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**Title:** Synthesis of bacterial oligosaccharides: developments in the construction of cis-glycosidic linkages  
**Date:** 2012-12-11
Chapter 1

General Introduction: Challenges and Strategies in Modern Synthetic Carbohydrate Chemistry

Introduction

Even though carbohydrates are classically typified by their function in the storage and transport of energy, they are involved in many more crucial biological processes such as fertilization, embryogenesis, cell–cell recognition, neuronal development, cell growth, tumor cell metastasis and immune defense. This functional variety reflects the structural diversity of these (polymeric) biomolecules and the monomeric residues they are built up from. Besides the unequalled amount of chiral stereocenters, in comparison with the other classes of biopolymers, carbohydrate ring size and regiochemistry in terms of interresidual connectivity are dimensions that contribute to the structural diversity. To unravel the mechanistic details concerning carbohydrate-mediated biological processes, pure and structurally well-defined oligosaccharides and glycoconjugates can serve as valuable tools. Extraction of carbohydrates or glycoconjugates from natural sources is often very difficult, if not impossible, or does not provide the samples in sufficient purity and/or quantity to allow for the establishment of their structure-activity relationships. Organic synthesis can provide a solution to these shortcomings.
One of the most challenging aspects in synthetic carbohydrate chemistry is the stereoselective introduction of glycosidic linkages.\(^1\) 1,2-\textit{Trans} bonds can generally be introduced in a reliable way by equipping a glycosyl donor with an acyl functionality on the C-2 position. Upon activation of the donor a transient dioxolenium ion is formed, directing the glycosylation event towards the 1,2-\textit{trans} product. 1,2-\textit{Cis} bonds however, are less straightforward to construct. By the hand of selected examples, this Chapter illustrates the challenges in modern synthetic carbohydrate chemistry and describes recently introduced strategies for the stereoselective introduction of \textit{cis}-glycosidic bonds.

Zwitterionic polysaccharides (ZPSs) are the only known class of polysaccharides capable of eliciting a T-cell dependent immune response.\(^2\) The availability of well-defined polysaccharide fragments can contribute to the elucidation of the mechanism of action at a molecular level. In addition, their structural complexity makes zwitterionic oligosaccharides attractive synthetic targets. Sp1 is a type 1 zwitterionic polysaccharide found on the outer layer of the cell wall of \textit{Streptococcus pneumonia}. The repeating unit of Sp1 \([\alpha-d-GalA-(1\rightarrow3)-\alpha-d-Fuc(4-N)NAC-(1\rightarrow4)-\alpha-d-GalA-(1\rightarrow3)]\) contains two negatively charged carboxylate groups on the GalA residues and one positively charged amino group on the rare 4-amino-N-acetyl fucosamine moiety. Bundle and co-workers were the first to synthesize monomeric and dimeric repeats of the Sp1 polysaccharide.\(^3\) Their synthetic approach to construct the 1,2-\textit{cis} glycosidic linkages entailed the use of C-6-O-acetyl esters as remote directing protective groups, during the glycosylation event (see Scheme 1). Although there is ongoing debate as to whether 6-O-acyl functions actually steer the stereochemistry of glycosylations, there are reports indicating the beneficial effect of remote acyl functionalities on the formation of \(\alpha\)-glycosidic linkages.\(^4\) The C-6-O-acetyl groups were also used as precursors for the GalA carboxylate functions to circumvent difficulties with the low reactivity of the GalA residues.\(^5\)

