-*O*-Methyl and C-4-deoxy congeners. These substrates for enzymatic alginate synthesis were assembled from the corresponding anomeric phosphates and a guanosine phosphoramidite building block. The target compounds were each generated in multi-milligram quantities. The GDP-ManA donors will be employed to fuel the mannuronic polymerase for the enzymatic assembly of polymannuronic acids. The generated C-4-capped and C-4-deoxygenated GDP-ManA donors will be explored as "chain stoppers" to gain control over the length of the growing polymannuronic acid chains.

The alginate biomachinery of *Pseudomonas aeruginosa* also incorporates acetyl groups on some of the mannuronic acid residues.[1] Therefore, it would be of interest to generate partially acetylated fragments to investigate binding of these to the biomachinery enzymes. Up to now, only de-acetyl alginate oligosaccharides have been synthesized and a logical extension of this work would be the assembly of partially acetylated fragments (the retrosynthesis is shown in Scheme 8.1). The flexible disaccharide acceptor **2** is chosen as key elongation unit to build oligosaccharide **5**, then remove temporary protecting group Nap to install Ac. After global deprotection, the spacer can be installed to generate target molecular.[2]

**Scheme 8.1** The retrosynthesis of partially acetylated fragments of mannuronic acid alginate.




Sulfated oligomannuronic acid alginates (SOMAs) have been studied for their potential anti-cancer, anti-HIV activity and as mimics for heparin.[3] All these studies have been conducted with mixtures of compounds and the assembly of sulfated mannuronic acids, following the strategy outlined above (with the sole difference of incorporating sulfate esters in stead of acetyl esters) would therefore be of interest.

In the syntheses of the mixed sequence alginates, it has been found that the acceptors' flexibility is very important for a successful glycosylation.[4] It would be interesting to further study this phenomenon using different donors and different acceptors (of varying length). In future glycosylations involving poor nucleophiles, the acceptors' flexibility can be an important factor to consider when optimizing the reaction.

**8.2 Synhesis of zwitterionic SP oligosaccharides**

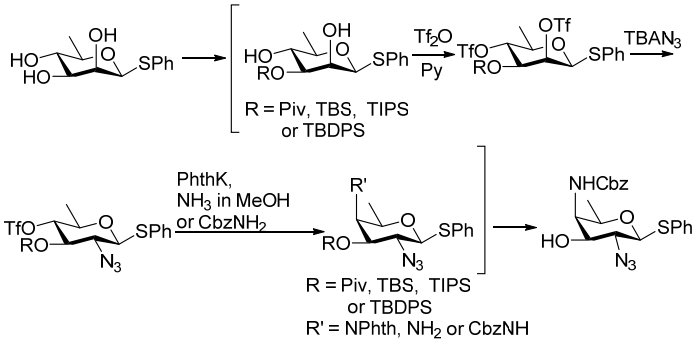
**Chapter 2** has provided a summary of the synthetic efforts undertaken so far in the assembly of zwitterionic polysaccharide fragments. These oligosaccharides all contain a glycuronic acid moiety. In **Chapter 6**, a new oxidation protocol for the selective oxidation of primary alcohol to carboxylic acids is introduced using a two-step one-pot TEMPO/BAIB-Pinnick oxidation sequence. This protocol was used in the oxidation of complex substrates, such a disaccharide synthon for the assembly of the *Staphylococcus aureus* Strain M Capsular Polysaccharide trisaccharide repeating unit and a SP1-model hexasaccharide fragment, that previously could not effectively be oxidized using the TEMPO/BAIB reagent combination. **Chapter 7**, reports the first synthesis of long zwitterionic Sp1 oligosaccharides. A Sp1-nonamer and dodecamer were assembled by combining a pre-glycosylation oxidation and post-glycosylation oxidation strategy. To guarantee complete stereoselectivity in the condensations of the trisaccharide building blocks, a silylidene galactose reducing end donor moiety was used. This required the simultaneous oxidation of three (for the nonasaccharide) or four (for the dodecasaccharide) primary alcohols at the end of the synthesis, which proved to be a major challenge. After the newly introduced TEMPO/BAIB-Pinnick oxidation protocol failed another improvement to the TEMPO/BAIB

