Chemical synthesis of fragments of galactosaminogalactan and pel polysaccharides

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Chapter 1

General Introduction

1. Introduction

Carbohydrates are one of the most structurally diverse biopolymers on earth. They play crucial roles in every corner of biology, besides as an energy source, in cell signaling, pathogen recognition, inflammation, modulation of innate immune response, etc.[1-5] To unravel the role of carbohydrates in biological processes, pure and well-defined carbohydrates are a prerequisite. However, isolation of carbohydrates from natural sources is often impractical because of the microheterogeneity and/or biological impurities. Chemical synthesis is therefore an important approach to provide these oligosaccharides. Although tremendous progress has been made in carbohydrate chemistry, the assembly of complex oligosaccharides and glycoconjugates continues to be a challenging task, requiring a huge

time and labor investment.[6-10] The stereoselective construction of glycosidic linkages is key to success in the synthesis of oligosaccharides. The glycosylation reaction, indeed a central theme of carbohydrate chemistry, usually involves the condensation of a donor with a leaving group at the anomeric position and a nucleophilic acceptor, under influence of a catalyst or promotor to yield a coupled saccharide.[11] The formation of α/β-mixtures during glycosylation often results in a time-consuming purification process, thus decreasing the efficiency of oligosaccharide assembly. While 1,2-*trans* glycosides can be reliably formed using neighboring group participation by acyl protecting groups, the construction of 1,2-*cis* linkages is more difficult. To overcome this issue, many strategies have been developed to stereoselectively introduce these glycosidic linkages, including intramolecular aglycon delivery[12-14], the use of six-membered ring containing chiral auxiliaries[15-18], conformational constrained glycosyl donors[19-26], additive controlled glycosylations[27-29], hydrogen bond-mediated aglycon delivery[30], *etc*. However, none of these methods represents a general solution to the problem, each having its distinct advantages and disadvantages.[31] In this context, the development of innovative methodologies to efficiently provide various glycoconjugates, is strongly desired.

Amino sugars, an important type of carbohydrates, are characterized by the replacement of at least one of its hydroxyl groups by a (substituted) amino group. 2-Amino-2-deoxy-glycosides, such as glucosamine (GlcN), galactosamine (GalN), *N*-acetyl-glucosamine (GlcNAc) and *N*-acetyl-galactosamine (GalNAc), are the most common D-aminosugars. Many of these aminosugars are found on cell surfaces to play a significant role as receptor ligands for macromolecules, participating in for example antibody-antigen interactions.[8, 32-35] This Chapter introduces two exopolysaccharides mainly composed of 2-amino-2-deoxy-glycosides, including *Aspergillus* galactosaminogalactan and *Pseudomonas* Pel polysaccharides. Also, it provides an overview of α-galactosaminylation and α-glucosaminylation methodologies developed to date.

2. Galactosaminogalactan (GAG)

Aspergillus fumigatus is an opportunistic fungal pathogen that causes invasive infections in immunocompromised patients.[36-37] *Aspergillus* spores are present in suspended dust all around us, both indoor and outside. Although antifungal agents are currently available, the mortality of invasive aspergillosis remains over 50%, highlighting the need for new therapies.[38] One strategy used by the mold *A. fumigatus* to establish and maintain pulmonary infection is the production of biofilms during invasive infection. Galactosaminogalactan (GAG), a cell wall component of *A. fumigatus*, has been identified as an important factor

during biofilm formation as well as infection/invasion of the host.[39-40] GAG is a linear polysaccharide composed of 1,4-linked galactose (Gal), galactosamine (GalN) and *N*-acetyl-galactosamine (GalNAc) residues that are interconnected through *cis*-glycosidic linkages (Figure 1).[37, 41-42] It hides the immunostimulatory β-glucans from the host immune system and functions as an immunomodulatory polysaccharide by inhibiting the generation of proinflammatory cytokines.[36] This feature suggests that GAG is a potential lead compound in the development of anti-inflammatory therapies.

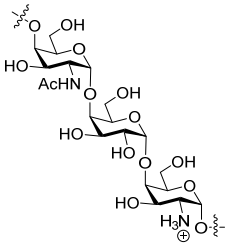


Figure 1. Structure of the GAG exopolysaccharide.

Sheppard's group provided a plausible biosynthetic pathway of GAG by comparative transcriptional analysis of *A. fumigatus* regulatory mutants deficient in the production of GAG.[37, 40, 43] The biosynthesis of GAG depends on a cluster of genes located on chromosome 3 encoding five carbohydrate-active enzymes.[44] Structural and biochemical studies indicated that GAG synthesis begins with the transformation of UDP-glucose and UDP-*N*-acetyl glucosamine into UDP-galactose and UDP-*N*-acetyl-galactosamine through the activation of epimerase Uge3 (Figure 2). Polymerization of the monosaccharides and transport across the membrane is supposed to be mediated by the glycosyl transferase Gtb3. Then GalNAc moieties within the newly secreted polymer are partially de-acetylated by the secreted protein Agd3. It has been found that the agd3-deficient mutant produces normal amounts of GAG, but this strain is impaired in its ability of biofilm formation and lacks cell wall decoration. The agd3-deficient strains also exhibit markedly lower virulence in a murine model of *A. fumigatus* infection compared to the wild-type strain, indicating Agd3 as a virulence factor.[45] After de-*N*-acetylation, the emerging polymer is thought to be cleaved by two glycoside hydrolases: an endo-α-1,4-*N*-acetylgalactosaminidase Sph3 and an endo-α-1,4-galactosaminidase Ega3. Recent studies have shown that these two hydrolases can degrade GAG, disrupt *A. fumigatus* biofilms, and attenuate fungal virulence in mice, suggesting that targeting these hydrolases holds promise for therapeutic applications in the treatment of *Aspergillus* infections.[46-47]

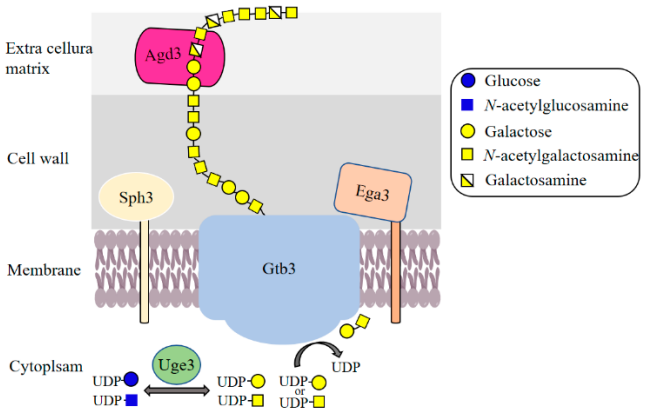
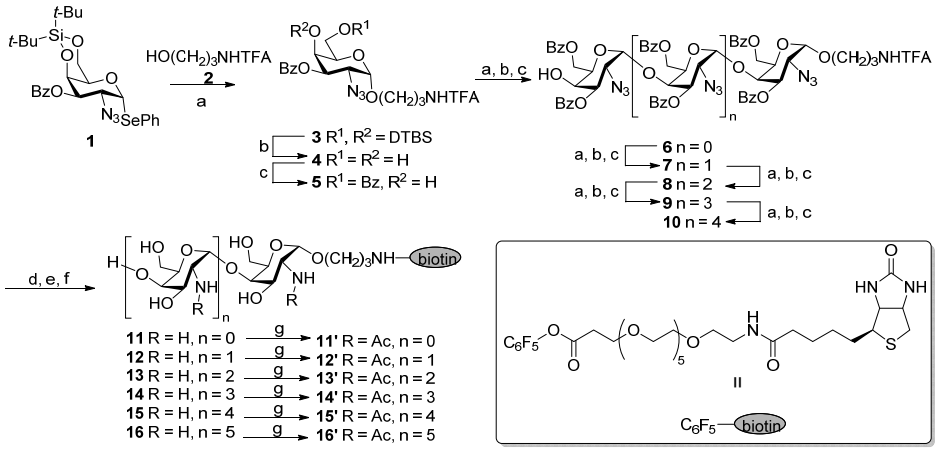


