Structure-reactivity relationships in glycosylation chemistry

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Chapter 1: General introduction

Introduction

Carbohydrates are a class of biopolymers with large variety in terms of both structure and function in both prokaryotes and eukaryotes. The main difference that separates carbohydrates from the other biopolymers (nucleotides and proteins) is that when two carbohydrate building blocks are linked together, a new stereocenter is introduced. The stereocenter is important for the properties of the carbohydrate, for example starch is a polymer of α 1→4 linked glucose and cellulose is a polymer of β 1→4 linked glucose (***Figure 1***).

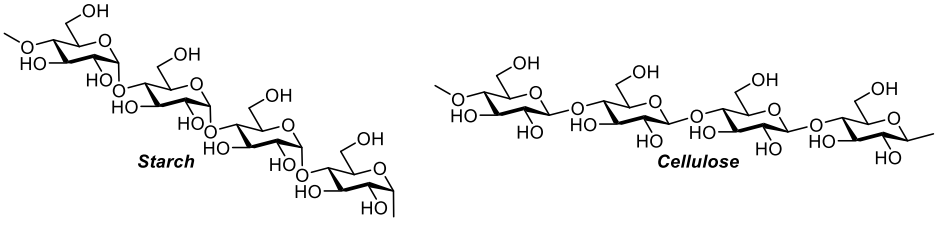


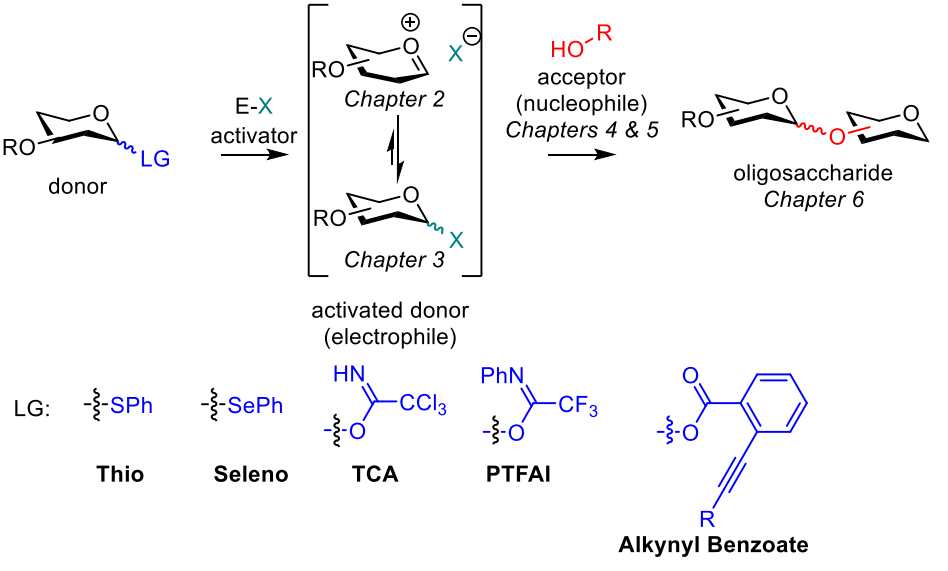
Figure 1: *Structure differences between starch and cellulose*

There are many (bio)medical applications of carbohydrates.[1-3] However, carbohydrates can be difficult to isolate from biological sources in sufficient quantify and purity. Therefore, chemical synthesis is an attractive alternative, because it allows for full control over the structure of the obtained product regarding properties including substitution pattern and chain length. The two main challenges in synthetic carbohydrate chemistry are regio- and/or chemoselective protection of hydroxyl- and other functional groups[4-7] and the stereoselective formation of a glycosidic bond.[8-12] This thesis will focus mainly on the latter.

Carbohydrate synthesis

A typical synthesis of a glycosidic bond is depicted in ***Scheme 1***. There are many variations in glycosylation protocols, but the vast majority of them follows this same scheme, where a donor with a latent leaving group reacts with an electrophilic activator. Examples of leaving groups include thio- or selenophenol, which are activated by a stoichiometric amount of an electrophilic promotor such as NIS/TMSOTf or a sulfoxonium ion, generated from, for example, Ph_2SO/Tf_2O[13-16]. A different class of leaving groups are the glycosyl imidates (most importantly the trichloroacetimidate (TCA) or *N*-phenyl trifluoroacetimidate (PTFAI) donors), which are activated by a catalytic amount of Lewis- or Brønsted acid such as $BF_3.OEt_2$, TMSOTf or TfOH.[17, 18] Another type of donor, introduced by Yu and coworkers, are the alkynyl benzoates, which can react under mild conditions catalysed by Au(I).[19] Upon activation of the

donor, a mixture of electrophilic species is generated, varying from oxocarbenium ions, which react via a S_N1 like mechanism, to covalent species which react via a S_N2 mechanism with a nucleophile (acceptor). These are attacked by the incoming nucleophile leading to either the α-product, the β-product or a mixture of the two.[18]



Scheme 1: *General overview of a glycosylation reaction and the contents of this thesis*

Stereoselective glycosylation methods

Many methods have been developed for the stereoselective formation of a glycosidic bond (***Figure 2***). 1,2-Trans glycosidic bonds are usually relatively easy to construct using neighbouring group participation (***Figure 2A***), where an acyl type protecting group, usually an ester, amide or carbamate, is placed on O-2 or N-2. Upon activation of the donor, the carbonyl group reacts to form a dioxolenium ion, which reacts with an acceptor to form a 1,2-trans glycosidic bond.[20, 21] Participation of protecting groups on other positions has also been observed and used for the control of stereochemical outcome (*vide infra*). More recently, it has also been reported that alkoxymethyl protecting groups can provide 1,2-trans glycosidic bonds via a similar mechanism.[22]
For the formation of 1,2-cis glycosidic bond no such universally applicable technique exists, making the synthesis of these linkages more challenging.[11, 23] Nevertheless, multiple methods for the construction of these glycosidic bonds have been developed and this chapter will discuss a few examples.