As a result, a strategy was developed in which the 4-amino-N-acetyl fucosamine moiety is constructed in a disaccharidic stage and the GalA carboxylate functions are introduced after the execution of all necessary glycosylation steps. Thus, galactoside acceptor 1 was glycosylated with diacetyl glucosazide imidate donor 3 using TMSOTf as promoter to provide disaccharide 5 in 60\% with excellent \(\alpha\)-selectivity. Because a trichloroacetimidate based glycosylation proved ineffective in the assembly of the second dimer 6, a dehydrative coupling between lactol 4 and thexyldimethylsilyl galactoside 2 was employed. In this event dimer 6 was formed in 73\% as a single anomer. Now, the glucosazide residue of both dimers was converted to a diamino fucose residue in 7 steps: azide reduction, \(N\)-acetylation, O-deacetylation, 6-O-mesylation, \(\text{NaBH}_4\) reduction, triflation and azide substitution. Deprotection of the PMB group, acetylation and deallylation gave acceptors 7 and 8 in 10\% and 20\% over 10 steps, respectively. Dimers 7 and 8 were glycosylated with thioglycosides 9 and 10, using NIS and a catalytic amount of silver triflate to provide trisaccharides 11 and 12, respectively. Trimer 11 was transformed into acceptor 12 by treatment with DDQ (73\%) and donor 14 was constructed from trisaccharide 13 by removal of the anomeric thexyldimethylsilyl (TDS) group and subsequent trichloroacetimidate formation. The union of the two trisaccharide parts required careful tuning of the reaction conditions (temperature,
Synthesis of a Sp1-hexamer fragment. **Reagents and conditions:** (i) TMSOTf, CH₂Cl₂, -15°C → rT, 60%; (ii) Ph₃SO, Tf₂O, TTBP, CH₂Cl₂, -25°C, 73%; (iii) H₂S, pyridine, H₂O, Et₃N; (iv) Ac₂O, pyridine, 74% over two steps for 5, 75% over two steps for 6; (v) NaOMe, MeOH; (vi) MsCl, pyridine, -15°C → 0°C, 71% over two steps (α-OMe), 95% over two steps (β-OTDS); (vii) DMSO, NaBH₄, 85°C, 80% (α-OMe), 85% (β-OTDS); (viii) Tf₂O, pyridine, CH₂Cl₂, -30°C; (ix) NaN₃, DMF, rT, 57% over two steps (α-OMe), 62% over two steps (β-OTDS); (x) α-OMe: TFA (1%) in CH₂Cl₂; β-OTDS: DDQ, CH₂Cl₂, H₂O; (xi) Ac₂O, pyridine, 80% over two steps (α-OMe), 80% over two steps (β-OTDS) dimer; (xii) PdCl₂, NaOAc, AcOH, H₂O, 53% (α-OMe), 66% (β-OTDS); (xiii) NIS, AgOTf, CH₂Cl₂, -30°C → rT, 79% (11), 66% (13); (xiv) CH₂Cl₂, H₂O, DDQ, 73%; (xv) (1) TBAF, HOAc, THF, 92%, (2) CCl₃CN, DBU, CH₂Cl₂, 71%; (xvi) TMSOTf, CH₂Cl₂, 85%; (xvii) (1) NaOMe,
MeOH; (2) TBABr, NaHCO₃, TEMPO, CH₂Cl₂, NaOCl; (3) HCl, tBuOH, 2-methylbut-2-ene, NaClO₂, NaH₂PO₄; (4) CsF, BnBr, DMF, 52%; (xviii) H₂, Pd(OH)₂, CH₂Cl₂, MeOH, H₂O, 53%.

 donor equivalents and amount of Lewis acid activator) and was accomplished in 85% yield. The fully protected hexasaccharide was deacetylated to give the tetraol, which was oxidized in a two-step procedure to provide the tetracarboxylate. Benzylation then gave hexamer 16 in 52% over the last steps. Hydrogenolysis of all benzyl ethers and esters and the two azide groups gave the zwitterionic hexasaccharide 17.

Polysaccharide A1 (PS A1) is a ZPS which is found on the capsule of the commensal bacterium Bacteroides fragilis. It consists of the tetrasaccharide repeating unit [−3]−α-d-Fuc(4-N)NAc−(1→4)[β-d-Galf−(1→3)]−α-d-GalNAc−(1→3)−β-d-Gal−(1→3)] bearing a positive charge on a diaminofucose residue and a negative charge on a pyruvate that spans positions 4 and 6 of a galactose residue. The presence of two 1,2-cis linkages, one of which is connected to an axially oriented galactoside C-4-OH, and the previously encountered diaminofucose residue make PS A1 a challenging synthetic target. The first synthesis of a protected PS A1 tetrasaccharide repeating unit was reported by van den Bos et al.⁶ and, more recently, the group of Seeberger described the synthesis of the repeating unit structure 28 (Scheme 2).⁷

