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Lewis acid catalysed Direct Glycosylation of N-Acetyl-D-Glucosamine

Tesi di Laurea Sperimentale

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ABSTRACT

Incorporation of the relevant monosaccharide N-Acetyl-D-glucosamine (GlcNAc) into synthetic oligosaccharides by chemical glycosylation is still a very challenging object of studies, since direct reactions are low yielding. This issue is generally ascribed to its low solubility in common solvents and to the formation of a poorly reactive oxazoline intermediate, which is typically bypassed by introducing extra synthetic steps to avoid the presence of the NHAc moiety during glycosylation. Recently, a new direct Lewis acids-catalysed GlcNAc-ylation protocol has been disclosed, with acylated donors appearing to hold potential for high yielding glycosylation reactions.

This master project focused indeed on a novel synthesis of promising 1-acyl GlcNAc donors, in order to test them in direct Lewis acid catalysed glycosylation without the need of N-protecting groups. Screening of various Lewis acids and reaction conditions with these acylated donors has been carried out, in presence of reactive primary alcohols as well as more challenging carbohydrate acceptor alcohols. These experiments demonstrated that the fine tuning of the leaving group combined with a suitable metal triflate could lead to a successful reaction outcome in the direct glycosylation.

Successful methodology of this kind would provide rapid access to naturally occurring N-glycan motifs, such as the highly relevant human milk oligosaccharides (HMOs).

Summary

1 Introduction

1.1 The biological relevance of carbohydrates

Carbohydrates are one of the four major biological macromolecules, together with polynucleotides, polypeptides and lipids, and are one of the main responsible for much of the information transfer in biological system. They have proven to be the most diverse class of macromolecules. The theoretical complexity of oligosaccharides outweighs that of DNA and proteins, even though mammals have only ten different monosaccharide building blocks[1].

Carbohydrates have been found to have a variety of different biological effects. They are central to cell adhesion and signalling and the cell walls of all organisms are encapsulated by a dense and complex coat of carbohydrates[2,3]. This is the reason why they constitute an obvious target in drug development [4]. Although scientists have been able to isolate many naturally occurring oligosaccharides from biological materials, these supplies are often contaminated or inadequate[5]. As a consequence, chemical synthesis must be resorted to. Compared to synthetic accessibility of other major biomolecules like peptides and oligonucleotides, which can be readily synthesised and sequenced[6,7], the synthesis of oligosaccharides is a less developed area. Where peptides and nucleic acids contain linear sequences of building blocks, carbohydrates frequently form branched glycosidic linkages that occur in different configurations (anomeric isomers). It is from this structural complexity and diversity that the synthetic methods to produce carbohydrates encounter additional obstacles [8]. To gain insight into biological processes, and for carbohydrate based drug development, access to pure, structurally well-defined carbohydrates is essential. Additionally, although remarkable developments have been made in automated solid-phase synthesis[9] and chemoenzymatic synthesis[10] every carbohydrate constitutes a unique target and no general synthetic strategy is yet available[11,12,13] .

1.2 N-acetyl-D-glucosamine

The monosaccharide N-acetyl-D-glucosamine (GlcNAc) is a glucose derivative bearing an acetamido functionality on the 2-position, as shown in **Figure 1**. This particular sugar is the most abundant monosaccharide building block in mammalian glycoconjugates

(31.8%), where it by far exceeds glucose occurrence (2.5%)[1]. It is frequently encountered in Nature as relevant repetitive unit, such as in the core pentasaccharide, nodulation factors and carbohydrate epitopes[14].

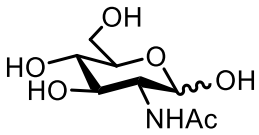


Figure 1: the monosaccharide N-acetyl-D-glucosamine, an important building block in mammalian glycoconjugates

Given its biological relevance and the great abundance in naturally recurring glycan motifs, incorporation of GlcNAc into synthetic oligosaccharides for biological studies is essential. However, its chemical manipulation is far from trivial due to solubility issues and to the fact that GlcNAc derivatives work poorly as both glycosyl donors[15] and acceptors[16,17]. Indeed, it hardly dissolves in most of the common solvents and poorly reacts in several widely-known and conventional protocols of carbohydrate chemistry. The principal reason of this unordinary behaviour is given by the presence of the NHAc functional group and it will be explained in more detail later in this work.