Historically, the β-mannosidic bond was one of the most difficult ones to synthesize.[24] One method to synthesize this kind of glycosidic bond was developed by the laboratory of Crich, in which a benzylidene is used as a protecting group for the C-4-OH and C-6-OH (***Figure 2B***).[25, 26] Evidence based on variable temperature NMR,[27-29] and a combination of kinetic isotope effect[30] and cation clock experiments[31] show that the β-mannosidic bond is formed through a S_N2 reaction with the α-triflate, which is formed when the donor is activated with a triflate based promotor.[32] However, there is also evidence that the stereoselectivity can be explained via an S_N1 reaction via an oxocarbenium ion adopting a $B_{2,5}$-conformation.[33]

Another method for generating a 1,2-cis glycosidic bond based on the use of a cyclic protecting group on the C-4-OH and C-6-OH, is the use of a di-tert-butylsilylene (DTBS) group on galactose and galactosamine (***Figure 2C***). Introduced by Kiso and coworkers in 2003, glycosylation with this type of donor stereoselectively yields the α-galactoside, even when a participating group is present on C-2.[34] In the mechanism that explains this selectivity, an oxocarbenium ion is generated where the bulky DTBS group shields the top-side from attack by a nucleophile, so that only an attack from the bottom of the oxocarbenium ion is possible, which yields the α-product.[35]

Participation of the protecting group on C-2 has also been used to introduce a 1,2-cis glycosidic bond via a chiral auxiliary (***Figure 2D***). In this system, a nucleophilic group (for example CO_2Et[36] or SPh[37]) on the protecting group on C-2 reacts with the oxocarbenium ion upon activation of the donor. The *S*-configuration of the protecting group is necessary for the stabilisation of the *trans*-decalin species to form the desired α-product. When the corresponding *R*-configured protecting group was used, the *cis*-decalin intermediate was generated, delivering the β-product.[36, 37] Of note, the use of a non-chiral, nucleophilic protecting group on C-2 for the formation of α-glucosidic bonds was also described.[38-40]

The final two methods discussed in this chapter rely on "directing" the acceptor in the right direction, either through hydrogen bond formation (hydrogen bond mediated aglycon delivery, ***Figure 2E***) or by covalently attaching the acceptor to a protecting group on C-2 (intramolecular aglycon delivery, ***Figure 2F***). In hydrogen bond mediated aglycon delivery, a protecting group (in this case a picoloyl) with a hydrogen accepting functional group forms a hydrogen bond with the nucleophilic alcohol, which is then positioned on the "right" side of the reactive species to ensure a stereoselective glycosylation. This method has delivered β-mannosides using a C-3-picolyl ester[41], α-glucosides and α-xylosides using a C-4-picolyl ester[42,43], and β-glycosides and β-fructosides using a C-6-picolyl ester[44,45]. With intramolecular aglycon delivery, the protecting group on C-2 first forms a covalent bond with the acceptor. In the next step, an activator is added, and the 1,2-cis-glycosidic bond is formed. In the depicted example, the PMB protecting group is first oxidised to form an acetal with the acceptor,

which in the next step forms the desired β-mannosidic bond. Other acetals, as well as silyl based protecting groups have also been described.[46, 47]

The reason that stereoselective glycosylation remains challenging is two-fold. For one part, solutions are usually target-oriented and the other reason is that there are multiple different mechanistic pathways that can lead to two different products and that the mechanistic pathways are influenced by external factors such as solvent, temperature and concentration, as well as properties inherent to the donor and the acceptor.[48] A better understanding of the mechanistic pathways and the factors that influence these makes it easier to rationalize and eventually predict the outcome of glycosylation reaction and as such will streamline the total synthesis of biologically relevant carbohydrates.