Since two routes of synthesis, comprising coupling of a protected diaminofucose derivative onto the poorly nucleophilic axially oriented galactoside C-4-OH in a trimeric and dimeric stage failed, an alternative order of glycosylation events was followed. In the first coupling, galactosazide acceptor 19 was united with fucosyl donor 18 bearing a non-participating C-2-azido group, to afford α-linked disaccharide 20. After DDQ mediated removal of the 2-naphthylmethyl (NAP) ether, the resulting acceptor 21 was coupled with galactofuranoside 22. Neighbouring group participation (NGP) ensured the formation of the β-linkage in trisaccharide 23. The anomeric tert-butylidimethylsilyl (TBS) group in this trimer was converted to an N-phenyl trifluoroacetamidate functionality to provide the requisite donor for the last glycosylation event. Since this donor proved to be ineffective for the construction of the tetramer, the imidate group was replaced by an anomeric thioethyl function. Several activation methods (NIS/AgOTf, MeOTf, Ph₂SO/Tf₂O) were examined to condense thioglycoside 25 and pyruvate galactoside 26 and eventually tetrasaccharide 27 was obtained in 58% when DMTST was used as a promoter. To complete the synthesis of fragment 28 the azido functions were first converted to acetamides by reaction with thiolacetic acid. Hydrogenation with Pearlman’s catalyst preceeded basic removal of the ester groups because reversal of the reaction sequence led to the formation of a cyclic carbamate. The final basic treatment leading to target tetrasaccharide 28 was effectuated in THF to prevent acetyl migration to the 4-amino group of the diamino fucose residue. The syntheses described above show that complex structures such as 17 and 28 can be assembled using state-of-the-art chemistry, but at the same time considerable optimization is required for the construction of the interglycosidic linkages. Although the in-
Synthesis of the tetrasaccharide repeating unit of PS A1. Reagents and conditions: (i) TMSOTf, CH₂Cl₂, 0°C, 74%; (ii) DDQ, MeOH, CH₂Cl₂, 23°C, 86%; (iii) TMSOTf, CH₂Cl₂, -30°C, 90%; (iv) (1) TBAF, AcOH, THF, 0°C; (2) F₃CC(NPh)Cl, Cs₂CO₃, CH₂Cl₂, 23°C, 82% over two steps; (v) EtSH, TMSOTf, CH₂Cl₂, 0°C, 96%; (vi) DMTST, TTBP, 0°C, 58%; (vii) AcSH, pyridine, 23°C, 67%; (viii) (1) H₂, Pd(OH)₂/C, MeOH, 23°C; (2) THF, then 0.5 M NaOMe in 1:1 MeOH/H₂O, 23 °C, 46% over two steps.
better heptopyranoside, cyano still set thioglycoside reactions involved uncommon position, stereoselective Plesimonas donor tetrasaccharide combination. trisaccharide use naphthylmethyl group. 

In synthesis of a tetrasaccharide subunit of a lipopolysaccharide from Plesimonas shigelloides, Crich et al. further extended the benzylidene β-directing principle (see Scheme 3). The assembly of tetrasaccharide comprised the incorporation of two uncommon β-linked heptose residues, one of which being a 6-deoxy moiety. Therefore the 1-cyano-2-(2-iodophenyl)ethylidene group was introduced as a 4,6-O-benzylidene surrogate set up for deoxygenation by radical fragmentation. The assembly of the target tetrasaccharide started off with the glycosylation of methyl rhamnoside with thioglycoside donor following a pre-activation protocol with the Ph2SO/Tf2O promoter combination. Because of the electron withdrawing cyano group on the benzylidene ketal the use of this promotor system was required, for it generates a somewhat more reactive electrophile in comparison to the BSP/Tf2O reagent system, originally developed by Crich and co-workers. Disaccharide was obtained in 86% yield with complete β-selectivity. Treatment of this disaccharide with tributyltin hydride and AIBN afforded a 6-deoxy-manno-heptopyranoside, which was transformed into acceptor by DDQ mediated removal of the 2-naphthylmethyl group. In the next preactivation based glycosylation event, this time using donor in combination with the BSP/Tf2O promoter system, the second 1,2-cis bond was introduced to provide the all cis-linked trisaccharide. Oxidative cleavage of the 2-naphthylmethyl group then set the stage for the final coupling step with thiorhamnoside. The α-directing nature of this donor had been previously established by the same group. Thus pre-activation of thiorhamnoside with BSP/Tf2O and subsequent addition of trisaccharide afforded tetrasaccharide as a single α-stereoisomer in 73% yield. Saponification followed by acid treatment gave a mixture of benzylidene protected and unprotected tetrasaccharides and in 85% combined yield. Hydrogenolysis of the individual tetrasaccharides gave target in 94% and 96% yield from and , respectively.

