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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. The introduction of 1,2-\textit{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-\textit{trans} product. The synthesis of 1,2-\textit{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-\textit{d}-galactose (AAT) building block is outlined. The unprotected progenitor hereof cannot be isolated from natural sources. The developed route of synthesis starts from \textit{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

\[
\begin{align*}
&\text{O} & \text{OTBDPS} & 1 & \xrightarrow{i} & \text{HN} & \text{Cl}_3\text{C} & \text{O} & \text{OTBDPS} & 2 \\
&\text{OH} & \text{O} & \text{OTBDPS} & 3 & \xrightarrow{i} & \text{HN} & \text{Cl}_3\text{C} & \text{O} & \text{OTBDPS} & 4 \\
&\text{HO} & \text{O} & \text{OTBDPS} & 5 & \xrightarrow{iv} & \text{Cl}_3\text{C} & \text{N} & \text{OTBDPS} & 6 \\
&\text{Nap} & \text{O} & \text{OTBDPS} & 7 & \xrightarrow{vi} & \text{H}_2\text{NCH}_2\text{CH}_2\text{NH}_2 & \text{BuOH} & 8 \\
&\text{Cl}_3\text{C} & \text{O} & \text{OTBDPS} & 9 & \xrightarrow{v} & \text{R}_2\text{NR}_1 & \text{OTBDPS} & 10 \\
&\text{R}_1 = \text{R}_3 = \text{H}, \text{R}_2 = \text{Cbz} & & & & & & & & & \text{R}_1, \text{R}_2 = \text{Phth}, \text{R}_3 = \text{Nap} & & & \text{R}_1 = \text{R}_2 = \text{H}, \text{R}_2 = \text{Nap}
\end{align*}
\]

Reagents and conditions: (i) Cl$_3$CCN, DBU, DCM, -13°C then Tf$_2$O, pyridine then DiPEA (4: 63%, 9: 24%); (ii) (1) AcOH, H$_2$O, EtOAc; (2) N-(benzylxycarbonyloxy)succinimide, triethylamine, DCM (5: 75%); (iii) (1) Bu$_2$SnO, toluene, reflux for 2 hours; (2) 2-(Bromomethyl)naphthalene, TBAI, toluene (57%); (iv) Tf$_2$O, pyridine, DCM; (v) potassium phthalimide, DMF (8: 48%, 10: 26%); (vi) H$_2$NCH$_2$CH$_2$NH$_2$, 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. was attempted (Scheme 1). Diol 1 was converted to C-3-O-methylnaphthyl ether 6 via an intermediate stanny 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 phthalamide, the resulting amine 11 can be functionalized as
please.

Recently, AAT was identified as a constituent of a polysaccharide found in the
human opportunistic pathogen Providencia alcalifaciens.3 Desesses 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 anomic TBDPS group to an N-phenyltrifluoracetimidoyl 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 synths 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).
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).
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 \( \text{para-nitrophenylsulfonyl triflate (p-NO}_2\text{PhSOTf)} \), \( N\)-phenylthio-\( \varepsilon \)-caprolactam-TMSOTf \) (or its tolyl derivative) \( \text{or dimethyl(methylthio)sulfonyl triflate (DMTST)} \) can be used. Judging from the insights gained in Chapter 4, it is advisable to use these promoters 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 anomic \( \beta \)-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, benzylxy 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 \( p\)-nitrophenylsulfonyl triflate shows, in 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 \( \alpha \)-selective glycosylation process, whereas an in-situ activation protocol leads to the formation of the \( \beta \)-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 \( \beta \)-triflate, which is substituted in a concerted fashion to provide the \( \alpha \)-product, an \( ^3\text{H}_4 \) oxocarbenium ion like species is substituted in the in situ activation experiment to provide the \( \beta \)-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, \( \text{para-nitrophenylsulfonyl triflate (p-NO}_2\text{PhSOTf)} \), \( N\)-phenylthio-\( \varepsilon \)-caprolactam-TMSOTf \) (or its tolyl derivative) \( \text{or dimethyl(methylthio)sulfonyl triflate (DMTST).} \)

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 \( \alpha \)-thioglycosyl counterpart of the studied galacturonic acid-3,6-lactone \( \beta \)-thioglycosyl donor 25. Most of the donors described in Chapter 4 reside in the \( ^1\text{C}_4 \) 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 $\sigma^*_{C1-S}$, 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$_2$PhSOTf, -60°C then acceptor 30; (ii) (1) TMSONa, DCM or MeOH, p-TsOH then H$_2$/Pd(C), tBuOH, H$_2$O, 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-deoxygalacturonic 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$_2$SO/Tf$_2$O 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-NO₂PhSOTf) 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 1C₄-sulfonium ion intermediate 34 is in equilibrium with the more reactive and β-selective mannosyl 3H₄-oxocarbenium ion and its α-selective 3H₃-conformer 35b. Oxocarbenium ion 35a places all ring-substituents in an electronically favorable position.

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 $\alpha$- and $\beta$-o-rhamnoses was synthesized.

During the assembly of the tetrameric backbone, it was observed that the second glycosylation towards $\beta$-linked products proceeded less selective than the first one (completely $\beta$-selective versus a 1/3 $\alpha/\beta$ 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**

10. (a) Kondakova, A. N.; Novototskaya-Vlasova, K. A.; Shashkov, A. S.; Drutskaya, M. S.; Senchenkova, S. N.; Shcherbakova, V. A.; Gilichinsky, D. A.; Nedospasov, S. A.; Knirel,