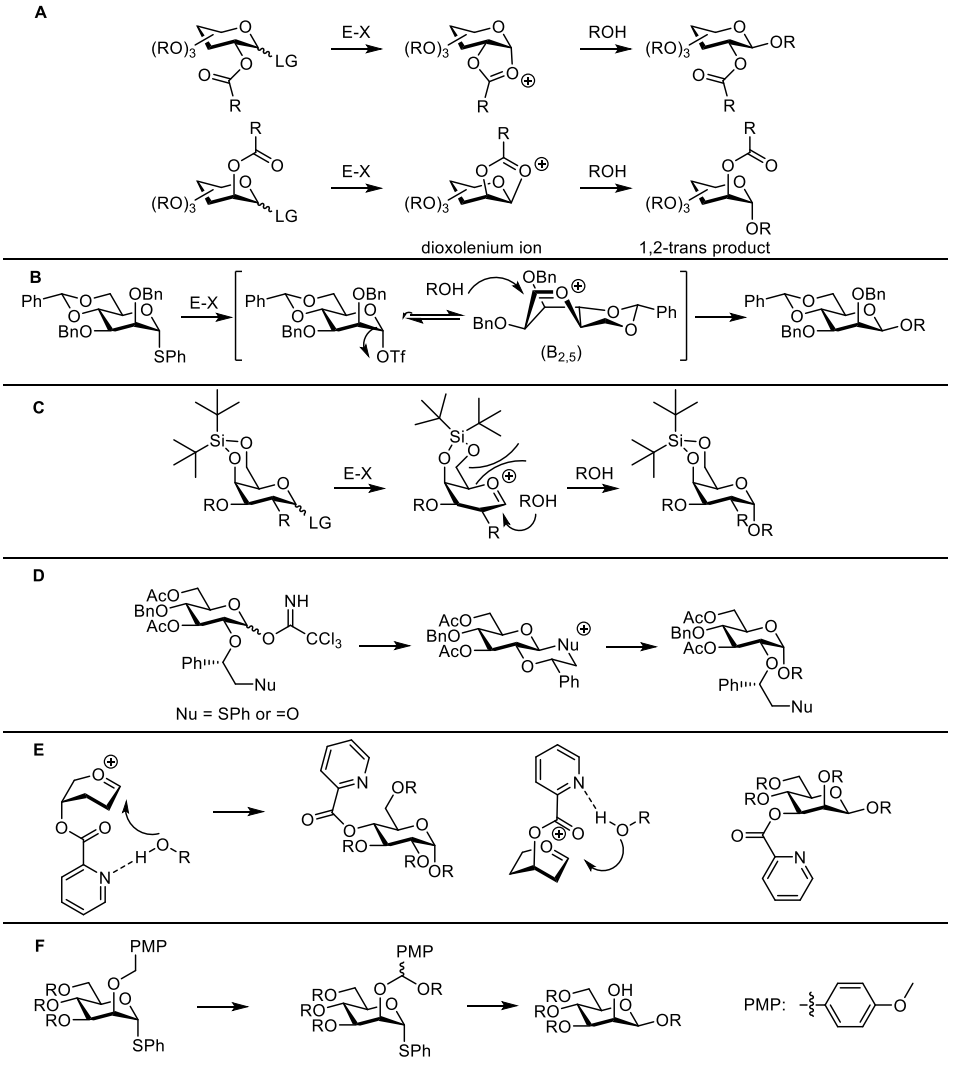


Figure 2: *Representative examples of methods for the stereoselective formation of glycosidic bonds. A: 1,2-Trans selective glycosylation through neighbouring group participation via a dioxolenium ion; B: β-Selective mannosylation via a conformationally restricted donor; C: α-Selective galactosylation using the bulky di-tert-butylsilylene (DTBS) group; D: α-Selective glucosylation using a chiral auxilary; E: 1,2-Cis selective glycosylation via hydrogen bond mediated aglycon delivery using the picoloyl (pico) protecting group; F) 1,2-Cis selective glycosylation via a covalent intermediate, using intramolecular aglycon delivery using the 4-methoxybenzyl (PMB) group as an example.*

Oxocarbenium ions in glycosylation reactions

In a S_N1 type glycosylation reaction, the acceptor reacts with an oxocarbenium ion to form a glycosidic bond. The stereochemical outcome of these reactions is determined by the conformation and reactivity of the corresponding oxocarbenium ion. Oxocarbenium ions typically take up either a $^3H_4$- or $^4H_3$-like conformation (***Figure 3***) and the relative stability of the two conformers depends on the substituents on the ring.[49] The two different conformations can give rise to opposite stereochemical outcomes of the glycosylation reactions. For a $^4H_3$ oxocarbenium ion, a top-faced attack will result in an unfavourable twist-boat like transition state, while a bottom-face attack proceeds through a favourable chair-like transition state, causing the $^4H_3$ oxocarbenium ions to be bottom-face selective. For the same reasons, $^3H_4$ oxocarbenium ions are top face selective (see ***Figure 3***).[50, 51] Consequently, this means that for D-configured hexoses, the $^4H_3$-configured oxocarbenium ion yields the α-product and the $^3H_4$-configured oxocarbenium ion yields the β-product. For L-configured hexoses the opposite is true.[52]

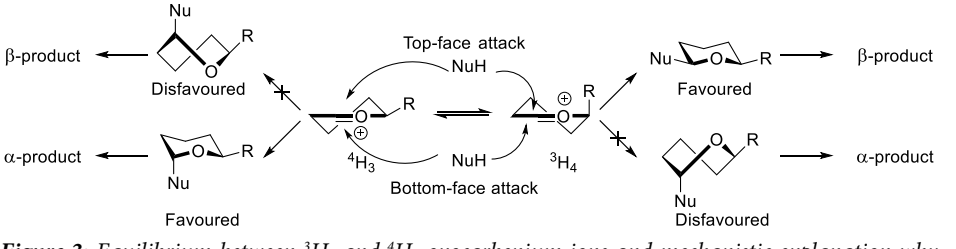


Figure 3: *Equilibrium between $^3H_4$ and $^4H_3$ oxocarbenium ions and mechanistic explanation why $^3H_4$-like oxocarbenium ions are Top-face selective and why $^4H_3$-like oxocarbenium are bottom face selective. Prediction for the formation of an α- or β-product from a conformation is based on D-configured carbohydrates, for L-configured carbohydrates, the opposite product is formed.*

The effects of single substituents on the $^4H_3/^3H_4$ equilibrium of oxocarbenium ions, and by extension the outcome of S_N1 reactions were first experimentally investigated by Woerpel and coworkers, who found that *O*-alkyl substituents on C-3 and C-4 prefer a pseudo-axial orientation in the oxocarbenium ion half chairs, due to electrostatic stabilisation of the positive charge, while alkyl substituents on the same positions prefer a pseudo-equatorial orientation because of steric reasons.[51, 53-55]

A more quantitative investigation of the reactivity of oxocarbenium ions was recently reported by Hansen *et al.*[56] A combination of computational chemistry, superacid NMR[57] and model glycosylations with deuterium nucleophiles was used to show that a S_N1 type glycosylation reactions with fully functionalised glycosyl donors are typically highly 1,2-cis selective.