Van den Bos et al. reported that mannnuronic acid donors can also be used for the stereoselective construction of 1,2-cis mannosyl linkages. Interestingly, the triflates generated from mannnuronic acid donors preferentially reside in a flipped \( ^1_4 \)-chair confor-
Scheme 3

29 \[\text{SPh} \] \rightarrow 30 \textit{i} \rightarrow 31 \textit{ii} \rightarrow 32 \textit{iii} \rightarrow 33 \rightarrow 34 \textit{iv} \rightarrow 35 \rightarrow 36

\text{38 R = benzylidene}
\text{39 R = H}
Synthesis of a tetrasaccharide subunit from *Plesimonas shigelloides*. Reagents and conditions: (i) 29, Ph$_2$SO, Tf$_2$O, TTBP, CH$_2$Cl$_2$, -20°C then 30, 86%; (ii) (1) Bu$_3$SnH, AIBN, xylene, Δ (2) DDQ, 57%; (iii) (1) 33, BSP, Tf$_2$O, TTBP, CH$_2$Cl$_2$, -60°C then 32 (2) DDQ, 6:1 CH$_2$Cl$_2$/H$_2$O, 88%; (iv) 35, BSP, Tf$_2$O, TTBP, CH$_2$Cl$_2$, -60°C then 34, 73%; (v) (1) NaOMe, MeOH (2) TFA, CH$_2$Cl$_2$ then tris (2-aminoethyl) amine polymer, 36% (38) and 49% (39); (vi) H$_2$, Pd(OH)$_2$/C, MeOH, 94% from 38, 96% from 39.

To date, the mannanuronic acid donors are amongst the most reliable donors to provide β-mannoside linkages,$^{15}$ and this was efficiently exploited in the automated solid phase synthesis of β-(1,4)-mannuronic acid alginate oligomers by Walvoort et al.$^{16}$ In the optimization process prior to the synthesis, the optimal reaction cycle, including the types of
reagents, the stoichiometry, the reaction times, the temperature and wash procedures were established.

It was also found that performing the repetitive glycosylations at 0°C gave a 1:3 α/β mixture of anomeric diastereomers. To improve this anomeric ratio and assure the intermediacy of α-anomeric triflates, the reaction temperature was lowered just below the decomposition temperature of the intermediate triflate (-40°C). This resulted in completely β-selective glycosylation reactions. The synthesis of dodecasaccharide 48 is outlined in Scheme 5. Butenediol-functionalized polystyrene resin 44 was subjected to 12 repetitive automated coupling/deprotection cycles. After release from the resin by cross-metathesis, the methyl esters of alcohol 46 were saponified. At this stage, the target dodecamer was separated from smaller oligomannuronic acid fragments. Dodecamer 47, featuring 12 cis-mannosyl linkages was obtained in 11% yield over 24 steps, representing an average yield of 90% per step. Hydrogenolysis catalyzed by palladium on charcoal gave the target propyl alginate 48.

Another alginate fragment, built up of L-guluronic acid -the C-5 epimer of D-mannuronic acid- monomers, was synthesized by Hung and co-workers. Their solution phase approach was hampered by the poor nucleophilicity of the axially oriented C-4-OH. To overcome this obstacle, Hung’s group resorted to the use of 1,6-anhydrogulopyranosyl 4alcohol 50 as a more reactive acceptor, because this rigid bicyclic building block places the 4-hydroxyl in an equatorial position. This key 1,6-anhydro bridge necessitated the construction of the target tetramer to be executed from the non-reducing to the reducing end (Scheme 6). Coupling of donor 49 with acceptor 50 gave the desired α-linked disaccharide in 70% yield along with 17% of the β-epimer. The authors ascribe the preferential formation of the α-linked product to the anomeric effect and nonparticipating nature of the O-2 benzyl group. However, other factors have been brought forward to account for the observed α-selectivity of gulose donors. The gulose substituents in the oxocarbenium-ion conformer generated upon activation are all positioned to favor the 3H₄ low energy conformer, which can be substituted from the -face leading to the 1,2-cis product (Scheme 7). The possible remote anchimeric assistance of the 6-O-acetyl group of donor 49 can also be of beneficial influence to the stereochemical outcome in this particular case. Next, opening of the anhydro-bridge followed by selective anomeric deacetylation with H₂NNH₂/AcOH gave lactol 52. Conversion of this hemiacetal to the corresponding trichloroacetimidate primed the dimer for another coupling reaction with 50. This glycosylation proceeded with complete α-stereoselectivity in 78% yield. Repetition of the last four steps gave tetramer 55 (coupling 68%, α product only). Again the bicyclic structure was disrupted under acidic conditions and the resulting diacetate was selectively deprotected. Initial attempts in a disaccharide stage to install an anomeric allyl functionality employing a TMSOTf catalyzed coupling with an imidate donor gave unsatisfactory results in terms of selectivity (8% α, 68% β). Since Williamson etherification afforded the desired α-product as a single isomer in good yield, this is the method of choice for the conversion of lactol 56 to allyl tetrasaccharide 57. It was suggested that the observed high stereoselectivity is induced by chelation of the potassium counterion with the C-1
Synthesis of a dodecasaccharide alginate. Reagents and conditions: (i) 12 times: (1) 45, TfOH, CH₂Cl₂, -40°C; (2) H₂NNH₂·HOAc, pyridine, AcOH; (ii) Grubbs 1 catalyst, H₂CCH₂; (iii) KOH, THF, H₂O; (iv) H₂, Pd/C, THF, H₂O, tBuOH.

