Chapter 6

Summary and Future Prospects

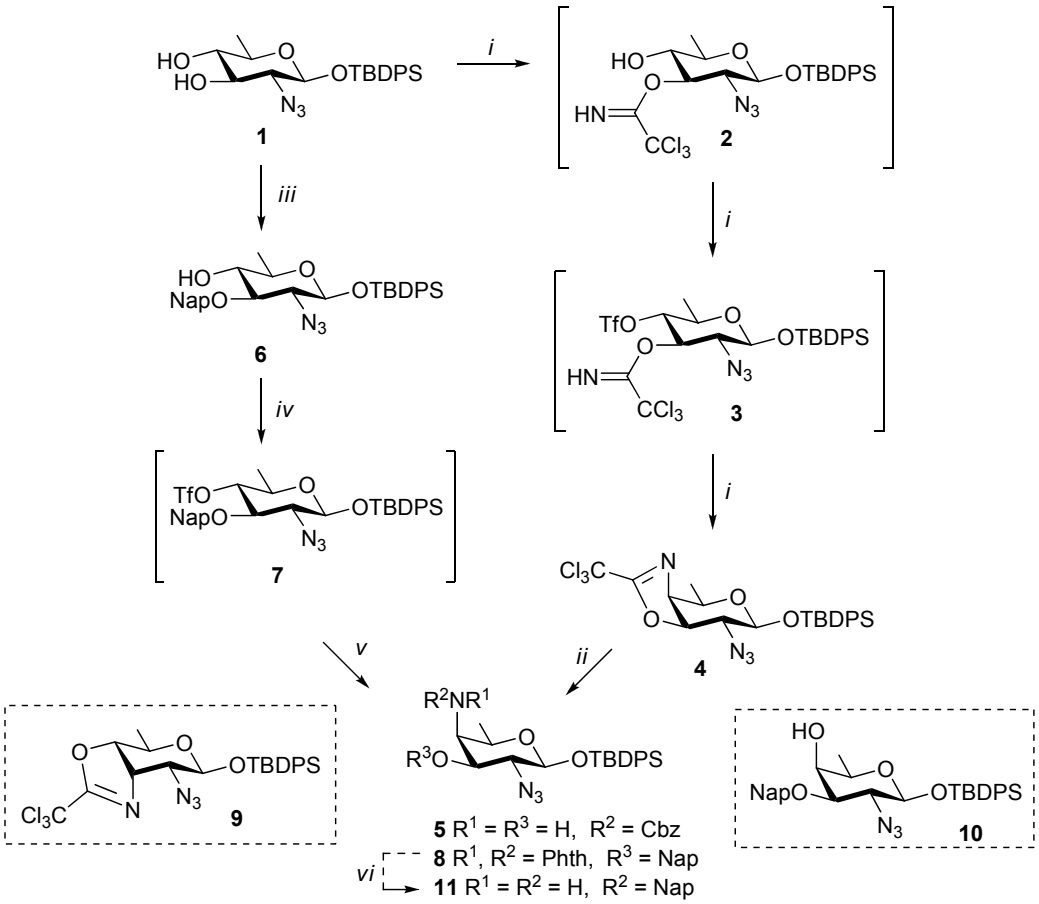
Outline and Perspectives

One of the most challenging aspects in synthetic carbohydrate chemistry is the stereoselective introduction of glycosidic linkages.[1] The introduction of 1,2-*trans* bonds is considered a straightforward matter. Equipping a glycosyl donor with an acyl functionality on the C-2 position leads to the formation of a transient acyloxonium ion upon activation. This directs the glycosylation event towards the 1,2-*trans* product. The synthesis of 1,2-*cis* configured bonds is more difficult and to this end various methods have been brought forward. By means of selected examples, recently introduced strategies for the stereoselective introduction of glycosidic bonds are described in **Chapter 1**.

In **Chapter 2** a synthesis of an orthogonally protected 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (AAT) building block is outlined. The unprotected progenitor hereof cannot be isolated from natural sources. The developed route of synthesis starts from D-glucosamine. Key features of the route are the regioselective installment of a C-3-*O*-imidate functionality, which is followed by the introduction of a C-4-triflate and subsequent oxazoline formation (**1**→**4** in Scheme 1). Even though treatment of diol **1** with trichloroacetonitrile at low temperature in the presence of DBU leads to the preferential formation of C-3-*O*-imidate **2**, the di-imidate and the C-4-*O*-imidate are also formed. The latter imidate, undergoing a

similar conversion as its C-3 counterpart **1**, eventually leads to the formation of the *allo*-configured sideproduct **9**. Due to structural similarities, the separation of oxazolines **4** and **9** by column chromatography is laborious and mixed fractions were occasionally encountered.