oxidation system was found in the addition of sodium bicarbonate to the reaction mixture to speed up the conversion of the intermediately formed aldehydes into the carboxylic acids. A benzylation protocol employing phenyldiazomethane proved crucial to obtain the desired dodecasaccharide in good yield. The final zwitterionic **SP1** oligosaccharides were obtained in multi mg quantities. Structural analysis of the zwitterionic oligosaccharides using $^{1}H$ NMR, $^{13}C$ NMR and 2D-Noesy experiments revealed that the structure of the hexamer began to resemble the structure of the native SP1, while the spectra for the nonamer and especially the dodecasaccharide matched the spectra for the native polysaccharide even better. This may indicate that the longest oligosaccharides generated start to take up a secondary helical structure, as has been reported for the native polysaccharide. Further structural studies by NMR, combined with molecular modeling will provide more insight into the three-dimensional structure of the assembled oligosaccharides. This in turn, may provide insight to understand their biological activity.

The synthesis of the zwitterionic SP1 oligosaccharides, has presented a few bottlenecks. Besides the oxidation of multiple primary alcohols to corresponding carboxylic acid, the synthesis of the rare 2,4,6-tri-deoxy-2,4-diamino-galactose (TDDAG) building block proved to be challenging as well as the final deprotection step, in which the double bond of the butenol spacer was partly reduced under the birch reduction condition. To optimize the synthesis route and obtain longer zwitterionic SP1 oligosaccharides improvements can be made to the synthesis of the TDDAG synthon. For example, bulky protecting groups (such as the Piv, TBS, TIPS and TBDPS) can be used to mask the C3-alcohol of 6-deoxy-1-thio-β-D-mannopyranoside to prevent the formation of the 2,4-di-azido byproduct and prevent acetyl migration to the C4-nitrogen, upon removal of the

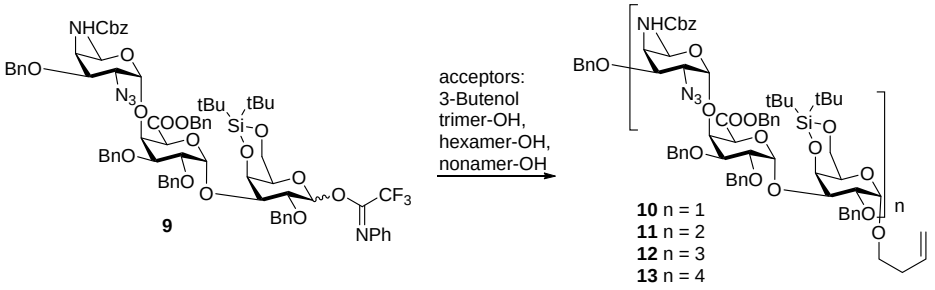
phthaloyl group. Also the nature of the nitrogen nucleophile in the second substitution reaction may be changed, to $NH_3$ or $CbzNH_2$, for a more profitable outcome (Scheme 8.2).[5]

**Scheme 8.2** Synthesis of 2,4,6-trideoxy-galactose building block.




To further streamline the assembly of larger oligomers, the C3-levulinoyl ester on the TDDAG may be replaced by a benzyl ether. This will make the global deprotection scheme shorter and therefore more effective (see Scheme 8.3).