Figure 2. Biosynthetic pathway of GAG polysaccharide

The chemical synthesis of GAG homo-oligomers of GalN and GalNAc was first reported by Nifantiev's group, which is presented in Scheme 1.[48] The key to the assembly of GAG oligosaccharides is the stereoselective glycosylation of the axial 4-OH groups in the galactosamine acceptors, which have relatively low reactivity. The DTBS-protected 2-azido-2-deoxy-galactoside **1** was used as glycosyl donor, as it precludes the formation of β-glycosylation products owing to the steric hindrance effect of DTBS group (*vide infra*). First, selenoglycoside **1** was coupled with the linker *N*-(3-trifluoroacetyl)-propanol **2** under the promotion of the dimethyldisulfide-methyl triflate (Me_2S_2-MeOTf) system, giving the desired α-linked product **3**. Removal of the DTBS group with HF afforded the diol **4**, which was regioselectively benzoylated to furnish the desired 4-OH acceptor **5**. Glycosylation of **5** with donor **1**, removal of the DTBS group, and 6-*O*-benzoylation then afforded disaccharide acceptor **6**. To elongate the chains, the three-step cycle was continued: 1) coupling reaction with donor **1**; 2) DTBS removal with HF/pyridine; and 3) selective 6-O-benzoylation. After repeating the three-step cycle several times, hexasaccharide **10** was generated. Deprotection of the synthesized oligomers was accomplished by $Pd(OH)_2$/C catalyzed reduction of the N_3 groups with H_2, in the presence of Boc_2O and Et_3N, and subsequent removal of benzoyl and trifluoroacetyl groups by a double base treatment. The free amine groups in the spacer of the generated *N*-Boc protected intermediates were biotinylated and the Boc groups were cleaved using acidic conditions, generating the biotinylated oligo-α-(1→4)-D-galactosamines **11**-**16**. Then these products were *N*-acetylated to provide GalNAc-containing conjugates **11'**-**16'** comprising from two to six monosaccharide units. Besides these synthesized GAG homo-oligomers, longer chains of GAG homo-oligomers and hetero-oligomers are still needed to elucidate their interaction with the host immune system as well as fungal biosynthesis enzymes.



Scheme 1. Chemical synthesis of oligo-α-(1→4)-galactosamine conjugates. a) **2**, Me_2S_2, MeOTf, MS 4Å, DCM, for **3**: 81%; **1**, Me_2S_2, MeOTf, MS 4Å, DCM, for **6-10**; b) 40% aq HF, pyridine; for **4**: 80%; c) BzCl, pyridine, 0 ºC, for **5**: 94%; for **6**: 71%; for **7**: 72%; for **8**: 72%; for **9**: 60%; for **10**: 55%; d) $Pd(OH)_2/C$, Et_3N, Boc_2O, EtOAc, atm. H_2; e) 1M NaOMe, DCM-MeOH (1:3), then 1 M NaOH; f) C_6F_5-biotin, Et_3N, DMF, then CF_3COOH, for **11**: 71%; for **12**: 62%; for **13**: 53%; for **14**: 48%; for **15**: 72%; for **16**: 53%; g) Ac_2O, Et_3N, MeOH, for **11'**: 90%; for **12'**: 97%; for **13'**: 95%; for **14'**: 88%; for **15'**: 80%; for **16'**: 87%.

3. Pellicle (Pel) polysaccharide

Pseudomonas aeruginosa is a widespread, opportunistic, biofilm-forming Gram-negative bacterium, which is well known for the chronic infections it causes in individuals with the genetic disease, cystic fibrosis (CF).[49-50] It can cause both acute and chronic infections in immunocompromised patients and can become resistant to antibiotics due to its ability to form a biofilm which complicates the treatment of *pseudomonas* infections. In biofilm formation, this bacterium is capable of synthesizing three distinct exopolysaccharides: alginate, the polysaccharide synthesis locus (Psl), and pellicle (Pel) polysaccharides.[51-52] Alginates are linear polysaccharides composed of β-1,4 linked D-mannuronic and L-guluronic acids, which contribute to increase the bacteria's resistance to antibiotics and evade the host defense mechanisms.[53] Psl is a neutral polysaccharide composed of a pentasaccharide repeating unit containing D-glucose, L-rhamnose and D-mannose, which is an essential matrix component required for biofilm formation.[54] Pel is a cationic linear polysaccharide composed of 1,4-linked α-GlcNAc and α-GalNAc residues, of which some of the residues have been de-acetylated to generate positively charged GlcN and GalN moieties (Figure 3).[55] The Pel polysaccharide plays an important role in maintaining cell-cell and cell-surface interactions in biofilms and affords biofilm protection by enhancing

resistance to aminoglycoside antibiotics.[56] It has been reported that deletion of genes responsible for Pel polysaccharide synthesis in *P. aeruginosa* can abolish biofilm formation and/or significantly compromise bacterial virulence.[52, 57] Understanding the production and mode of action of Pel polysaccharides will pave the way for the development of new therapeutics to combat *Pseudomonas* infections.

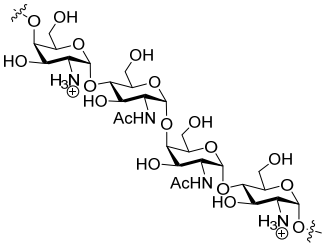


Figure 3. Putative structure of Pel polysaccharides.

Although the exact composition of the Pel polysaccharide remains to be definitively established, its biosynthesis machinery has been described as shown in Figure 4.[51, 56-58] The essential proteins involved in Pel biosynthesis are encoded by seven genes, *pelA* to *pelG*. Pel polymerization is proposed to begin with the predicted glycosyltransferase PelF, which is regulated by the binding of secondary messenger c-di-GMP to the cytoplasmic domain of the inner membrane protein PelD. After polymerization, Pel is predicted to be transported across the inner membrane by PelD in conjunction with the inner membrane proteins PelE and/or PelG. Once being shipped across the inner membrane, Pel is partially deacetylated by the periplasmic deacetylase PelA. After de-acetylation, the resulting polymer is exported across the outer membrane by the outer membrane proteins PelB and PelC. To date, the details of the Pel synthesis remain largely unknown, such as the characteristics and functions of the enzymes involved in Pel polymerization and transport across the inner and outer membranes. Accordingly, chemical synthesis of well-defined Pel polysaccharides is highly needed to study their biosynthesis and unravel their role in biofilm formation.