Scheme 1



Reagents and conditions: (i) Cl_3CCN, DBU, DCM, -13°C then Tf_2O, pyridine then DiPEA (**4**: 63%, **9**: 24%); *(ii)* (1) AcOH, H_2O, EtOAc; (2) *N*-(benzyloxycarbonyloxy)succinimide, triethylamine, DCM (**5**: 75%); (*iii*) (1) Bu_2SnO, toluene, reflux for 2 hours; (2) 2-(Bromomethyl)naphthalene, TBAI, toluene (57%); (iv) Tf_2O, pyridine, DCM; (v) potassium phthalimide, DMF (**8**: 48%, **10**: 26%); (*vi*) $H_2NCH_2CH_2NH_2$, BuOH.

With the aim to develop more straightforward procedures for this part of the route towards an AAT building block, a different approach inspired by a route developed by Pedersen *et al.*[2] was attempted (Scheme 1). Diol **1** was converted to C-3-*O*-methylnaphthyl ether **6** *via* an intermediate stannyl ether. Straightforward column chromatography allowed the procurement of pure regioisomer **6** in 57%. Conversion of the remaining alcohol to triflate **7** and subsequent nucleophilic displacement by a phthalimide furnished galactoside **8**

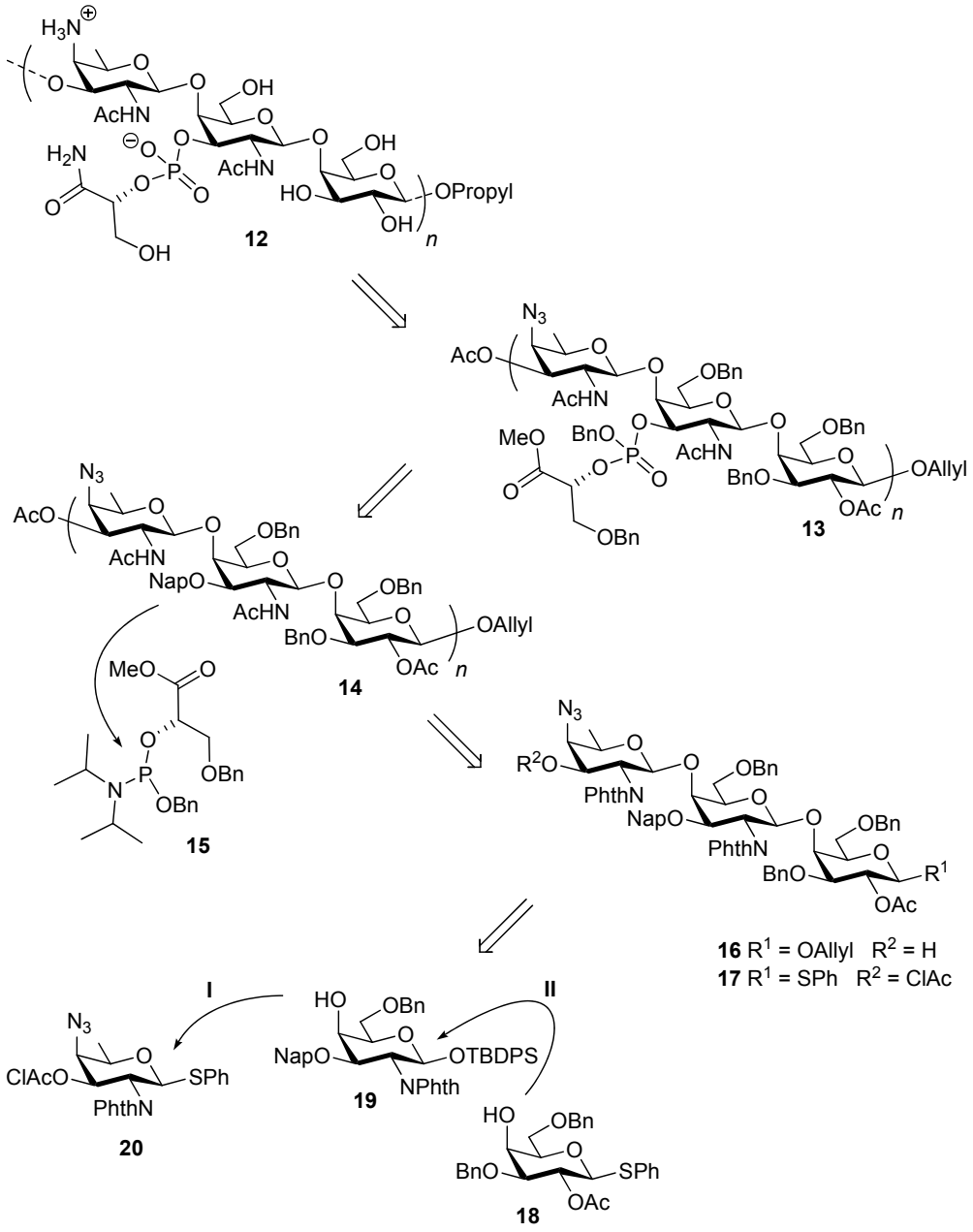
in 48% yield. Although this alternative route is lower-yielding than the initial sequence, it does provide an appropriately configured building block in a practically straightforward manner. Furthermore, sideproduct **10**, resulting from substitution of triflate **7** by water, was obtained in 26%. This indicates that there is ample room for improvement of the efficiency. After deprotection of the phthalimide, the resulting amine **11** can be functionalized as pleased.