alkoxide and the lone-pair electrons of O-2, in a 1,2-cis constellation. Cleavage of the acetyl groups in 57 unmasked the primary alcohols which were oxidized to carboxylic acids with TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy free radical) employing BAIB (bis-acetylxyiodobenzene) as a co-oxidant to give partially protected tetramer 58. A hydrogenolysis step completed the synthesis of the all-cis-linked guluronic acid tetramer 59.
Synthesis of a guluronic acid containing alginate tetrasaccharide. Reagents and conditions: (i) cat. TMSOTf, CH$_2$Cl$_2$, -86°C, 1 h, 70% (51a), 17% (51b); (ii) 1.TFA, Ac$_2$O, 0°C, 16 h; 2. H$_2$NNH$_2$·AcOH, DMF, 0°C  →  rT, 6 h, 79% (52), 72% (toward 55), 66% (56) in two steps; (iii) CCl$_3$CN, K$_2$CO$_3$, CH$_2$Cl$_2$, rT, 5 h, 89% (53), 89% (toward 55); (iv) 50, cat. TMSOTf, CH$_2$Cl$_2$, -86°C, 3 h, 78% (54), 68% (55); (v) tBuOK, H$_2$C=CHCH$_2$Br, tBuOH, 0°C → rT, 2 h, 71%; (vi) 1. NaOMe, MeOH, rT, 2 h; 2. TEMPO, BAIB, CH$_2$Cl$_2$, H$_2$O, rT, 1 h, 51% in two steps; (vii) H$_2$, 10% Pd/C, MeOH, H$_2$O, AcOH, rT, 12 h, 93%. 

Scheme 6
The same factors that render the stereoselective introduction of 1,2-cis mannosidic and the aforementioned manno-heptopyranosidic linkages difficult have to be dealt with in the synthesis of β-rhamnosides. Crich and Li recently reported a solution to this problem with an adaptation of their benzylidene mannoside strategy. In this approach the C-6 oxygen was replaced by a C-6-sulfur atom to give a benzylidene-thio acetal, which provided the necessary -directing effect and allowed for the straightforward installation of the rhamnosyl C-5 methyl functionality by Raney nickel reduction of the thioether. An alternative approach to 1,2-cis rhamnoside linkages has been reported by Ito and co-workers who made use of an intramolecular aglycon delivery (IAD) strategy. This method, originally developed by Hindsgaul in 1991 for the introduction of β-mannosidic linkages, entails the tethering of a glycosyl acceptor to the C-2 hydroxyl of a donor. In a subsequent intramolecular glycosylation step the acceptor is delivered from the same face of the donor as the C-2 hydroxyl, yielding a 1,2-cis linkage. The IAD method initially employed a dimethyl acetal for tethering, but nowadays, different acetal and ketal groups have been developed and the methodology has been shown to be compatible with different anomic leaving groups and glycosylation conditions. In addition to β-mannosides other anomic linkages have been synthesized by IAD, including α-glucosides, α-glucofuranosides and β-arabinofuranosides. Ito and co-workers used an IAD approach to construct the β-rhamnose bond in their synthesis of a trimeric substructure of a polysaccharide from Sphaerotilus natans as depicted in Scheme 8.

A mixed naphthylmethylidene linkage was formed between alcohol 62 and C-2-O-naphthylmethyl donor 61 under oxidative conditions. Next, activation of the resulting thiomethylglycoside 63 with MeOTf afforded disaccharide 65 via an IAD pathway and subsequent trapping of the benzyl cation by the neighboring silyl ether. Regioselective reductive opening of the naphthylmethylidene gave acceptor 66 in 87% yield. Straightforward introduction of an α-rhamnose linkage employing 2-O-acetyl donor 67 led to a fully protected trisaccharide 68. Global deprotection was done in two steps, basic deacetylation and hydrogenation, to give target 69.