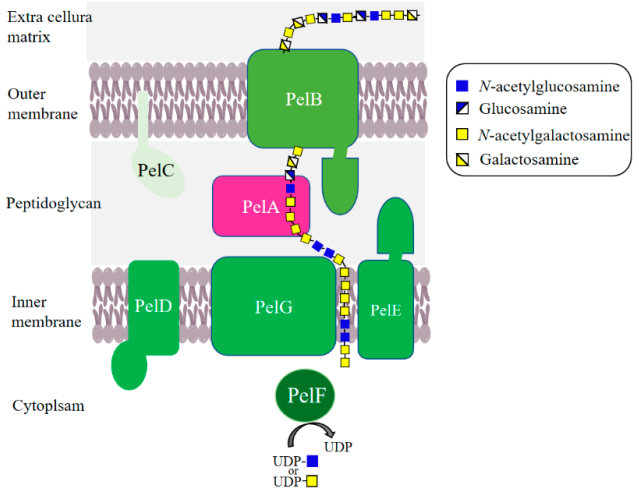
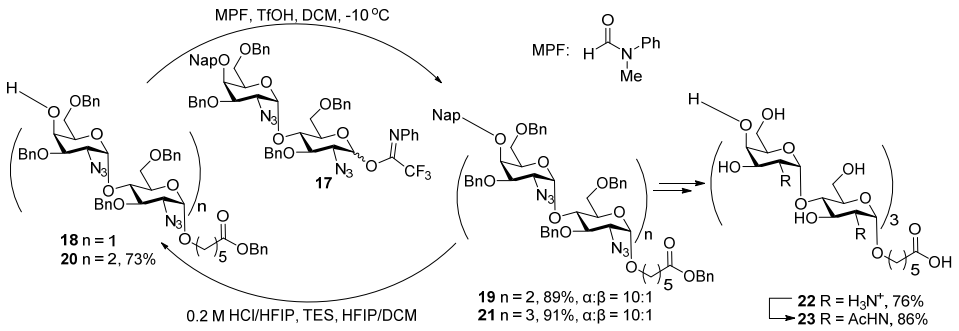


Figure 4. Plausible biosynthetic pathway of Pel polysaccharide.

Recently, Wang *et al.* reported the synthesis of the (GalN-GlcN)$_3$ Pel fragments **22** and **23** (Scheme 2).[59] The key challenge in the generation of these hexasaccharides is the stereoselective construction of two kinds of *cis*-glycosidic linkages, namely the α-GlcN-(1→4)-GalN and α-GalN-(1→4)-GlcN connections. The α-GalN$_3$ linkages can be introduced with DTBS-directed α-galactosylation methodology, while the α-GlcN$_3$ linkages were stereoselectively constructed using a new additive, methyl(phenyl)formamide (MPF), controlled glycosylation method. A [2 + 2 + 2] strategy was developed for the assembly of the hexasaccharides. The [2+2] glycosylation using MPF as additive at −10 °C at a 0.2 M concentration afforded the tetrasaccharide **19** in 89% yield with 10:1 α/β ratio. Next, the Nap ether was cleaved using HCl and triethylsilane in DCM/HFIP to give the tetrasaccharide acceptor **20**, which was coupled with donor **17** under modulation by MPF to generate hexasaccharide **21** in high yield and α-selectivity. Reduction of the azides and removal of the benzyl ester and ethers were achieved in a one-step reduction to provide compound **22**, of which the amino groups were acetylated to afford the Pel structure **23**.



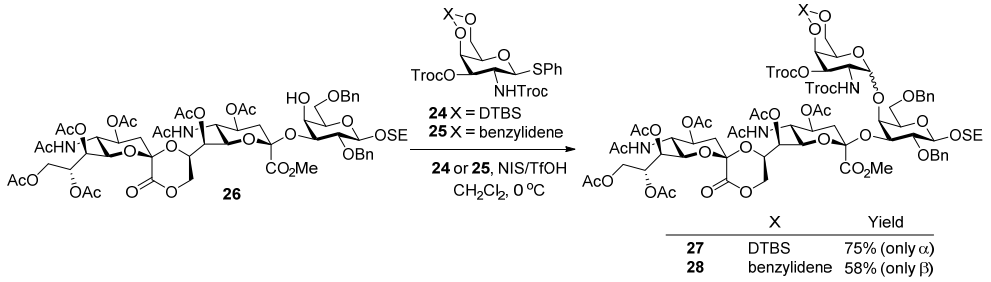
Scheme 2. Chemical synthesis of well-defined Pel oligosaccharides.

4. Stereoselective synthesis of α-galactosamines

The 1,2-*cis*-selective formation of 2-amino-2-deoxy-glycosides remains a considerable challenge, because of the requirement for a non-participating amino protecting group and the lower reactivity of glycosamine donors. To improve the stereoselectivity of glycosylation reactions, many strategies have been developed in recent years. Below some methods are presented that can be used for the formation of 1,2-*cis*-galactosamine linkages, including the previously introduced di-*tert*-butylsilylene (DTBS)-directed α-galactosylation methodology[24, 48], reagent controlled glycosylations[27, 60], the use of 2,3-oxazolidinone protected glycosyl donors[61-62] and glycosylations based on Nickel-catalyzed reactions of C(2)-*N*-substituted benzylidene galactosamine donors[32].

4.1 DTBS-directed α-glycosylation

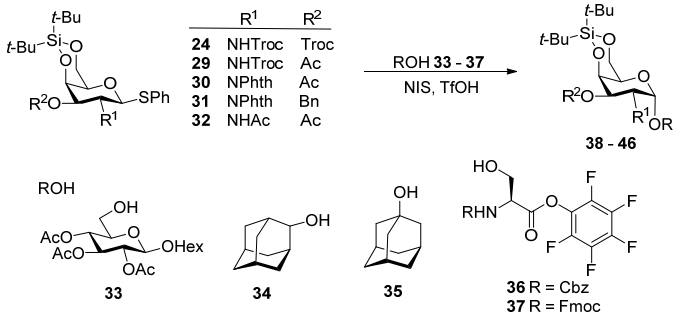
The unusual α-galactosylation using DTBS-protected galactosides as donors was discovered by chance in Kiso's group during a synthetic study towards b-series gangliosides (Scheme 3).[24] In the study, the 4,6-*O*-DTBS protected donor **24** exhibited excellent α-selectivity in the coupling reaction with trisaccharide acceptor **26**, affording tetrasaccharide **27** in 75% yield. In contrast, the corresponding 4,6-*O*-benzylidene protected donor **25** afforded β-product **28** exclusively. This indicates that 4,6-*O*-DTBS-protection predominantly leads to α-galactosylation.



Scheme 3. First encounter of 4,6-*O*-DTBS controlled α-galactosylation.

Notably, the α-directing capacity of this galactosylation method is independent of the reaction temperature, solvent and protecting groups even in the presence of participating acyl groups, such as NHTroc, NPhth and NHAc groups at C2 (Table 1).[24, 63-64] What's more, the DTBS-directed approach is tolerant to different types of acceptors. The α-selectivity is almost completely independent of the nucleophilicity of the acceptor hydroxyl, which can be a primary, secondary or tertiary alcohol. Besides employment in the stereoselective synthesis of α-galactosides[24, 63-69], the DTBS-group has been used to direct the stereoselectivity on the construction of different biologically relevant glycans, including β-arabinofuranosides[25, 70-72], α-galactofuranosides[73-74], α/β-glucosides[75-76], β-mannosides[21], β-glucuronides[77], α-sialosides[78] and α-kdo glycosides[79].

Table 1: α-Selective glycosylations of GalN donors with various acceptors.