Recently, AAT was identified as a constituent of a polysaccharide found in the human opportunistic pathogen *Providencia alcalifaciens*.[3] Deseases that are associated with *Providencia* strains include urinary tract infections and enteric diseases such as travelers' diarrhea. The repeating unit structure of the polysaccharide is depicted in Scheme 2 (compound **12**) and consists of a trisaccharide branched with a d-glyceramide (GroAN) 2-phosphatyl group: [→4)-(d-GroAN-2-P-3-)-β-d-GalNAc-(1→4)-β-D-Gal-(1→3)-β-d-FucNAc4N-(1→].

All three glycosidic linkages are of the 1,2-*trans* type and can thus be introduced by means of a participating C-2 acyl functionality. Retrosynthetic analysis shows that the fully deprotected target structure **12** can be accessed from protected oligosaccharide **13** by hydrogenation and conversion of the methylester to an amide with concomitant deacetylation (Scheme 2). Oligosaccharide **13** can be obtained by deprotection of the 2-naphtylmethyl ethers in **14**, coupling of the resulting alcohols with phosphoramidite **15** and ensuing oxidation of the intermediate phosphite triester. Oligosaccharide **14** can be obtained by the repetitive extension of acceptor **16** with donor **17** followed by dechloroacetylation of the growing chain. Trisaccharide **16** can in turn be accessed by glycosylation of trisaccharide **17** with allyl alcohol and subsequent dechloroacetylation. Trisaccharide **17** can be synthesized by the union of AAT imidate donor **20** and protected galactoside **19**, two step conversion of the anomeric TBDPS group to an *N*-phenyltrifluoroacetimidoyl group and an acid catalyzed coupling of the resulting disaccharide donor with alcohol **18**. Imidate donor **20** is accessible by chloroacetylation of its known C3-OH analogue.[4]

Chapter 3 describes a modular approach towards the synthesis of all possible trimer repeating units of the type 1 capsular polysaccharide of *Streptococcus pneumonia*, Sp1. The trisaccharide repeats are composed of two galacturonic acid monomers and an AAT residue. All monomeric constituents are linked through *cis*-glycosidic bonds. The difficulty associated with the efficient stereoselective introduction of the α-galacturonic acid bonds was overcome by employing galacturonic acid-[3,6]-lactone building blocks. These synthons performed well when used as donor galactosides and also showed to be reactive acceptor glycosides, when equipped with a free hydroxyl function. All three frame-shifted trimer repeats were constructed *via* highly stereoselective glycosylation reactions, with one exception. The epimeric mixture of trisaccharides, formed in the non-selective glycosylation event, could be readily separated after global deprotection using High Performance Anion Exchange Chromatography (HPAEC).