As described above, mannuronic acid donors have been employed in an automated solid phase setting for the introduction of 1,2-cis mannosidic linkages on-resin. Boons and co-workers have recently reported on the stereoselective construction of 1,2-cis- glucosyl and
Synthesis of a tetrasaccharide subunit from *Plesimonas shigelloides*. Reagents and conditions: (i) DDQ, CH₂Cl₂, 93%; (ii) MeOTf, DTBMP, (CH₂Cl)₂; (iii) DIBAL-H, toluene, 87%; (iv) TMSOTf, Et₂O, -30°C, 3h, 95%; (v) (1) NaOMe, MeOH, CH₂Cl₂, 98%; (2) H₂, Pd(OH)₂/C, MeOH, AcOH, 96%.

1,2-cis-galactosyl linkages on-resin. Boons’ stereoselective induction of 1,2-cis linkages is based on the use of a (S)-(phenylthiomethyl)benzyl chiral auxiliary at the C-2-OH.²² Upon activation of the anomic leaving group the auxiliary forms a trans-decalin β-
sulfonium ion with the phenyl substituent placed in an equatorial position. The \(\alpha\)-sulfonium ion would possess an axially oriented phenyl group that would suffer from sterically unfavorable interactions with H-3. Electron- withdrawing acyl functionalities on the remaining alcohol groups are needed to promote the formation of a sulfonium ion intermediate through suppression of oxocarbenium ion generation. Substitution by alcohols of the \(\beta\)-sulfonium species occurs in an \(S_n2\)-like manner leading to the desired 1,2-cis stereochemistry (Scheme 9).

\[\text{Scheme 9}\]

![Scheme 9 diagram]

Anchimeric assistance by Boons’ \((S)-\text{(phenylthiomethyl)}\)benzyl chiral auxiliary.

This method was applied in the assembly of an \(\alpha\)-glucan pentasaccharide repeating unit found in \(A.\ \text{carmichaeli}\).\(^{23}\) The synthesis is shown in Scheme 10 and commenced with the TMSOTf catalyzed union of polystyrene resin-bound alcohol 74 and donor 75. The glycosylation was carried out by pre-activation of the donor with a stoichiometric amount of TMSOTf at -40°C. The formed intermediate sulfonium ion was added to the resin bound acceptor. Alloc deprotection of the resulting disaccharide furnished the C-3-OH acceptor, which proved to be rather inreactive because of significant steric shielding, as revealed in model studies. Therefore, the \((S)-\text{(phenylthiomethyl)}\)benzyl group was converted to an acetyl function with \(\text{Ac}_2\text{O}\) and \(\text{BF}_3\cdot\text{OEt}_2\) prior to the Alloc removal and ensuing glycosylation event, in which pre-activated donor 78 was condensed with resin bound dimer 77. After Fmoc deprotection, the same glycosylation protocol was followed using donor 81 to construct the third \(\alpha\)-glycosidic bond. Again removal of the Fmoc preceded the final coupling toward the fully protected resin-bound branched pentaglucan 84. Conversion of the chiral auxiliaries to acetyl functions, Fmoc deprotection, release of the glucan from the resin under Zemplén conditions and reacetylation gave, after size exclusion chromatography, a pentasaccharide as the major product with its mono-debenzylated counterpart as a side product. From this mixture the stereochemical integrity of the introduced glycosidic linkages could be determined and no anomeric \(\beta\)-isomers were detected. After 13 steps on resin the overall yield was 25%, corresponding to a yield of 90% per step. Finally, deacetylation and hydrogenation gave the target pentamer 85. In addition, the authors showed that the same methodology could be used to construct a galactose containing analogue of pentasaccharide 85.
Solid supported synthesis of an α-glucan pentasaccharide repeating unit found in A. *carmichaeli*. 

Reagents and conditions: (i) 75, 78 or 81, TMSOTf, CH$_2$Cl$_2$, MS 4 Å, 15 min, -40°C then added to 74, 77 or 80, DTBMP, CH$_2$Cl$_2$, MS 4 Å, 16 h, -40°C → rT; (ii) BF$_3$•Et$_2$O, Ac$_2$O, CH$_2$Cl$_2$; (iii) Pd(PPh$_3$)$_4$ (40 mol%), THF, AcOH; (iv) piperidine, DMF; (v) NaOMe, MeOH, CH$_2$Cl$_2$; (vi) Ac$_2$O, pyridine; (vii) Pd(OH)$_2$/C (20 wt%), H$_2$, EtOH/H$_2$O (1/1, v/v); PS, polystyrene.