Entry	Donor	Acceptor	Solvent	*T* (°C)	Product	Yield (α:β) (%)
1	**24**	**33**	CH_2Cl_2	0	**38**	96:3
2	**24**	**33**	*n*-hexane	RT	**38**	58:3
3	**24**	**33**	Toluene	0	**38**	91:7
4	**24**	**33**	$MeNO_2$	0	**38**	93:6
5	**24**	**33**	MeCN	0→40	**38**	23:0
6	**24**	**34**	CH_2Cl_2	0	**39**	91:7
7	**24**	**35**	CH_2Cl_2	0	**40**	90:0

8	**24**	**36**	CH_2Cl_2	0	**41**	78:0
9	**24**	**37**	CH_2Cl_2	0	**42**	90:5
10	**29**	**33**	CH_2Cl_2	0	**43**	96:0
11	**30**	**33**	CH_2Cl_2	0	**44**	90:5
12	**31**	**33**	CH_2Cl_2	0	**45**	94:0
13	**32**	**33**	CH_2Cl_2	0	**46**	50:0

The reaction mechanism for the DTBS-directed α-galactosylation has been elucidated by a combination of experimental and computational studies, and is shown in Figure 5.[26] Upon activation of the glycosyl donor **47**, the intermediate oxocarbenium **48** is formed, of which the conformation of the sugar ring is restricted to the half-chair $^4H_3$ conformer. The fused ring system formed by the DTBS group hampers the other conformers. Subsequently, the $^4H_3$ conformer can undergo nucleophilic attack by the alcohol acceptor, either from the *exo* side (α-face) or the *endo* side (β-face). With the *endo* attack on the β-face being blocked by the substantial steric hindrance of the *tert*-butyl group, nucleophilic attack predominantly takes place from the *exo* side (α-face). *Endo*- and *exo* attack take place through different transition states. To maximize orbital overlap between the incoming alcohol acceptor and the developing lone pair on oxygen, the transition state of the former attack features a twist-boat-like conformation **49** while the transition state of the latter proceeds with a more favorable chair-like conformation **50**. Thus, the twist-boat-like conformer **49** is kinetically disfavored and suffers from an unfavorable steric clash between the approaching acceptor and the *tert*-butyl group. Therefore, nucleophilic attack predominantly occurs via *exo* attack through the more stable chair-like conformer **50** giving the α-product **52**.

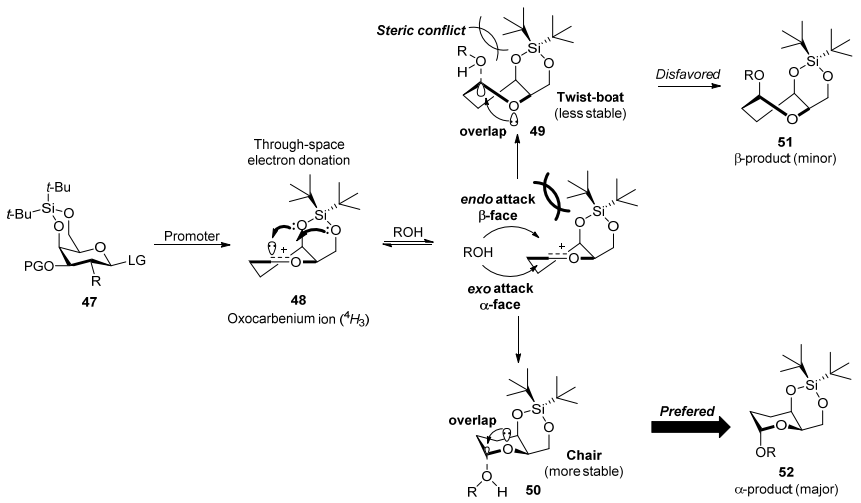
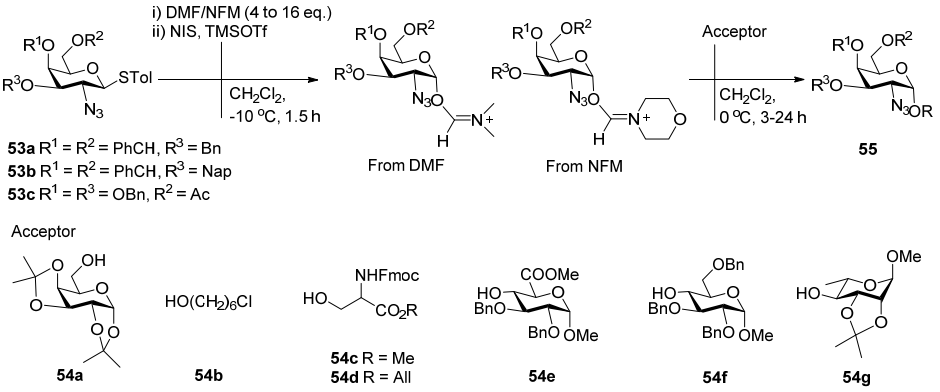


Figure 5. Proposed mechanism of DTBS-directed α-galactosylation.

4.2 Reagent controlled synthesis of α-galactosamine

Reagent controlled glycosylation methodology is an effective approach for stereoselective construction of *cis*-glycosidic bonds. In 2011, Mong's group first reported the DMF-modulated glycosylation strategy for α-galactosaminylation using 2-azido-2-deoxy thioglycosyl donors (Table 2).[27] Following a preactivation glycosylation procedure, the 2-azido-2-deoxy galactoside donors **53a** and **53c** were activated with NIS and TMSOTf in the presence of 6 equiv of DMF, followed by addition of primary acceptors **54a**-**54c**, affording the products in excellent selectivity (7:1 to α only, Table 2, entries 1, 2 and 3). However, further studies showed that glycosylations of 2-azido-2-deoxy-glycosyl donors with secondary glycosyl acceptors were impractically slow.[60] In formamide modulated glycosylations, the formation of a glycosyl imidinium ions is the key step. To modulate the reactivities of these adducts, *N*-formyl morpholine (NFM), *N*,*N*-diisopropyl formamide (DIPF), *N*-formyl piperidine (NFP), tetramethylurea (TMU), dimethylacetamide (DMA), as well as other additives, such as diphenyl sulfoxide (DPSO) and triphenylphosphine oxide (TPP) were used as nucleophilic additives. In these evaluation studies, NFM was found to be an effective modulator for glycosylations of 2-azido-2-deoxy-glycosyl donors **53a** and **53b** with primary and less reactive secondary acceptors **54d**-**54g**, providing the disaccharides in 13:1 to α-exclusive α/β ratios (Table 2, entries 4-7). Similar to this methodology, the MPF-modulated glycosylation has been successfully applied for the assembly of Pel fragments as described above (see Scheme 2).

Table 2. DMF/NFM-modulated glycosylation with 2-azido-2-deoxy thiogalactosyl donors.

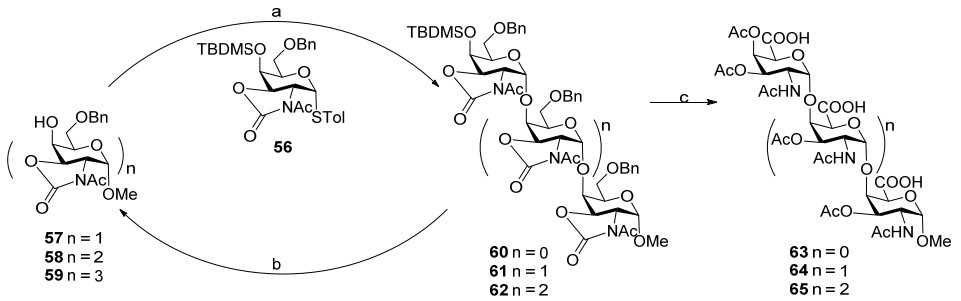


Entry	donor/acceptor	Additive (equiv.)	Yield	α:β
1	**53a**/**54a**	DMF (6)	66%	10:1
2	**53a**/**54b**	DMF (6)	65%	α only

3	**53c**/**54c**	DMF (6)	80%	7:1
4	**53a**/**54d**	NFM (16)	90%	α only
5	**53a**/**54e**	NFM (16)	89%	α only
6	**53b**/**54f**	NFM (16)	83%	13:1
7	**53b**/**54g**	NFM (4)	82%	32:1