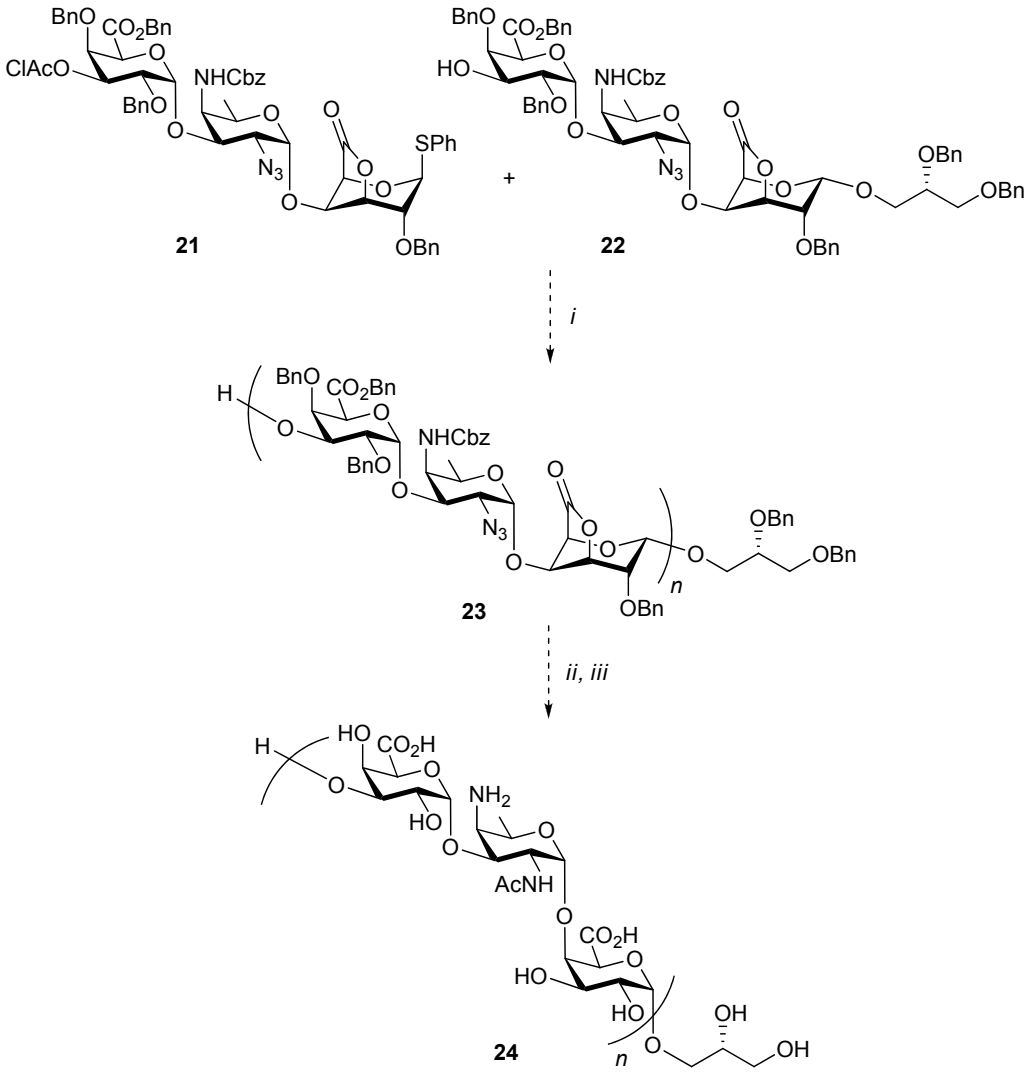
Scheme 2



Retrosynthetic analysis of a trisaccharide from *Providencia alcalifaciens*.

Now that efficient routes toward trisaccharide donor **21** and trisaccharide acceptor **22** have been developed, the synthesis of longer fragments lies within reach (Scheme 3).

Scheme 3



Reagents and conditions: (*i*) n times: (1) **21**, Ph_2SO, Tf_2O, DCM, TTBP, -60°C then acceptor **22**; (2) thiourea, EtOH, pyridine, 65°C; (*ii*) AcSH/pyridine (1/1 v/v); (*iii*) TMSONa, DCM, then H_2/Pd(C), *t*BuOH, H_2O, HCl.