Another technique used to characterise glycosyl cations is infrared ion spectroscopy (IRIS).[58, 59] In this technique, glycosyl cations are generated in the gas phase, for example by ion collision. The cations are then measured using IR spectroscopy. This technique has been used to study the formation of oxocarbenium and dioxolenium ions, formed by attack of the carbonyl group of one of the protecting groups (as is well described for neighbouring group participation from C-2, *vide supra*). The structures of dioxolenium ions were characterised using IRIS by Pagel and coworkers (***Figure 4A***), showing that the glucosyl 1,2-dioxolenium ion takes up a $^{3}S_{1}$ conformation, the mannosyl dioxolenium ion a $B_{O,3}$ conformation, and the galactose dioxolenium ion forms a mixture of ^{4}E and $^{1}S_{3}$ conformers.[21]

Acyl protecting group participation from other positions than C-2, has been less well understood than neighboring group participation by C-2-acyl groups. IRIS has been used to better understand remote participation of acyl groups on other positions than C-2.[58, 59] Rijs, Boltje and coworkers used a combination of NMR and IRIS to investigate the mechanisms behind the stereoselectivity of 3,6-uronic acid lactone donors. The high β-selectivity of the mannuronic acid 3,6-lactone donor was explained by participation from the acetyl group on C-4 via a dioxolenium ion with a $B_{1,4}$ conformation (***Figure 4B***).[60] To prove that the increased α-selectivity of 4-O-acetyl galactosyl donors (compared to per-benzyl donors) is due to remote participation, Marianski, Pagel and coworkers used IR spectroscopy to characterise a $^{1}S_{5}$ configured dioxolenium ion that is formed when a 4-O-acetyl galactosyl donor is activated (***Figure 4C***).[61]

Boltje, Codée and coworkers studied the stabilisation of uronic acid glycosyl cations through participation from a C-4 acetyl protecting group or participation from the ester on C-5 using IRIS. It was concluded that stabilisation happens mainly through participation of the C-5 carboxyl, when C-2 and C-5 are *cis*, as is the case with mannuronic and taluronic acid. When C-2 and C-5 are *trans*, as is the case for glucuronic and galacturonic acid, stabilisation mainly happens trough participation from the acetyl group on C-4. (***Figure 4D***) [62]

Finally, through a combination of experimental glycosylations, DFT-calculations and IR-spectroscopy, it was found that remote participation is particularly strong from the C-3 position of mannose, making glycosylations with these donors highly α-selective. The order of remote participation was found to be as follows (strongest to weakest) 3-Ac-Man » 4-Ac-Gal > 3-Ac-Glu ≈ 3-Ac-Gal > 4-Ac-Glu > 4-Ac-Man ≈ 6-Ac-Glc/Gal/Man.[63]

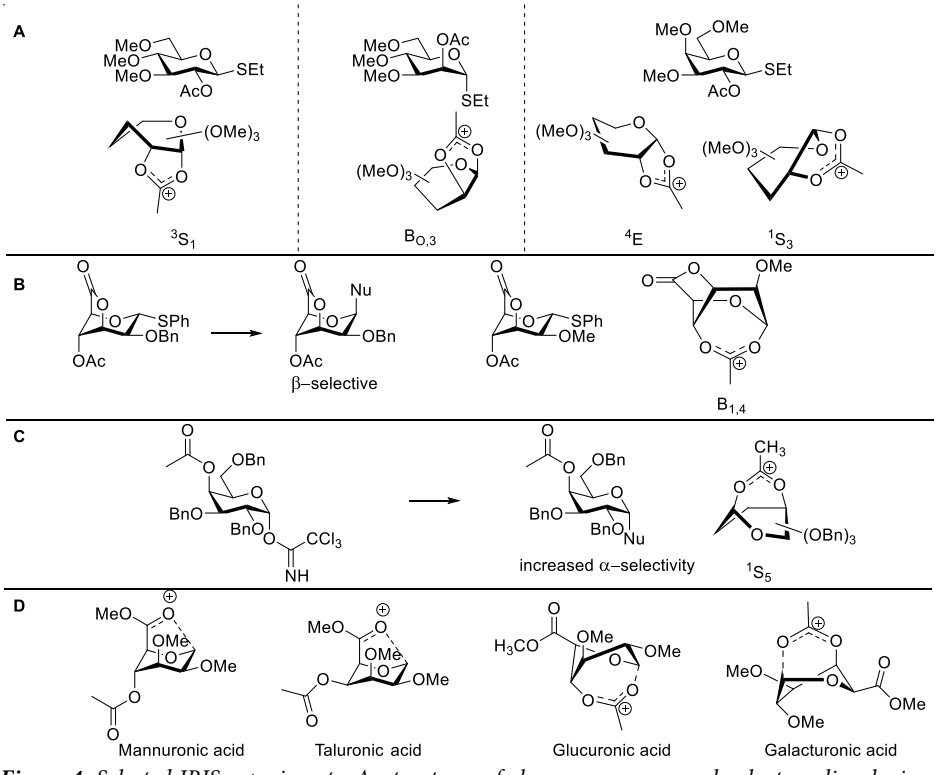


Figure 4: *Selected IRIS experiments. A: structures of glucose, mannose and galactose dioxolenium ions with neighbouring group participation.[21] B: the 3,6-mannuronic acid lactone donor is β-selective by participation of the acetyl group on C-4.[60] C: remote participation of the C-4-O-acetyl group on a galactose donor via a $^1S_5$ dioxolenium ion.[62] D: the structure of the dioxolenium ion of 4-O-acyl glucuronic acid donors is mainly determined by the stereochemistry of C-2. Mannuronic acid and taluronic acid show participation via C-5, while glucuronic acid and galacturonic acid show participation via C-4-O-acetyl.[62]*

Covalent species in glycosylation reactions

On the other side of the mechanistic spectrum of glycosylation reactions, are S_N2-like reactions, in which covalent reactive intermediates play a decisive role. Through *in-situ* anomerisation, first described by Lemieux and coworkers, a relatively stable and unreactive α-species is in equilibrium with a more reactive and less stable β-species. The equilibrium leans towards the side of the α-species, but the more reactive β-species can selectively react with an acceptor in an S_N2 reaction to form an α-glycosidic bond (***Figure 5A***).[64] Examples using this phenomenon have been described for bromides[65] and iodides[66-69] using a source of X^- ions, for example TBABr or TBAI.