4.3 2,3-Oxazolidinone-protected galactosamine donors

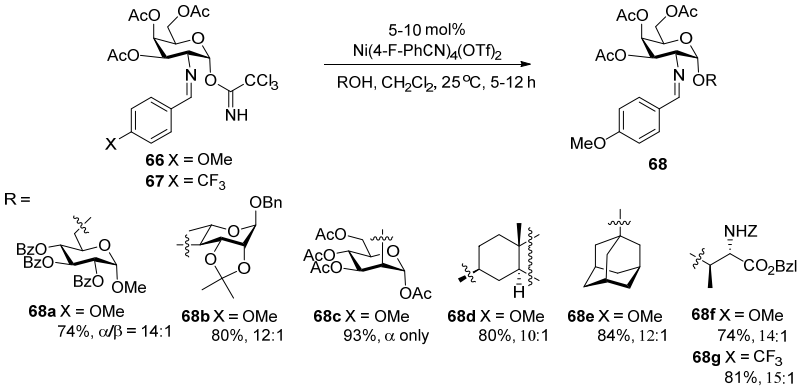
Oxazolidinone-protected glucosamine as an α-selective glycosyl donor was first reported in 2001 by the group of Kerns[80], and subsequent investigation of its *N*-acetyl or *N*-benzyl analogues confirmed that the ring-fused oxazolidinone moiety is an effective nonparticipating group for the stereoselective construction of α-glucosamine linkages.[20, 61, 81] This methodology has been successfully used to introduce the α-galactosamine moiety in the synthesis of fragments of the Vi antigen from *Salmonella typhi* (Scheme 4).[62] The Vi polysaccharide is a linear homopolymer of 1,4-linked *N*-acetyl-α-galactosaminuronic acid with *O*-acetylation at C3. The key feature of the synthesis of Vi antigen depicted in Scheme 4 is the two-step chain elongation cycle, consisting of 1) glycosylation reaction with the *N*-acetyl-2,3-oxazolidinone glycosyl donor; and 2) removal of the TBDMS group. A pre-activation strategy, comprising the use of a combination of diphenyl sulfoxide (Ph_2SO), triflic anhydride (Tf_2O) and the hindered base TTBP, was used to activate the donor. The selectivity of all glycosylation reactions was excellent, while the yields of isolated α-products decreased (72% for the dimer, 61% for the trimer; 53% for the tetramer) as the reactivity of 4-OH group decreased with the elongation of the chain. To form the final products, the oxazolidinone group was hydrolyzed in a NaOH solution, and at the same time the TBDMS group was cleaved. Subsequent acetylation and a tandem hydrogenolysis and oxidation furnished the uronic acid in moderate to good yields.



Scheme 4. Synthesis of oligosaccharide fragment of the Vi antigen. a) Ph_2SO, Tf_2O, CH_2Cl_2, -72 °C to RT, 3 h, yields for **60**: 72%; **61**: 61%; **62**: 53%. b) TBAF/THF, RT, 10 min, yields for **58**: 90%; **59**: 90%. c) i) NaOH (aqueous)/1,4-dioxane (1:1), 40 °C, 2-5 h; ii) Ac_2O, DMAP, pyridine, 0 °C to RT, 2–10 h; iii) H_2, Pd/C, THF/AcOH/H_2O (4:2:1), 2-5 h; iv) $NaIO_4$, $RuCl_3 \cdot xH_2O$, $CCl_4/CH_3CN/H_2O$ (2:2:3), overnight, yields for **63**: 67%; **64**: 53%; **65**: 47%.

4.4 Nickel-catalyzed glycosylations of C(2)-*N*-benzylidene galactosamine donors

Ni-catalyzed stereoselective glycosylation with C(2)-*N*-benzylidene galactosamine trichloroacetimidates for the formation of α-galactosamine was first reported by Nguyen's group.[32] Coupling of α-galactosamine trichloroacetimidates **66** and **67** with primary, secondary, and tertiary acceptors in the presence of 5-10 mol % of $Ni(4\text{-F-PhCN})_4(OTf)_2$ at 25 °C provided the desired products in high yields (74-93%) and with excellent α-selectivity (10:1 to α-only, Scheme 5). The α-selectivity of the nickel method relies on the nature of the nickel-complex, while the reactivity of the nucleophiles and protecting groups on acceptors have little effect on the stereoselectivity. This methodology has also been applied for the synthesis of α-glucosamines, which will be discussed in the next section.



Scheme 5. α-Selective coupling with *N*-substituted benzylidene galactosamine imidates.

Two plausible mechanisms for the nickel-catalyzed α-selective glycosylation are described in Figure 6.[32] In pathway I, the seven-membered ring complex **A** is first formed through the reversible coordination of $L_nNi(OTf)_2$ to both the trichloroacetimidate nitrogen and benzylidene protected nitrogen in donor **69**. Ionization of **A** leads to the corresponding complex **B**, facilitated by the hydrogen bonding between the incoming hydroxy nucleophile and the trichloroacetamide. Next, ligand exchange and dissociation of trichloroacetamide gives the ion pair **C**, which recombines to afford the favorable five-membered ring

intermediate **D**. Dissociation of the nickel species from **D** provides α-glucosamine/galactosamine **70**. In pathway II, the Lewis acid $L_nNi(OTf)_2$ coordinates to the trichloroacetimidate nitrogen of **69** to form the complex **E**, which is transformed into the oxocarbenium intermediate **F** after ionization. Ligand exchange followed by coordination of nickel to the benzylidene nitrogen of **F** furnishes the ion pair **C**, which finally yields the 1,2-*cis*-2-amino glycoside **70**. It has been verified that the α-orientation of the trichloroacetimidate leaving group and the presence of the external alcohol nucleophile are essential for the ionization of glycosyl imidate donors. Furthermore, the substituted benzylidene group at the C(2) amino position in the glycosyl donors is pivotal for the high α-selectivity.

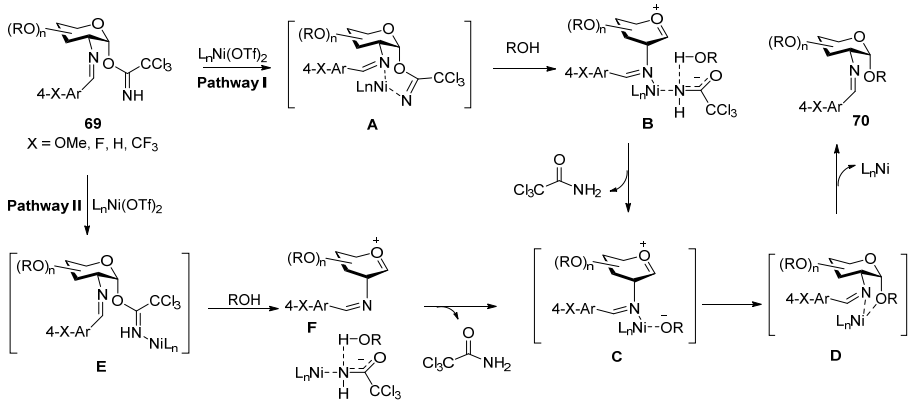


Figure 6. Plausible mechanism of nickel-catalyzed α-selective glycosylation.