Successive block couplings of trisaccharide thioglycoside donor **21** onto acceptor **22**, followed by dechloroacetylation of the resulting oligosaccharide can give protected oligomers **23**. In view of the varying results obtained with similar glycosylations, as shown in Chapter 3, it is well conceivable that this 1,2-*cis* block coupling strategy will need substantial optimization. Data obtained in Chapter 4, however, suggests that favoring the formation of an intermediate anomeric β-triflate is beneficial for the creation of a 1,2-*cis* linkage. All attempts to steer the selectivity might provide valuable clues that lead to a deeper

mechanistic understanding. Besides exerting the glycosylation at various temperatures, different promoter systems like *para*-nitrophenylsulfenyl triflate (*p*-NO_2PhSOTf),[5] *N*-phenylthio-ε-caprolactam-TMSOTf[6] (or its tolyl derivative)[7] or dimethyl(methylthio)sulfonium triflate (DMTST)[8] can be used. Judging from the insights gained in Chapter 4, it is advisable to use these promotors in a pre-activation glycosylation protocol. The addition of more triflic acid (and a hindered base, such as TTBP) might also promote the formation of an anomeric β-triflate. Furthermore, altering the nucleophilicity of acceptor **22** through the formation of a stannyl ether might influence the stereochemical outcome of the glycosylation. Next, oligosaccharide **23** can be converted to the fully deprotected oligosaccharide **24**. A treatment with thiolacetic acid and pyridine can convert the azides to acetamides. The lactone bridges can be opened with TMSONa. Finally, hydrogenolysis of the remaining benzyl ester, benzyloxy carbamate and benzyl groups can provide the fully deprotected target oligosaccharide.

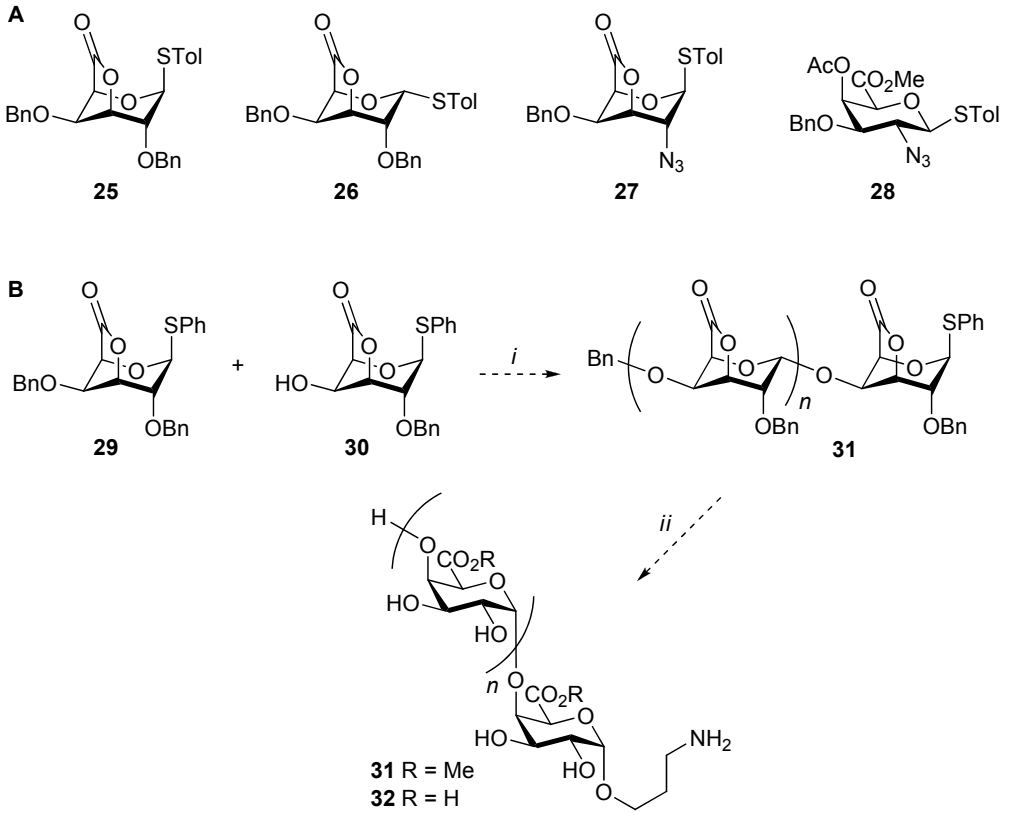
Chapter 4 describes a study of the reactivity and stereoselectivity of a galacturonic acid-3,6-lactone thioglycosyl donor in comparison with protected galacturonic acid and galactose donors using a series of competition experiments and condensation reactions with different thiophilic activator systems. It was revealed that the relative reactivity of different thioglycosides depends on the activator system used and that *p*-nitrophenylsulfenyl triflate shows, in an in situ protocol, overall attenuated reactivity differences with respect to the commonly used *N*-iodosuccinimide-triflic acid promoter system. With respect to the stereoselectivity of the studied galacturonic acid-3,6-lactone thioglycosyl donor, it was revealed that a pre-activation based glycosylation system gives rise to an α-selective glycosylation process, whereas an *in-situ* activation protocol leads to the formation of the β-product with good selectivity. It was hypothesized that these opposing stereoselectivities are the result of different product-forming intermediates. Where pre-activation of the donor leads to the formation of an intermediate β-triflate, which is substituted in a concerted fashion to provide the α-product, an $^3H_4$ oxocarbenium ion like species is substituted in the *in situ* activation experiment to provide the β-linked product.