Based on this mechanism, the use of nucleophilic additives to modulate the stereochemical outcome of the glycosylation reaction has been explored (***Figure 5B***). West and Schuerch used dimethylsulfide, triethylamine and triphenylphosphine for the modulation of methanolysis of glucosyl bromides. They proposed that through the formation of a β-adduct with the additive, the α-product is formed through an S_N2 mechanism. Dimethylsulfide gave a 6:1 α:β ratio, where triethylamine and triphenylphosphine gave full α-selectivity.[70] Amides are also used as glycosylation modulator. Koto and coworkers introduced *N,N*-dimethylacetamide (DMA) as an additive for the stereoselective synthesis of α-glucosides.[71-73] More recently *N,N*-dimethylformamide (DMF) was introduced as a glycosylation modulator by Mong and coworkers for the synthesis of α-glucosides[74] and α-2-deoxy or 2,6-deoxy sugars.[75] DMF was, however, less successful in the modulation of less reactive 2-azido-glycosyl donors. Therefore, *N*-formylmorpholine (NFM) was introduced to overcome the low reactivity issues. With NFM α-2-azido-glucosides and α-2-azido-galactosides could be synthesised with good yield and selectivity.[76] Of note, the amide additives work well for stereoselectivity modulation with secondary alcohols, but not that well with more reactive primary alcohols.

Based on their success with sulfur containing chiral auxiliaries (see above) Boons and coworkers investigated phenyl ethyl sulfide (PhSEt) and thiophene as additive for 1,2-cis glycosylation of α-2-azido-glucoside donors. With a disarmed donor, these additives gave good yield and selectivity. With an armed donor, significantly less selectivity was obtained.[77] Diphenyl sulfoxide was first introduced for dehydrative glycosylations by Gin and coworkers,[13] but is was also used as an additive for the glycosylation with sialic acid donors by Crich and Li (see also chapter 7), where it was shown that the sulfoxonium adduct plays an important role in ensuring a productive outcome of the glycosylation reaction.[78] Bennett and coworkers used 2,3-bis(2,3,4-trimethoxyphenyl)-cyclopropene-1-thione for stereoselective glycosylation 2,6-dideoxy sugars with a quaternary carbon on C-3 in the total synthesis of the antibiotic saccharomicin B.[79]

Like West and Schuerch, Ye and coworkers investigated phosphines for the synthesis of α-glucosides and galactosides. In a system based on urea catalysed glycosylation of chloride donors, they found tri-(2,4,6-trimethoxyphenyl)-phosphine to be the most effective.[80] The use of phosphine oxides was first described by Bogusiak and Szeja, who used HMPA as an additive for the stereoselective synthesis of α-xylofuranoses.[81] Later, Mukaiyama and coworkers further investigated the effect of different phosphine oxides with glycosyl bromides, iodides and acetates for the synthesis of α-glucosides. The inherent instability of glucosyl iodides was overcome by using TMSI and phosphine oxide on glucosyl acetates to generate the glucosyl iodide *in situ*.[82-84] Phosphine oxides were also used in the synthesis of α-ribofuranosides,[85] α-2-deoxyglycosides[86] and β-3-amino-2,3,6-trideoxy sugars.[87] A representative example which shows the synthetic utility of glycosylation modulated by nucleophilic additives was published by Wang *et*

al. (***Figure 5C***). For secondary alcohols, DMF was used as an additive, while TMSI/Ph_3P=O was used in glycosylations with primary alcohols in the stereoselective synthesis of the α-glucosidic bonds using an *in-situ* anomerisation mechanism.[88-90] In a similar way, methyl(phenyl)formamide was used for glucosazide donors.[76, 91]

The extent to which in-situ anomerisation plays a role in pre-activation based glycosylations which generate triflates as reactive species remains somewhat controversial, but recently, sophisticated NMR studies have also shown that in situ anomerisation can also play a role in glycosylations which have triflates as reactive intermediates (see below)