5. Stereoselective synthesis of α-glucosamines

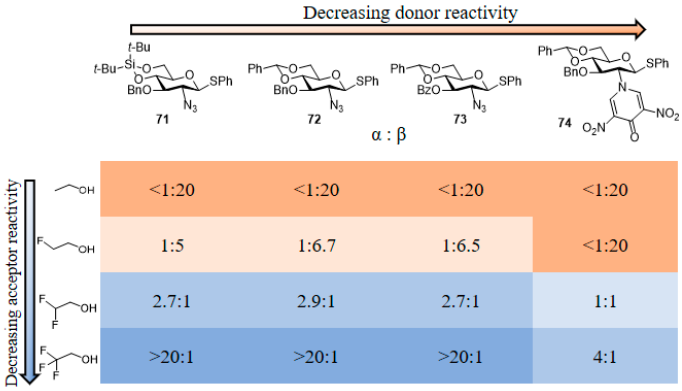
Glucosamine is a key component in various natural polysaccharides and glycoconjugates. While β-glucosamines can be facilely synthesized, no general solution exists for the stereoselective construction of α-glucosamines. Here some strategies are presented that can be used for the α-selective formation of glucosamine linkages.

5.1 4,6-Tethered glucosazide donors

To stereoselectively construct α-glucosamines, a C2-azido group is most commonly used in glucosamine donors as a non-participating group. In 2017, van der Vorm *et al.*[82] systematically evaluated a set of glycosylation reactions between a series of 4,6-tethered glucosazide donors and a panel of acceptors with decreasing nucleophilicity (Table 3). The DTBS-protected donor **71** was found to be more reactive than benzylidene-protected donors **72** and **73**, while donor **74**, carrying the strongly electron-withdrawing dinitropyridone

(DNPY) group proved to be the least reactive. The nucleophilicity of the acceptors, used in this study, gradually decreased from ethanol to monofluoroethanol (MFE), difluoroethanol (DFE) to trifluoroethanol (TFE). The glycosylation reactions, which were undertaken using the Ph_2SO/Tf_2O preactivation procedure, present two major trends. First, with the decreasing reactivity of the donors, the glycosylations provided a larger proportion of the β-products, with the least reactive donor **74** being the most β-selective of the donors listed above. Secondly, decreasing acceptor nucleophilicity corresponds to an increase in the α/β ratio. This trend is apparent for all donors, with the most reactive acceptor, ethanol, offering least α-linked product while the least reactive acceptor, TFE, provided most α-linked product.

Table 3. Glycosylations of 4,6-tethered glucosazide donors with (partially) fluorinated ethanols



The reactive intermediates and plausible reaction pathways for 4,6-tethered glucosazide donors are indicated in Figure 7. The following kinetic scenario emerges. The relatively stable α-triflate, which can be observed by low-temperature NMR spectroscopy, is in equilibrium with the more reactive β-counterpart and if the acceptor is nucleophilic enough, the triflate can be directly displaced. For instance, the glucosazide donors react with ethanol and MFE in an S_N2-like substitution reaction pathway, forming the products with a high β : α-ratio. The stronger electron-withdrawing DNPY group in donor **74** can lead to a more stable covalent α-triflate and favors an associative displacement mechanism, giving a further increase in β-selectivity. For the weaker nucleophiles, such as DFE and TFE, the glycosylation is less likely to proceed in the S_N2-like pathway. The high α-selectivity for these acceptors can be explained by the involvement of more electrophilic intermediates such as the glycosyl oxocarbenium ion-like species. Conformationally restricted by the benzylidene and silylidene protecting groups, the intermediate oxocarbenium ion preferentially adopts a $^4H_3/^4E$-like conformation. A $B_{2,5}$-like structure such as **77** is

significantly less favorable because this puts the C-2-azide in a flagpole position. The $^4H_3/^4E$-conformer is attacked from the bottom face to generate the α-products through a chair-like transition state. The more reactive donors more readily dissociate to form an oxocarbenium ion-like species, which accounts for the increased α-selectivity for those donors.

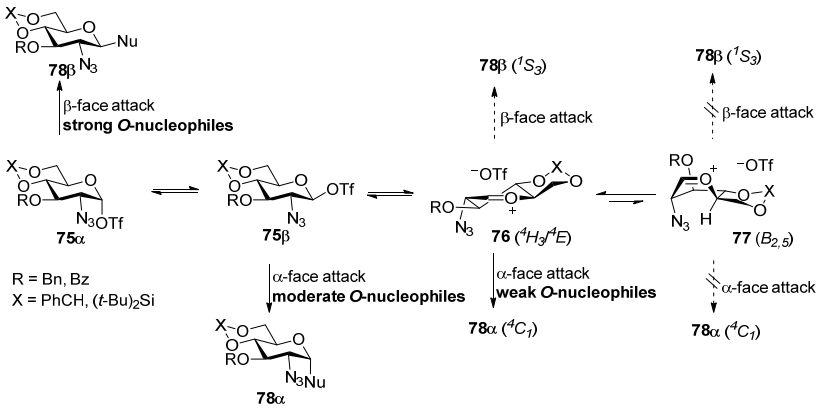
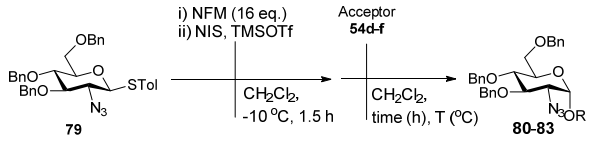


Figure 7. Reactive intermediates and reaction pathways for 4,6-tethered glucosazide donors.

5.2 Reagent controlled α-glucosaminylation methodology

As described above, the reactivity of both the donor and acceptor has a great influence on the stereoselectivity of a glycosylation reaction. Therefore, additive controlled glycosylation methodology, in which donor reactivity can be modulated to match the reactivity of the acceptor alcohols, is attractive and gaining increasing interest for the stereoselective construction of 1,2-*cis*-glycosidic linkages.[83-84] Different additives have been investigated to accommodate the reactivity difference between different donors and acceptors.[85-90] DMF-modulated glycosylations were first developed and these have been applied for the synthesis of various oligosaccharides, such as a branched α-glucan with an α-(1,4)-linked backbone from *Mycobacterium tuberculosis* and α-(1,3)-glucans from *Aspergillus fumigatus*.[29] Mong and co-workers found that glycosylations mediated by DMF didn't proceed with satisfactory stereoselectivity for the construction of 1,2-*cis*-glucosamine and galactosamine linkages.[60] They introduced NFM to modulate the reactivity of $GalN_3$ (*vide supra*, section 4 and Table 2) and $GlcN_3$ donors, showing better stereoselectivity compared to DMF (Table 4). With the strong electron-withdrawing azide group in the C2-azido donors, their reactivity is lower in comparison to their 2-*O*-benzyl counterparts. This lower reactivity can be counterbalanced by the use an additive, that is less capable of supporting the positive charge at the imidinium ion, resulting in a better leaving group, thereby explaining why NFM outperforms DMF in these glycosylations.

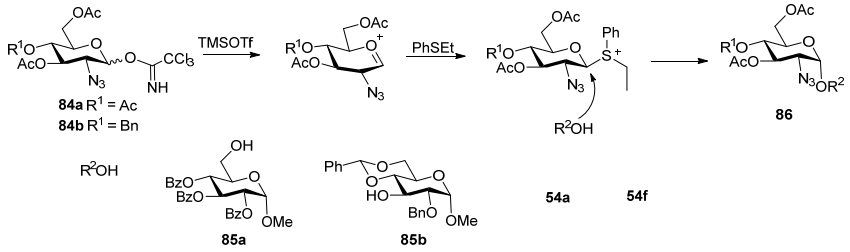
Table 4. NFM-modulated glycosylation with 2-azido-2-deoxythioglucosyl donor



Entry	Acceptor	Time (h), T (°C)	Product	Yield (%), α:β
1	**54d**	12, -5	**80**	81, 11:1
2	**54e**	12, -5	**81**	75, 16:1
3	**54f**	18, -5	**82**	70, 19:1
4	**54g**	12, -10	**83**	84, 19:1

Besides the formamide additives, thioethers, such as PhSEt, and thiophene were explored as additives for stereoselective glycosylation of 2-azido-2-deoxy-glucosides by Boons's group.[91] Glycosylations of $GlcN_3$-trichloroacetimidates **84a** and **84b** provide excellent α-selectivity, promoted with TMSOTf at a relatively high temperature (0 °C) in the presence of PhSEt or thiophene (10 equiv, Table 5). Mechanistic studies indicated that a β-anomeric sulfonium ion is formed after activation of the imidate donor in the presence of PhSEt. Subsequent displacement of the β-anomeric sulfonium ion by an acceptor alcohol then affords an α-linked product.