Recently several methods have been introduced for the stereoselective formation of furanosyl 1,2-cis-linkages. For example, the group of Lowary has found that 2,3-anhydropentosyl thioglycosides and sulfoxides can be used as stereoselective 1,2-cis directing donors.$^{24}$ The mechanistic principle behind the selectivity was addressed through computational chemistry and low-temperature NMR spectroscopy, which identified anomeric triflates as glycosylating species.$^{25}$ Following glycosylation, the epoxide can be opened to provide the desired cis-furanoside. A recent and illustrative example of this approach is the synthesis of trisaccharide 94 from 2,3-anhydro-d-gulofuranosyl sulfoxides, as depicted in Scheme 11.$^{26}$ This trisaccharide is structurally related to an antigenic polysaccharide from *Eubacterium saburreum* strain T19. By varying the protective group pattern on the key 2,3-anhydro-d-gulofuranosyl sulfoxide donor it was found that a benzoyl protected building block gave the best results in terms of stereoselective coupling and subsequent epoxide opening. Thus, the assembly of the target trisaccharide started with the coupling of anhydro donor 86 with acceptor 88. This was done using conditions which ensured the intermediacy of triflate 87 and S$_2$N$_2$-like substitution of this intermediate gave dimer 89 with complete α-selectivity. Opening of the epoxide with LiOBn and (−)-sparteine and concomitant benzoyl deprotection gave triol 90 in 69% yield along with 5% of its regioisomer.

Although the mechanistic details underlying the regioselectivity the epoxide opening have not been determined, a specific lithium-sparteine-epoxide complex has been proposed to account for the observed regiochemistry. Acid-mediated installment of an isopropylidene acetal on dimer 90 gave alcohol 91, which was subjected to the next cis-furanosylation event.
Synthesis of a trisaccharide related to *Eubacterium saburreum* strain T19. Reagents and conditions: (i) 86, Tf₂O, DTBMP, CH₂Cl₂, -78°C 10 min, then -40°C 20 min, then 88 or 91, 30-60 min, 72% (89), 59% (92); (ii) LiOBn, (-)-sparteine, BnOH, 75°C, 69% (90), 61% (93); (iii) (CH₃)₂C(OCMe)₂, acetone, p-TsOH, rt, 89%; (iv) AcOH, H₂O, 50°C, 81%; (v) H₂, Pd(OH)₃/C, MeOH, rt, (quant.).

Prolonged reaction times were necessary for the glycosylation of acceptor 91, which most probably is due to the sterically hindered nature of the acceptor. Opening of the epoxide in 92 gave triol 93 in 61% alongside 13% of the regioisomeric product. Acidic hydrolysis of the
acetal and catalytic hydrogenation took place uneventfully to afford the desired target trisaccharide 94.

Besides stereospecific reactions on anomic triflate intermediates, furanosyl oxocarbenium ions have also been exploited for the introduction of cis-furanosidic linkages. Boons and co-workers described that L-arabinofuranosyl donors and the corresponding oxocarbenium ions can be locked in a \( E_3 \) conformation. A computational study revealed that the L-arabinofuranosyl oxocarbenium ion can take up two possible low-energy ion conformers \( ^3E \) and \( E_3 \) (Scheme 12). Attack on the \( ^3E \) conformer by a nucleophile proceeds preferably from the diastereotopic face that leads to 1,2-\( \text{trans} \) product. This is due to unfavorable interactions with the eclipsed C-2 substituent on the \( Re \)-face of the oxocarbenium ion and the presence of only staggered substituents on the \( Si \)-face.