1.2.1 Relevant examples of GlcNAc function in Nature: Human milk oligosaccharides

Human milk oligosaccharides (HMOs) are a family of structurally diverse and complex glycans that are present at a very high concentration in human milk but not in infant formula[18]. Oligosaccharides represent indeed the third most abundant component in human breast milk (5-15 mg/L, after lactose and lipids) and a high amount and structural diversity of these glycans are unique to humans[19]. Research has firmly established that breastfeeding is known to reduce the risk of enteric and other infectious diseases in infancy[20], not only providing essential nutrients for the infant, but also dispensing specific components, such as HMOs, which are able to give health benefits beyond traditional nutrients[21]. Indeed, they apparently cannot be digested by the infant, and seem to be instead targeted to its gastrointestinal microbiota[19].

Evidences accumulated from *in vitro* and *in vivo* studies in the last few years[18,22], combined with epidemiological associations and correlations, suggest that HMOs are beneficial to infants through multiple mechanisms and in a variety of clinical contexts. To date, research

has demonstrated that HMOs serve as prebiotics that help shaping microbiota composition and act as soluble decoy receptors to block pathogens from attaching to host cells and causing disease[18]. Moreover, researches showed that they have direct antimicrobial effects to halt pathogen growth and survival, preventing adhesion and infection by certain viral strains as well as by pathogenic bacterial strains[23]. In addition, HMOs may modulate epithelial and immune cell responses and contribute to the maturation of the immune system[22].

1.2.1.1 Structure of HMOs

As previously mentioned, HMOs consist of oligosaccharides in varying shape and size. More than hundreds different HMOs have been identified so far. However, not every woman bio-synthesizes the same set of oligosaccharides and in the same concentration during the different stages of lactation [24,22]. Human milk oligosaccharides are composed of five monosaccharide building blocks: glucose (Glc), galactose (Gal), N-acetylglucosamine (GlcNAc), fucose (Fuc), and sialic acid (Sia). As illustrated in **Figure 2**, all HMOs carry lactose (Galβ1–4Glc) at the reducing end [23], which can be elongated by up to 15 disaccharide units of either lacto-N-biose (Gal β1–3-linked to GlcNAc) or N-acetyllactosamine (Gal β1–4-linked to GlcNAc). In addition, HMO can be fucosylated in α1–2, α1–3, or α1–4 linkage or/and sialylated in α2–3 or α2–6 linkage. A β1-6 linkage between two disaccharide units introduces branching, leading to *iso*-HMOs instead of *para*-HMOs (with linear structures only)[18].

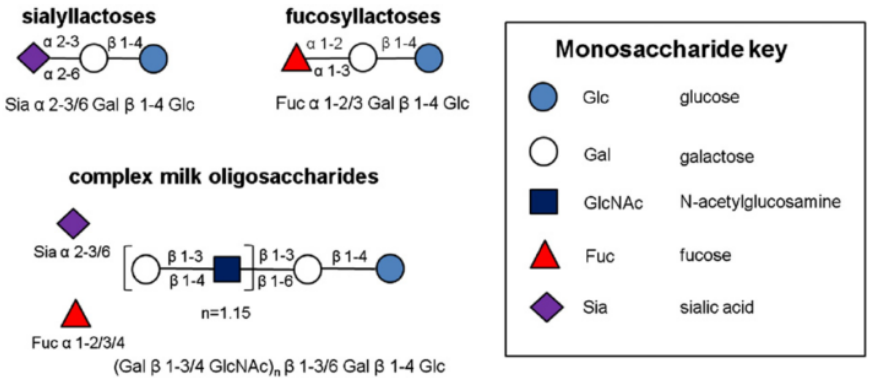


Figure 2*:* Structural composition of human milk oligosaccharides (HMOs). HMOs can be grouped into short-chain trisaccharides (sialyllactose or fucosyllactose) and complex high-molecular-weight oligosaccharides

1.2.1.2 Lack of HMOs availability and possible solutions to HMOs supplement

Until recently, research on HMOs has been limited by an insufficient availability of HMOs. Most of them are found uniquely in human milk, and thus far it has been prohibitively tedious and expensive to isolate and synthesize them[23].