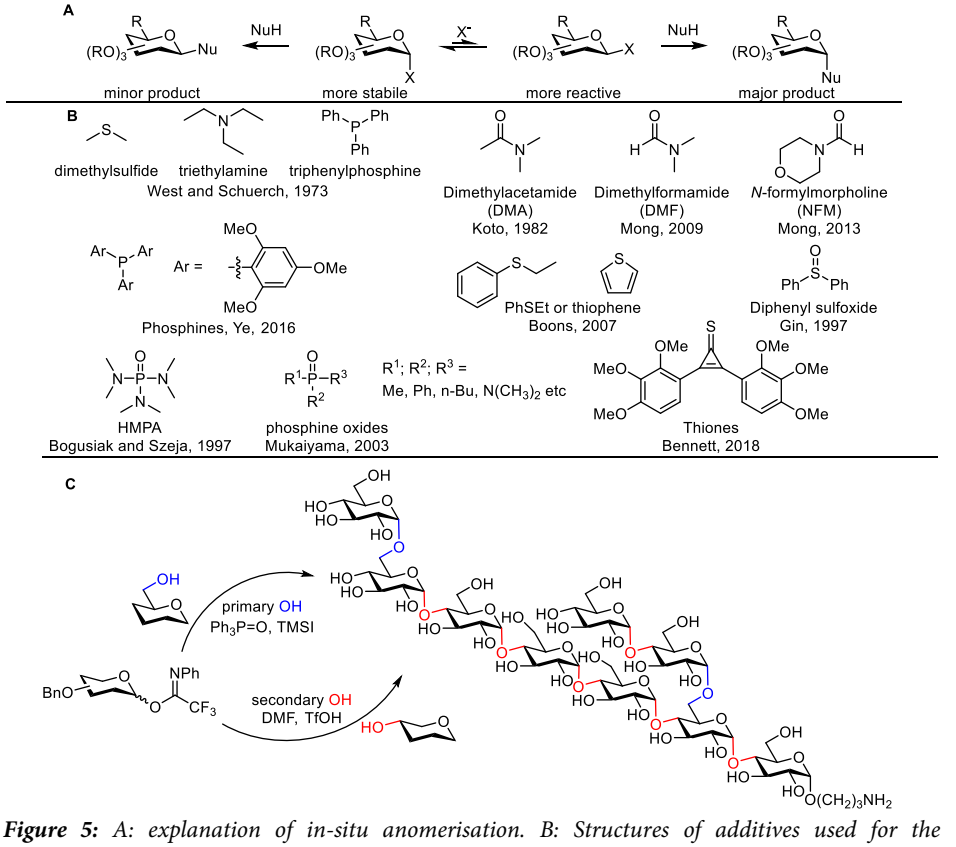


Figure 5: *A: explanation of in-situ anomerisation. B: Structures of additives used for the modulation of stereoselectivity in glycosylation reactions. C: representative synthesis of an oligosaccharide using additives by Wang et al.*[88]

Variable temperature NMR (VT –NMR) is a versatile technique to investigate the covalent reactive intermediates in glycosylation reactions. In this method, a donor and an activator are reacted in an NMR tube to enable the recording of NMR spectra of the reaction mixture at low temperature to characterise the reactive species that are formed. Many reactive intermediates, mostly triflates, have been characterised using this method. Frihed, Bols and Pedersen have provided an extensive review on glycosyl triflates[92] and a few representative examples will be highlighted here.

When trichloroacetimidates (TCA) are used as donor, the reactive intermediates that are formed are determined by the nature of the activator (***Figure 6A***). By using a combination of 1-H NMR, 19-F NMR and DOSY NMR, Qiao *et al.* showed that when triflate based activators are used, α-triflates can be formed, which eventually decompose to provide a mixture of α- and β-trichloroacetamides. With BF_3-Et_2O, the β-fluorides were formed, which isomerise to the α-anomer at higher temperature. When bistriflimide activators are used, the silylated donor was found to be formed as a reactive intermediate, which decomposes by an intramolecular Friedel-Crafts type reaction of the C-2-benzyl group.[93]

During the synthesis of mannuronic acid and 2- and 2,3-deoxy amino mannuronic acid containing bacterial oligosaccharides, Walvoort *et al.* characterised the triflates that are formed when the thio donors are activated with the Ph_2SO/Tf_2O reagent combination (***Figure 6B***). The α-triflate was formed exclusively in all cases, but two species were observed by VT-NMR, because the triflates were present as a conformational mixture of $^4C_1$ and $^1C_4$ conformers.[94-96]

During the total synthesis of a complex oligosaccharide from *Mycobacterium marinum* by Hansen *et al*, VT-NMR was used in the optimisation of glycosylations with rare caryophyllose donors (***Figure 6C***). When the alcohols on the side-chain on C-4 were protected with benzyl groups, the glycosylation reactions were unsuccessful due to the rapid formation of a bicyclic side product. When the benzyl groups were replaced by carbonate protecting groups, the caryophyllose β-oxosulfonium triflate was found as the main reactive intermediate, which successfully react to form the glycosylation products.[97]

Santana *et al*. have used VT-NMR to show that β-triflates play a role in glycosylation reactions of benzylidene protected glucosyl, mannosyl and allosyl donors, (***Figure 6D***). Normally, β-triflates are not visible in VT-NMR experiments due to their low concentration, but though a series of sophisticated 1D and 2D experiments it was possible to confirm their presence.[98]

The aforementioned formation of dioxolenium ions that occurs when there is a participating protecting group on C-2 can also be observed using VT-NMR (***Figure 6E***). When a peracetylthio glucosyl donor was activated with AgOTf/p-TolSCl in $CDCl_3$ at -60 ℃, a 1:1 mixture of anomeric triflates and dioxolenium ions was obtained. Upon warming to -20 ℃, the dioxolenium ion was converted into the α-triflate. When the

mixture was recooled to -60 °C, the mixture of triflates and dioxolenium ions was formed again.[99]