**Scheme 8.3** Synthesis of ZPS –SP1 oligosaccharides.




To prevent the difficult oxidation step at the end, a fully oxidized trisaccharide building block could be explored. For example, the trisaccharide uronic acid donor **14** could serve

as elongation unit to produce ZPS-oligosaccharides. The glycosylating properties of this building block will have to be established however as it is very likely that the generation of anomeric mixtures during the glycosylations will be a major problem. To investigate this type of coupling reaction, the monosaccharide and disaccharide uronic acid donor **15** and **16** can be investigated in the condensation with acceptor **17** (Figure 8.1). Donor **16** was shown to be a proper α-selective donor in the coupling with phenyl 2-*O*-benzyl-4,6-di-*tert*-butyl-silylidene-1-thio-β-D-galactopyranoside, as described in Chapter 7.

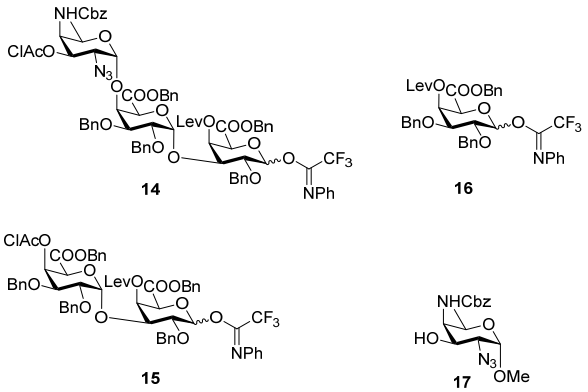
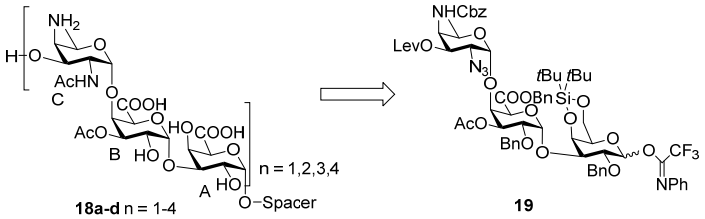



Figure 8.1 Pre-glycosylation oxidation strategy.

The naturally occurring capsular polysaccharide of *S. pneumoniae* type 1 is partly acetylated and it has been proposed that the C3-OH of the galacturonic acid reside B (see Scheme 8.4) carries the ester.[6] Therefore it would be interest to synthesize these partly acetylated zwitterionic oligosaccharides **18** (for example a trimer, hexamer, nonamer and dodecamer). This could be accomplished using trisaccharide donor **19**. These oligosaccharides can then be studied for their 3D structure and biological activity. By

comparing them with the zwitterionic SP1 oligosaccharides lacking the acetate (compounds **1**, **2**, **3** and **4** in Chapter 7).




Scheme 8.4 The structure of partly acetylated zwitterionic SP1 oligosaccharides.

## 8.3 References


[1] G. SkjÅk-Bræk, H. Grasdalen, B. Larsen, *Carbohydr. Res*, **1986**, *154*, 239-250.

[2] a) A. R. Prudden, L. Liu, C. J. Capicciotti, M. A. Wolfert, S. Wang, Z. Gao, L. Meng, K. W. Moremen, and G.-J. Boons, *Proc. Natl. Acad. Sci. U. S. A*, **2017**, *114*, 6954-6959; b) S. Munneke, J.R. C. Prevost, G. F. Painter, B. L. Stocker, M. S. M. Timmer, *Org. Lett*. **2015**, *17*, 624-627.

[3] H. Ronghua, D. Yumin, Y. Jianhong, *Carbohydr. Polym*, **2003**, *52*, 19-24.

[4] In chapter 3 and chapter 4.

[5] A. R. Podilapu and S. S. Kulkarni, *Org. Lett*, **2017**, *19*, 5466-5469.

[6] a) B. Lindberg, B. Lindqvex, J. Lönngern, D. A. Powel, *Carbohydr. Res,* **1980**, *78*, 111-117; b) R. C. E. Guy, M. J. How, M. Stacey, M. Heidelberger, *J. Biol. Chem*, **1967**, 242, 5106-5111.