Scheme 12

L-Arabinofuranosyl oxocarbenium low-energy ion conformers.
Synthesis of a fragment of arabinogalactans. Reagents and conditions: (i) NIS, AgOTf, DCM, MS 4Å, -20 °C, 100 (67%), 102 (89%); (ii) H₂NNH₂, DCM/MeOH, 80%; (iii) TBAF, THF; (iv) NaOMa, MeOH, 50 °C; (v) Pd/C, H₂, pyridine; (vi) Pd(OH)₂, H₂, AcOH, H₂O, 34% (over 4 steps).
Through the same line of reasoning it becomes evident that attack of the E₃ conformer occurs preferably from the Si-face, giving the 1,2-cis product. To lock the arabinofuranosyl donor in the E₃ conformation, 3,5-di-tert-butylsilane protected thiodonor 95 was designed. The donor places C-5 and O-3 in pseudoequatorial positions, resulting in a perfect chair conformation of the protecting group. The efficiency of the methodology was illustrated by the synthesis of an arabinogalactan fragment, a constituent of the primary plant cell wall. A part of the synthesis is shown in Scheme 13. Trisaccharide 99 was constructed in 4 steps using thioglycoside donors and following an approach from the reducing to the non-reducing end. Introduction of the first arabinose moiety was accomplished by a NIS/AgOTf mediated coupling employing silylidene donor 95. The tetrasaccharide product was obtained in 67% yield with complete β-selectivity. Liberation of the non-reducing end C-6-OH by levulinoyl deprotection afforded acceptor 101. The second arabinose residue was coupled to this acceptor using as the same promoter system and pentamer 102 was obtained as a single diastereomer. Global deprotection of this product was achieved in four steps, entailing removal of the silyldiene groups with TBAF, saponification of the acetyl and benzoyl esters under Zemplén conditions, reduction of the azide moiety to an amine and final catalytic hydrogenolysis of the benzyl ethers, giving the fully deprotected target pentasaccharide 103. It is interesting to note that in the benzylidene mannopranosyl system, a ketal functionality is used to favor formation of an anomeric triflate, whereas the silylidene ketal in donor 95 serves to promote the formation of a single oxocarbenium ion intermediate.

Conclusion

Glycosylation reactions can proceed via a multitude of pathways, passing through a variety of reactive intermediates. Because all these intermediates have their specific reactivity and associated selectivity, predicting and controlling the stereochemical course of glycosylation reaction can be a precarious undertaking. And although our understanding of the stereoelectronic effects, controlling the stereochemistry in the formation of the glycosidic bond, is continuously growing, optimization of a glycosylation reaction is often still a game of trial and error. This chapter has described some recent developments aimed at effecting stereoselective glycosylations in the context of complex carbohydrate synthesis. From the presented examples it becomes clear that there is a broad pallet of reaction intermediates that can be summoned to achieve this goal. The key to success in these approaches are to promote one reactive intermediate over another and the judicious tuning of the carbohydrate core.

Outline of this Thesis

As described above 2-acetamido-4-amino-2,4,6-trideoxy-β-D-galactose (AAT) is a rare carbohydrate residue present in polysaccharides of various infectious bacteria. To gain more
insight into the roles played by these polysaccharides in pathologic pathways, access to pure fragments of polysaccharides is of importance. Therefore the synthesis of these polysaccharides and AAT, as a consequence, has attracted quite some attention. One of the obstacles in these syntheses is presented by the procurement of sufficient amounts of an AAT-building block. Chapter 2 describes the synthesis of an orthogonally protected AAT-building block on multigram-scale from d-glucosamine. A key feature of the synthetic strategy is the introduction of the C-4 amino substituent, which is accomplished by a one-pot three-step procedure, involving regioselective C-3-O-trichloroacetimidate formation, C-4-O-triflation, and intramolecular substitution. The constructed AAT-building block is used in syntheses of all possible trimer repeating units of the type 1 capsular polysaccharide of *Streptococcus pneumoniae*, Sp1, which are described in Chapter 3. Key feature of all assemblies is the introduction of the required 1,2-cis galacturonic acid linkages by employing α-selective galacturonic acid-[3,6]-lactone building blocks. These synthons do not only perform well when used as donor galactosides, they also show to be reactive acceptor glycosides when equipped with a free hydroxyl function. All but one of the three frame-shifted trimer repeats was constructed via highly stereoselective glycosylation reactions. The epimeric mixture of trisaccharides, formed in the unselective glycosylation event, could be readily separated after global deprotection using high performance anion-exchange chromatography (HPEAC). An investigation of both the reactivity and the stereoselectivity of the used galacturonic acid-3,6-lactone thioglycosides is described in Chapter 4. Herein a series of competitive glycosylation experiments using different thiophilic activator systems are described and it is shown that the relative reactivity of different thioglycosides depends significantly on the activator system used. With respect to the stereoselectivity of the studied galacturonic acid-3,6-lactone thioglycoside donor, it was found 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. Chapter 5a describes the assembly of mannosyl donors, equipped with different thio ether linkages at C-6. Activation of these donors leads to the formation of bicyclic sulfonium ions, which serve as a reservoir for their more reactive monocyclic oxocarbenium ion counterparts. Nucleophilic attack of these species preferentially gives 1,2-cis linked products. This finding is exploited in Chapter 5b, where a synthesis of a tetrasaccharide found in *Xanthomonas campestris* is described. In addition to the stereoselective formation of 1,2-cis glycosidic linkages the synthesis features the reduction of C-6 thio ethers to gain access to rhamnoses.

References and notes

Chapter 1


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