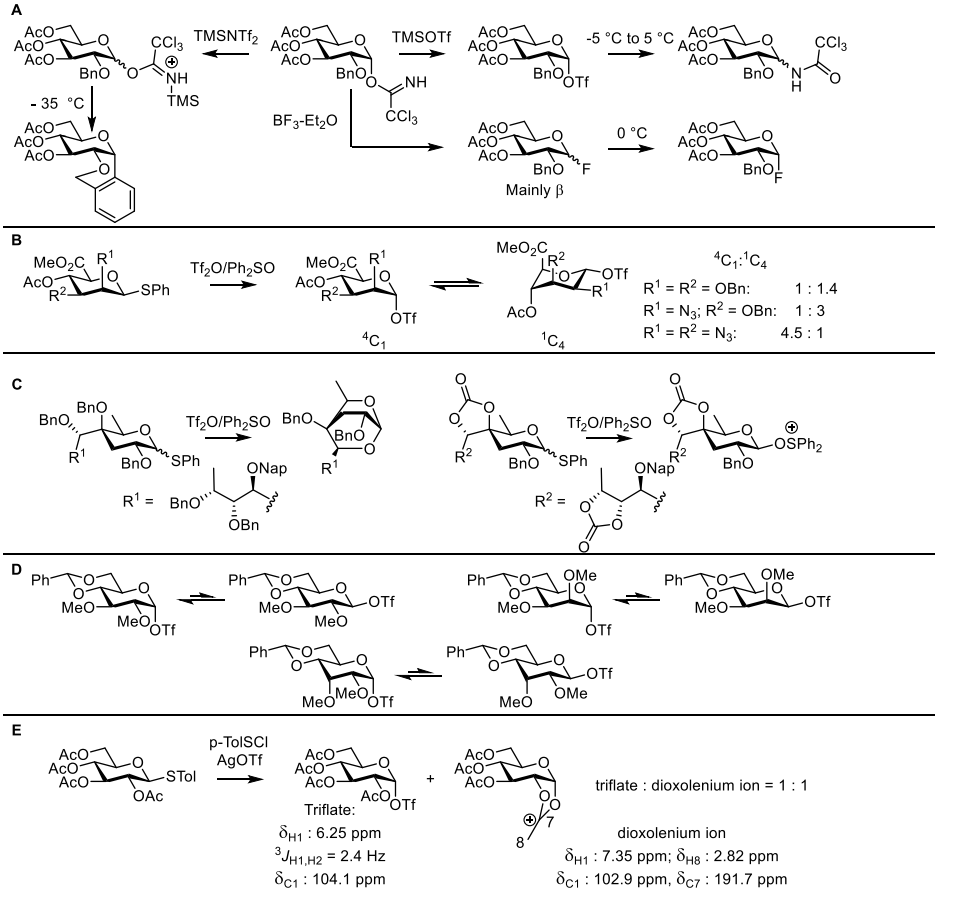


Figure 6: *Examples of VT-NMR used to investigate reactive intermediates formed in glycosylation reactions. A: Reactive species and decomposition products from a trichloroacetimidate donor with different activators.[93] B: Conformational equilibrium of triflates that form upon activation of the mannuronic acid donors.[95] C: Donor optimisation in the total synthesis of a Mycobacterium marinum Lipooligosaccharide.[97] D: In-situ anomerisation studied with VT-NMR.[98] E: The equilibrium between dioxolenium ions and anomeric triflates, observed by VT-NMR.[99]*

Acceptor reactivity

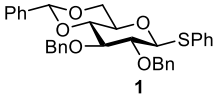
Influence of acceptor reactivity on the glycosylation reaction

The reactivity of the acceptor plays a crucial role in the outcome of a glycosylation reaction. The extent to which the reactivity of the acceptor affects the outcome depends heavily on other factors such as the reaction conditions and the donor. To understand the influence of acceptor reactivity on the outcome of glycosylation reactions, a series of model acceptors with varying nucleophilicity was introduced, with ethanol (EtOH) being the most nucleophilic and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) the least.[100] The acceptors in this set are not chiral, so that any difference in stereochemical outcome is due to differences in nucleophilicity and not due to diastereomeric effects.[101] ***Table 1*** shows a few key results in recent studies.

Glucose, mannose and mannuronic acid donors (Donors **1**, **2** and **4**) were used by van der Vorm *et al.* to investigate the influence of acceptor nucleophilicity on the reaction mechanism of glycosylation reactions. This set of ethanol acceptors together with some carbohydrate acceptors show that more nucleophilic acceptors react via a S_N2 type mechanism, while less nucleophilic acceptors react via a mechanism with more S_N1 character.[102] In another study, it was shown that the stereoselectivity of conformationally restricted glucosazide donors **3** strongly depends on the nucleophilicity of the acceptor. This donor is more β-selective than its glucose counterpart, because the electron withdrawing azide on C-2 stabilises the α-triflate more, making a S_N2-type glycosylation more favourable.[103]

Hagen *et al.* have used the set of model acceptors to map the relation between acceptor nucleophilicity and stereoselectivity for 2-azidofucosyl donors (*e.g.* using donor **5**).[104] The set of fluorinated acceptors was also used by Hansen *et al.* to generate more insight into the reactivity of rare building blocks used in the synthesis of a complex oligosaccharide from *Mycobacterium marinum* (donors **6** and **7**).[97] Finally, this set of nucleophiles was also used in the aforementioned study on remote participation.[63]

Table 1: *Model glycosylations by van der Vorm et al. (**1-4**)[102, 103], Hagen et al. (**5**)[104] and Hansen et al. (**6** and **7**)[97] n.d. = not determined*



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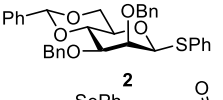

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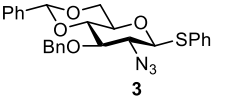

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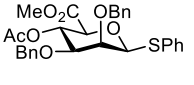

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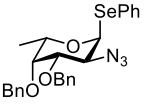

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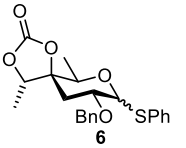

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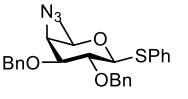
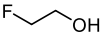
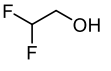