Table 5. α-Selective glucosylations in the presence of PhSEt or thiophene.



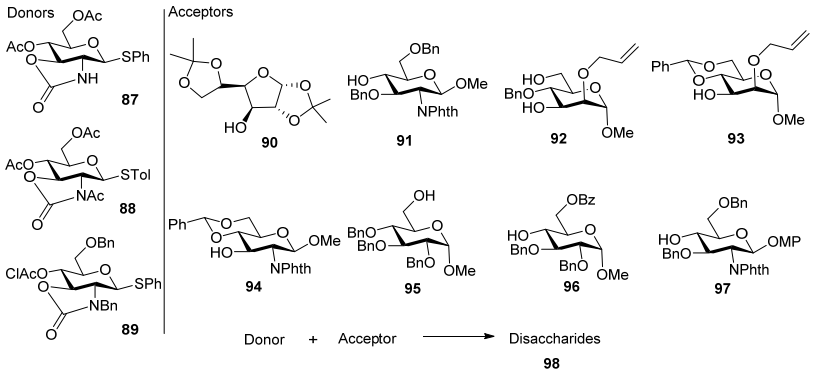
Donor	Acceptor	T (°C)	thioether	Yield (%), α:β
84a	**85a**	-78	none	91, 2:1
84a	**85a**	-78	PhSEt	83, 5:1
84a	**85a**	0	none	92, 8/1
84a	**85a**	0	PhSEt	94, 20/1
84a	**85a**	0	thiophene	91, α-only
84a	**85b**	0	thiophene	60, 15:1
84a	**54a**	0	PhSEt	92, 5:1

84a	**54a**	0	thiophene	95, 14:1
84a	**54f**	0	thiophene	43, α-only
84b	**85a**	0	thiophene	93, 20:1
84b	**85b**	0	thiophene	50, 15:1
84b	**54a**	0	thiophene	96, 15:1
84b	**54f**	0	thiophene	37, α-only

5.3 Oxazolidinone-containing glucosamine donor

The non-*N*-acetylated oxazolidinone protected 2-amino-2-deoxy-glucose **87** was first employed as a donor for the formation of α-linked glycosides by the group of Kerns.[80] The oxazolidinone **87** glycosylated primary and secondary glycosyl acceptors under the promotion of phenylsulfenyl triflate (PST) at -78 °C to give disaccharides in high yields and with excellent α-selectivity (Table 6). Nevertheless, the use of non-*N*-acetylated oxazolidinone-protected donors has several limitations: 1) some thioglycoside donors are difficult to activate, requiring at least 2 equiv of PST, as 1 equiv is lost to *N*-sulfenylation, and 2) *N*-glycosylation has been observed in oligosaccharide synthesis. To avoid the side-reactions, the *N*-acetylated donor **88** and *N*-benzylated donor **89** were prepared.[20, 92] With oxazolidinone **88** as the donor and BSP-Tf_2O as mild promotor, a selectivity-reactivity relationship was observed for the stereoselectivity of the glycosylations of various acceptors. Acceptors of low nucleophilicity gave mainly the α-products, while acceptors with intermediate reactivity led to α/β mixture, and the β-products were obtained with reactive acceptors. Afterwards, Ito and co-workers reported *N*-benzyl-2,3-oxazolidinone **89** as donor and PhSOTf or *N*-(phenylthio)-ϵ-caprolactam as promotor, furnishing disaccharides with high α-selectivity. Furthermore, Ye[93-94] and Oscarson[81] found that the stereoselectivity of *N*-acetylated-2,3-oxazolidinone-protected donors towards glycosylations can be significantly influenced by additives. Thiophene and AgOTf were found to be the best α-directing additives. It has been described that the stereoselectivity can also be controlled by the use of (Lewis) acidic reaction conditions, as the glycosylated β-products can isomerize to the corresponding α-products. The ring strain imposed on the system by the *trans*-fused oxozolidinone can lead to rapid ring opening upon protonation of the glucosamine endocyclic *O*-atom. Rotation around the C1-C2 bond and subsequent ring closure provides the thermodynamically more stable α-linked products.[81]

Table 6. Glycosylation reactions with ring-fused oxazolidinone of glucosamine



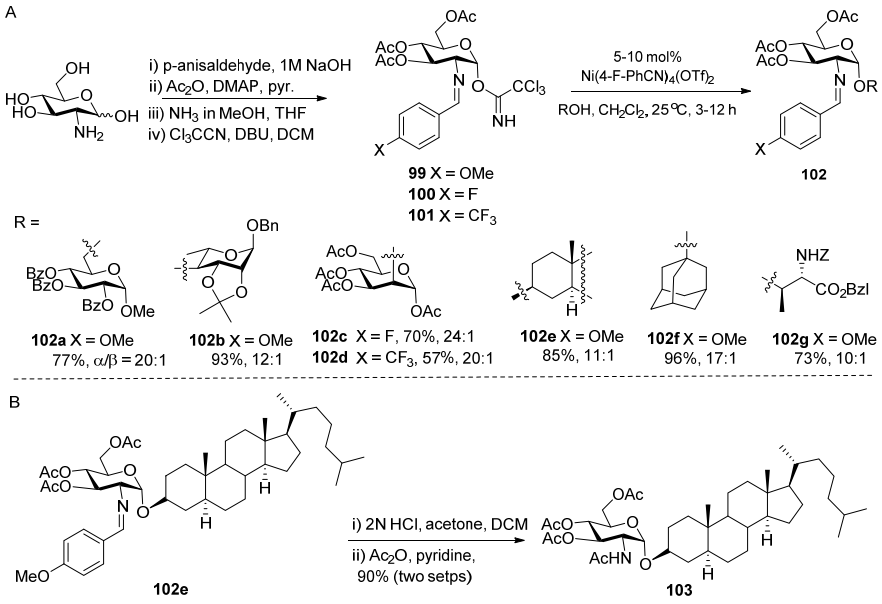
Entry	Donor	Acceptor	Condition	Product (%)	α:β
1	**87**	**90**	A	**98a** (97%)	α-only
2	**87**	**91**	A	**98b** (75%)	α-only
3	**87**	**92**	A	**98c** (90%)	α-only
4	**87**	**93**	A	**98d** (95%)	α-only
5	**88**	**90**	B	**98e** (95%)	β-only
6	**88**	**94**	B	**98f** (75%)	1:4.5
7	**88**	**91**	B	**98g** (81%)	α-only
8	**89**	**95**	C	**98h** (88%)	10:1
9	**89**	**96**	D	**98i** (52%)	α-only
10	**89**	**97**	D	**98g** (54%)	α-only

Conditions: A) PST, CH_2Cl_2, -78 °C. B) BSP, Tf_2O, TTBP, CH_2Cl_2, -60 °C. C) AgOTf, PhSCl, DTBMP, toluene/1,4-dioxane (3:1), 0 °C to rt. (D) *N*-(phenylthio)-ϵ-caprolactam, Tf_2O, CH_2Cl_2, rt.