It has thus been established in Chapter 4 that the relative reactivity of different thioglycosides depends on the type of activator system. A more direct relationship between the galacturonic acid-3,6-lactone thioglycosyl donor reactivity and the used promoter requires more data. This can be attained by running more competition experiments using different promoter systems, such as: IDCP,[9] *para*-nitrophenylsulfenyl triflate (*p*-NO_2PhSOTf),[5] *N*-phenylthio-ε-caprolactam-TMSOTf[6] (or its tolyl derivative)[7] or dimethyl(methylthio)sulfonium triflate (DMTST).[8]

To gain more insight in the reactivity of different glycosyl donors more building blocks can be incorporated in the investigated series. Three examples (**26**-**28**) are provided in Scheme 4A. Thioglycosyl donor **26** is the α-thioglycosyl counterpart of the studied galacturonic acid-3,6-lactone β-thioglycosyl donor **25**. Most of the donors described in Chapter 4 reside in the $^1C_4$ conformation and bear an equatorially oriented aglycon. Although expulsion of the aglycon upon activation of the donor is expected to occur more rapidly in

the case of donor **25** due to a better orbital overlap of the O5 lone pairs with the $\sigma^*_{C1\text{-}S}$, the equatorially oriented aglycon in **26** should be more prone to react with the promoter, owing to its increased accessibility. This reactivity difference can be assessed by indirect reactivity comparison using two separate competition experiments with a competing donor, such as **28**, since both galacturonic acid-3,6-lactone thioglycosyl donors **25** and **26** will give the same dimer upon reaction with an identical acceptor.

Scheme 4



Reagents and conditions: (*i*) n times: **29**, *p*-NO_2PhSOTf, -60°C then acceptor **30**; (*ii*) (1) TMSONa, DCM or MeOH, *p*-TsOH then H_2/Pd(C), *t*BuOH, H_2O, HCl.

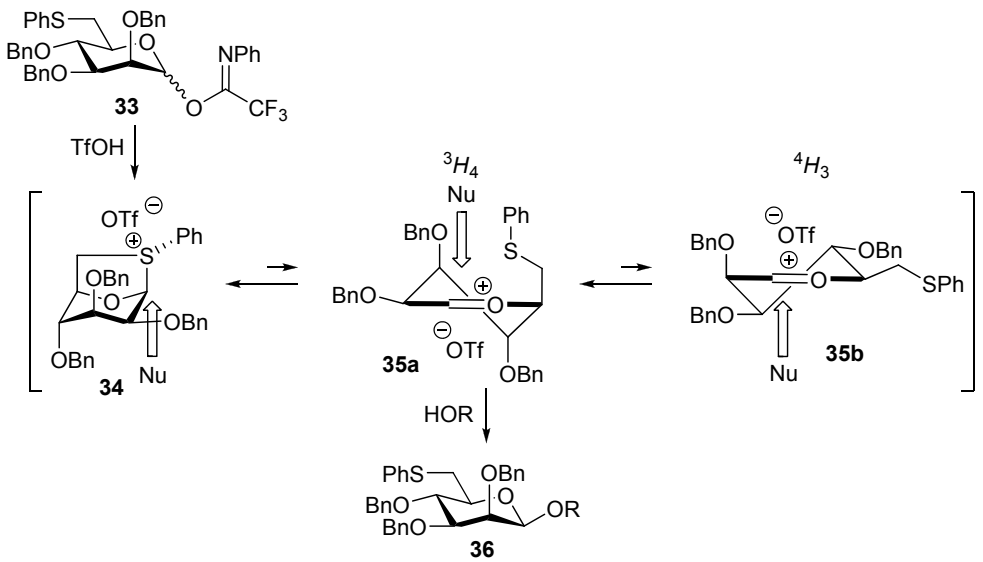
The effect of an azide functionality at the C2 position of galacturonic acid lactone **27** and galacturonic acid ester **28** is of interest as well, in particular since 2-acetamido-2-deoxy-galacturonic acid residues are found in several natural polysaccharides.[10]