Being the most similar formula to human milk among mammals, bovine milk has been a focus of research for several years: since it could be isolated in large volumes from dairy streams, it would be a possible alternative precursor for HMOs chemoenzymatic synthesis. Nevertheless, bovine milk formula is quite simpler than the human one: it contains oligosaccharides concentration much lower than in human milk, and not all HMOs are present in bovine milk (*e.g.*, it contains only trace amounts of fucosylated oligosaccharides and the typical polylactosamine backbone is not always present)[25]. Moreover, bovine milk contains several non-human oligosaccharides, which compatibility and functions in human body are still unclear[23]. Given all the disadvantages of bovine milk usage, researchers are losing interest in finding new ways to its purification.

Therefore, since HMOs are mostly absent from infant formula, formula-fed infants may miss the potential benefits attributed to HMOs. To overcome this issue in absence of an efficient supplement to human oligosaccharides, several infant formula-producing companies searched for inexpensive alternatives: they developed mixtures of galactooligosaccharides (GOS, **Figure 3a**) and fructooligosaccharides (FOS, **Figure 3b**) or inulin, that mimic the prebiotic effects of human milk and promote a bacterial microflora that closely resembles that of breastfed infants[22]. GOS and FOS or inulin, however, are structurally very different from the naturally occurring oligosaccharides in human milk. Considering that most of the postulated properties of HMOs are highly structure-specific, infant formula oligosaccharides may have different effects than HMOs[21]. To provide formula-fed infants with the same benefits that breastfed infants receive with their mother's milk, HMO-like glycans are needed as formula supplements.

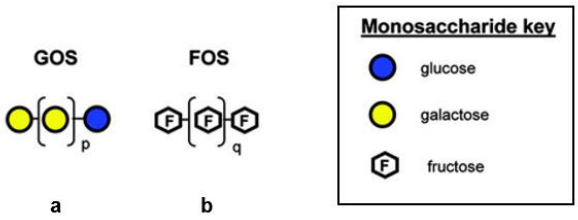


Figure 3*:* Structural composition of infant-formula oligosaccharides. Galactooligosaccharides (GOS) consist of one to seven Gal units with Glc at the non-reducing end. Fructooligosaccharide (FOS) is a β1-2 fructose polymer

While the complete mechanism has not been fully elucidated yet, studies have shown that human milk oligosaccharides are responsible for many of the positive effects associated with breastfeeding. It is thus reasonable to consider HMOs as a major part of an innate immune system by which breastfeeding mothers protect their infants from disease[22]. Therefore, to give insight into the function of HMOs in infant microbiota, it is essential to get chemical or biological access to these short-chain oligosaccharides, in order to create libraries of synthetic HMOs and consequently manage to produce effective infant-formula on an industrial scale. Two aspects must be considered:

-The production via enzymatic synthesis of HMOs has gained increasing interest in the past few years. For the synthesis, bacterial cultures are bioengineered into expressing or over-expressing specific enzymes that catalyse the formation of glycosidic bonds[26,27]. However, this requires bioengineered bacterial cultures and, above all, purification from the cells afterwards, which is particularly tedious: the separation of HMOs from large volumes of fermentation broth might be challenging if their concentration is low[23]. Additionally, biosynthesises can produce just a limited set of HMOs at a time and often require expensive chemicals.

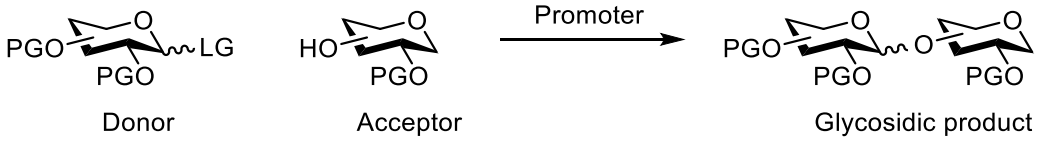
-Chemically, the large variety of identified HMOs poses a challenge. Specific properly protected building blocks should be synthesised suitable to be connected to other HMOs. Moreover, the N-acetyl functionality present in many human oligosaccharides is quite problematic from a glycosylation chemist point of view. For its efficient involvement in a glycosylation reaction, it usually requires manipulations with bulky protecting groups to convert it into a better assisting group. Alternatively, with a less efficient glycosylation performed with the free N-acetyl moiety, the purification of the product from unreactive intermediates is required[28,29].