5.4 Nickel-catalyzed stereoselective glycosylations of *N*-benzylidene protected donors

Similar to the nickel-catalyzed stereoselective α-galactosaminylation (*vide supra*), C2-*N*-substituted benzylidene glucosamine donors **99-101** were developed and found to be viable donors for the synthesis of α-linked glucosamines (Scheme 6).[32] Preparation of the trichloroacetimidate **99** was achieved by treatment of commercially available D-glucosamine with *p*-anisaldehyde under basic condition, followed by acetylation, selective deacetylation and coupling with Cl_3CCN (Scheme 6A). Condensation reactions with a variety of primary, secondary and tertiary alcohols with the trichloroacetimidate donor furnished disaccharides **102a**-**102g** with excellent α-selectivity (10:1 to 20:1). This method has been employed for the synthesis of a number of trisaccharides and tetrasaccharides with satisfactory α-selectivity using relatively unreactive disaccharide donors and acceptors. Removal of the benzylidene groups can be achieved under acidic condition (5 N HCl), after which *N*-acetyl or other

desired functionalities can be readily installed at the liberated nitrogen. For acid-sensitive oligosaccharides and glycoconjugates, the benzylidene groups can be cleaved with 1.1 equivalents HCl at 25 ºC for 5 minutes. For instance, treatment of **102e** with 2 N HCl, followed by acetylation of the generated amine afforded the glycoconjugate **103** in 90% yield.



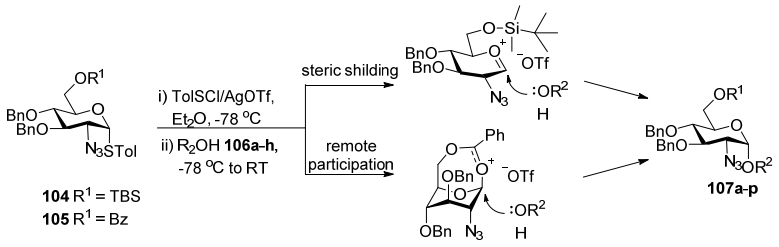
Scheme 6. A) α-Selective glycosylation with *N*-substituted benzylidene glucosamine donors. B) Removal of *N*-substituted benzylidene group

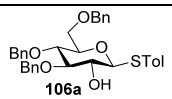
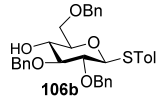
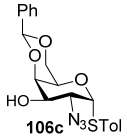
5.5 Remote participation in α-selective glucosaminylation reactions

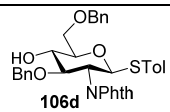
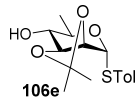
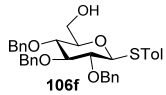
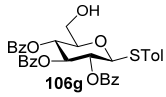
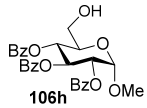
It has been reported that acyl groups located at more distant positions than *O*-2 or *N*-2 in glycosyl donors can affect the stereoselectivity of glycosylation reactions via remote participation.[95-101] This long range effect is heavily debated[102] and in general, glycosylations with donors bearing remote participating groups are not as stereoselective as donors bearing C-2-neighboring participating groups and the degree of the remote stereo-directing effect varies with the type of donor and the position of the remote participating group. For instance, 3,6-*O*-acyl groups in glucosyl donors have been reported to favor α-glycosylation with modest to good selectivity.[96, 99] Moreover, glucosyl donors with bulky substituents at the 6-*O*-position, such as tert-butyl diphenylsilyl (TBDPS) and trityl groups, also favor α-glycosylation owing to the steric shielding influence on the β-face.[100, 103]

Recently Gao *et al.* reported an efficient strategy to achieve α-selective glucosaminylation based on the combined α-directing effects of the TolSCl/AgOTf promotion system and the protecting groups at the 6-*O*-position in donors.[104] Table 7 presents the glycosylation of 6-*O*-TBS-2-deoxy-2-azido-thioglucoside **104** and 6-*O*-Bz $GlcN_3$ donor **105** with various primary and secondary alcohols. All of the glycosylation reactions were executed using a pre-activation protocol. The donor was activated with 1.0 equiv of TolSCl/AgOTf in diethyl ether at -78 °C, after which the acceptor was added to the reaction mixture and the mixture was slowly warmed to room temperature. The authors argued that the participating Bz group or the bulky TBS group at the *O*-6-position would block the β-attack of the glycosyl acceptor through either remote group participation or steric hindrance, thus facilitating the formation of the α-products. Glycosylation of $GlcN_3$-donors **104** and **105** with secondary acceptors **106a-e** afforded the desired disaccharides in good yields (78-86%) and with excellent α-selectivity (15:1 to α-only, Table 7, entries 1-5). However, coupling of these donors with reactive primary acceptor **106f** generated the products in 5:1 and 3:1 α/β ratio (Table 7, entry 6). The less reactive Bz-protected acceptors **106g** and **106h** gave better results in terms of α-glycosylation selectivity (16:1 to 19:1, Table 7, entries 7 and 8).

Table 7. Glycosylation of 6-O-TBS and Bz protected $GlcN_3$ donors



Entry	Acceptors	Products	Yield	α:β
1	 **106a**	**107a** R^1 = TBS	80%	α-only
		107b R^1 = Bz	81%	α-only
2	 **106b**	**107c** R^1 = TBS	81%	>19:1
		107d R^1 = Bz	83%	15:1
3	 **106c**	**107e** R^1 = TBS	86%	α-only
		107f R^1 = Bz	83%	α-only

4	 **106d**	**107g** R^1 = TBS	78%	>19:1
		107h R^1 = Bz	84%	α-only
5	 **106e**	**107i** R^1 = TBS	81%	α-only
		107j R^1 = Bz	85%	>19:1
6	 **106f**	**107k** R^1 = TBS	80%	5:1
		107l R^1 = Bz	84%	3:1
7	 **106g**	**107m** R^1 = TBS	87%	>19:1
		107n R^1 = Bz	85%	>19:1
8	 **106h**	**107o** R^1 = TBS	87%	>19:1
		107p R^1 = Bz	88%	16:1

6. Outline of the thesis

This Thesis reports the assembly of a library of GAG fragments from *Aspergillus fumigatus* and a library of Pel oligomers of *Pseudomonas aeruginosa*, using DTBS-directed α-glycosylation methodology. In the introductory **Chapter 1** a concise overview is presented on the recent progress of the stereoselective introduction of α-galactosamine and α-glucosamine glycosidic linkages. Information is given on the structure, occurrence, properties and (bio)synthesis of both GAG exopolysaccharides and Pel polysaccharides. **Chapter 2** describes the synthesis of homopolymers of Gal, GalN and GalNAc. Two nonasaccharides composed of Gal or GalN moieties as well as a dodecasaccharide containing GalNAc moieties were constructed with high yields and complete α-selectivity. **Chapter 3** shows the assembly of heteropolymers of Gal, GalN and/or GalNAc moieties. A DTBS-trichloroacetamide donor was used, alongside a DTBS-protected galactosazide donor to introduce the α-GalNAc linkages, overcoming the neighboring group participation effect, and effectively discriminating the two nitrogen functionalities. **Chapter 4** deals with the successful synthesis of a GAG-heptasaccharide with an 2-azido group on the galactose sugar ring of the non-reducing end. The azido group was introduced to provide the heptasaccharide with a biorthogonal conjugation handle, which will benefit the study of the biosynthesis pathway of GAG. **Chapter 5** describes the optimization of glycosylation reactions towards the stereoselective introduction of α-GlcN linkages. With the aid of the optimized condition, a library of Pel heptasaccharides was assembled. **Chapter 6** provides a summary of the obtained results described in the foregoing chapters and an outlook for future research.

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