Considerable experience has been gained concerning the glycosylations with donors **25** and **29**, the construction of pectin fragments **31**/**32** can be attempted (Scheme 4B). In a preliminary experiment donor **29** and acceptor **30** were coupled using Ph_2SO/Tf_2O as a promoter system. This led to the procurement of dimeric lactone **31** (n=1), albeit in low yield.

Furthermore a large excess of donor **29** had to be used to obtain this result. Usage of *para*-nitrophenylsulfenyl triflate (*p*-NO_2PhSOTf) as activator employing a pre-activation based protocol can provide a means to increase the efficiency of this glycosylation. A repetition of this coupling can give larger structures. The reason why this proposal elongates the chain from the reducing to the non-reducing end stems from the observation that the spacer-capped α-linked galacturonic acid lactone acceptors are intrinsically labile. Coupling of oligosaccharide **31** onto a suitable spacer, azidopropanol for example, hydrolysis or methanolysis of the lactone bridges followed by catalytic hydrogenation provides the target pectin oligosaccharides **31**/**32**.

Chapter 5a describes the application of a panel of C-6 thioether mannosyl donors, a C-6-selenoether donor and a C-6-iodide *N*-phenyltrifluoroacetimidate mannosyl donor in a series of condensation reactions. While all of these donors preferentially provided 1,2-*cis* linked disaccharides, a 2,3,4-tri-*O*-benzyl-6-deoxy-6-*S*-phenyl-6-thio-D-mannopyranosyl donor **33** showed the best potential as a 1,2-*cis*-mannosylating agent (Scheme 5). Variable temperature NMR experiments showed the formation of bridged sulfonium ion **34** upon activation of donor **33**. The stereoselectivity in the *cis*-mannosylation reaction can be rationalized with a Curtin-Hammett kinetic scenario in which the quasi-stable bicyclic $^{1}C_4$-sulfonium ion intermediate **34** is in equilibrium with the more reactive and β-selective mannosyl $^{3}H_4$-oxocarbenium ion and its α-selective $^{3}H_4$-conformer **35b**. Oxocarbenium ion **35a** places all ring-substituents in an electronically favorable position.

Scheme 5

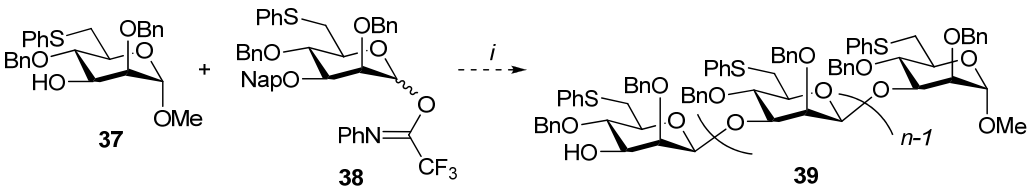


Mechanistic rationale for the formation of 1,2-*cis* mannosidic linkages from a C-6 thioether mannosyl donor.

The applicability of the 1,2-*cis*-mannosylating agent described above is the subject of **Chapter 5b**. Upon condensation of 6-thio-6-deoxy-mannosyl donors reductive removal of the 6-thio functionality provides 1,2-*cis* rhamnosides. Following this methodology a backbone tetrasaccharide containing alternating α- and β-D-rhamnosides was synthesized.

During the assembly of the tetrameric backbone, it was observed that the second glycosylation towards β-linked products proceeded less selective than the first one (completely β-selective versus a 1/3 α/β ratio). To investigate the influence of an elongating C-6 thiophenyl ether acceptor on the stereoselectivity of the glycosylations, the modular synthesis depicted in Scheme 6 is proposed.

Scheme 6



Reagents and conditions: (*i*) n times: (1) **38** and elongating **37**, cat. TfOH, DCM, -80°C; (2) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DCM, H_2O.

In conclusion, the development and application of different methods for the construction of 1,2-cis glycosidic bonds has been described in this thesis.

References and notes

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