7

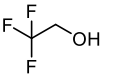
EtOH



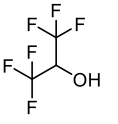
MFE



DFE



TFE



HFIP

Donor	Acceptor (product, **α:β,** yield)				
	EtOH	**MFE**	**DFE**	**TFE**	**HFIP**
	1A	**1B**	**1C**	**1D**	**1E**
1	**1:10**	**1:2.8**	**5:1**	**>20:1**	**>20:1**
	(83%)	(70%)	(90%)	(80%)	(65%)
	2A	**2B**	**2C**	**2D**	**2E**
2	**1:5**	**1:5**	**1:5**	**1:4**	**3:1**
	(70%)	(86%)	(90%)	(78%)	(56%)
	3A	**3B**	**3C**	**3D**	**3E**
3	**<1:20**	**1:6.7**	**2.9:1**	**>20:1**	**>20:1**
	(83%)	(90%)	(64%)	(94%)	(53%)
	4A	**4B**	**4C**	**4D**	**4E**
4	**1:8**	**1:6**	**1:5**	**1:2.5**	**1:1**
	(95%)	(70%)	(87%)	(85%)	(52%)
	5A	**5B**	**5C**	**5D**	
5	**1:1**	**1:1**	**2:1**	**19:1**	**n.d.**
	(88%)	(72%)	(81%)	(80%)	
	6A	**6B**	**6C**	**6D**	**6E**
6	**1:1**	**2:1**	**4:1**	**>20:1**	**>20:1**
	(60%)	(76%)	(100%)	(77%)	(28%)
	7A	**7B**	**7C**	**7D**	**7E**
7	**1.8:1**	**1:1.1**	**3.3:1**	**>20:1**	**>20:1**
	(87%)	(100%)	(91%)	(70%)	(69%)

Reactivity of carbohydrate acceptors

The glycosylations with fluorinated model acceptors highlight the importance of acceptor reactivity on the outcome of glycosylation reactions. But in contrast to glycosyl donors, where the structure-reactivity relations are relatively well described thank to spectroscopic and computational studies (see above) as well as measurement of relative reactivity values of thioglycosides,[105-107] factors influencing the reactivity of glycosyl acceptors are less well studied.[108] As described above, the stereochemical outcome of benzylidene glucose and glucosamine donors **1** and **3** highly depends on the nucleophilicity of the acceptor. This property was used to "measure" the reactivity of a large set of acceptors, all with systematic variations in stereochemistry and protecting group patterns. Screening of systematically varied sets of acceptors using this system shows how seemingly small changes on in the protecting group pattern of the acceptor can drastically change the reactivity of an acceptor. For example, when the C-3 protecting group of a C-4-OH glucose acceptor was changed from a benzyl to a sterically similar benzoyl group (effectively substituting a CH_2 group for a C=O group) the outcome of the glycosylation reaction changed from being unselective to fully α-selective and from β-selective to α-selective (***Figure 7***).[109]

A more systematic understanding of which factors influence the reactivity of the acceptor could also help developing a quantitative measurement system for the reactivity of acceptor reactivity, as proposed by Wong, Wang and coworkers.[110] This can in turn be used to measure the influence of acceptor reactivity on the outcome of glycosylation reactions.

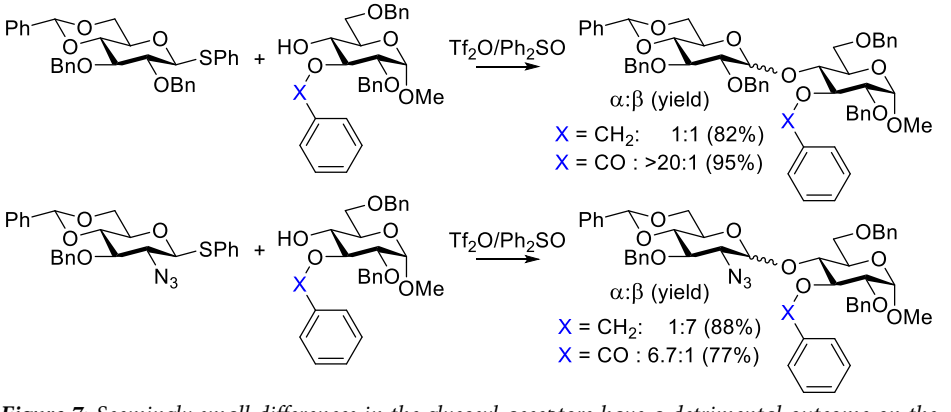


Figure 7: *Seemingly small differences in the glycosyl acceptors have a detrimental outcome on the glycosylation reaction in terms of stereoselectivity. Changing a CH_2 group into a C=O group on an acceptor reverses the stereoselectivity of the glycosylation reaction.*[109]

Contents

The aim of this thesis is to increase the understanding of several aspects of the glycosylation reaction and the influence of these on the (stereochemical) outcome, and as such make rational design of a synthesis route towards a target oligosaccharide possible.

Chapter 2 describes the synthesis of glycosyl donors of all the eight diastereomers of phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-pyranosyl donors, as well as their 6-deoxy analogues and studies the behaviour in S_N1-like reactions via computational and experimental chemistry.

Chapter 3 deals with the characterisation of reactive covalent species in S_N2 like reactions with VT-NMR and the investigation of the influence of nucleophilicity of oxygen nucleophiles on the mechanism of the glycosylation reaction.

Chapter 4 reports the structure-reactivity relationships of ether and ester protected carbohydrate acceptors using the stereoselectivity of two conformational restricted donors.

Chapter 5 builds on chapter 4 and outlines the influence of azides, trifluoro- and trichloroacetamides on the reactivity of different glycosyl acceptors.

Chapter 6 presents an application of the research as described in chapter 2-5 for the synthesis of an oligosaccharide with an unusual structure found in *Acinetobacter Baumannii*, an antibiotic resistant pathogen, through the mapping of the reactivity and selectivity of 2,3-di-*N*-acetlyglucuronic acid and bacillosamine donors and acceptors using VT-NMR and model glycosylations.

Chapter 7 provides a brief summary and gives some suggestions for further research.

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