Therefore, it is essential now for researchers to optimise a good synthetic approach which guarantees access to these short-chain oligosaccharides, finally providing an efficient and unexpansive source of HMOs which would improve maternal and infant health.

1.3 Chemical oligosaccharides synthesis: general glycosylation mechanisms

The formation of O-glycosidic linkages for the preparation of oligosaccharides is at the heart of carbohydrate chemistry. Since the advent of the first glycosylation at beginning of XX century[30], scientists have made great progress in the search for alternative oligosaccharides synthesis methods, milder or more selective. However, despite the fact that many different types of glycosyl donors are now available, no general approach for glycosylation reaction currently exists[31]. Nevertheless, it is possible to make some general comments about the mechanism operative in glycosylation reactions.

The two principal species taking part in a glycosylation reaction are defined as the glycosyl donor and the glycosyl acceptor. The donor is the carbohydrate which donates its glycosyl residue. It is usually fully protected and bears a good leaving group on the anomeric carbon. The glycosyl acceptor, which has at least one free hydroxyl group, is the saccharide that accepts the residue[32]. Glycosylations always require a chemical promoter to occur, which function is to activate the donor[28] (**Scheme 1**).

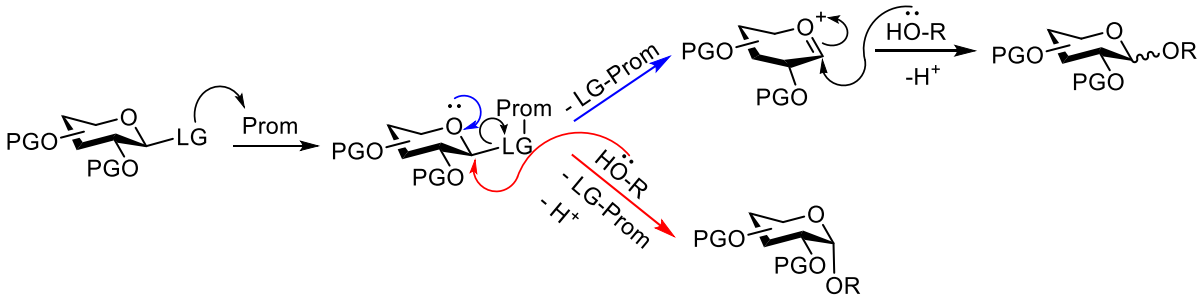


Scheme 1*:* General reaction scheme for the glycosylation reaction. PG: protecting group, LG: leaving group

The mechanism by which glycosyl donor and acceptor react together depends on the presence of a neighbouring participatory group in the glycosyl donor.

Firstly, the donor is always activated from the promoter species, increasing anomeric carbon electrophilicity (and thus the leaving group ability). Then, if the neighbouring group in 2-position is not a participatory group, the reaction proceeds through the formation of an oxacarbenium ion intermediate, as shown in the mechanism in **Scheme 2**, blue pathway. The oxacarbenium ion is stabilized by resonance and could now be attacked

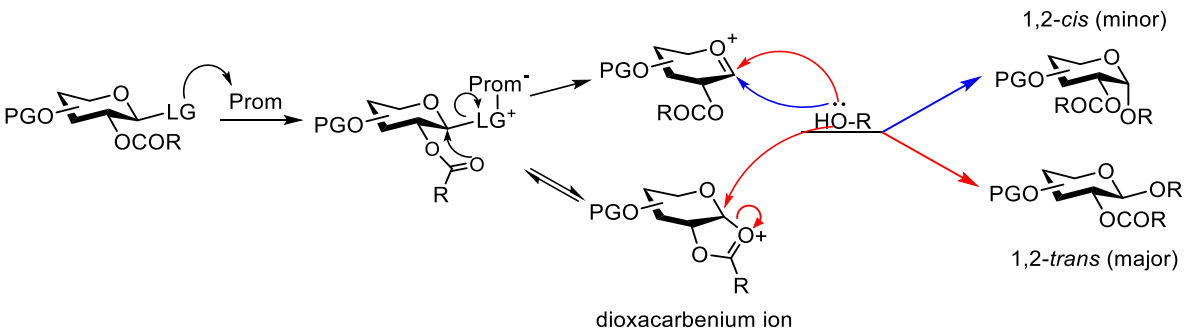
by the acceptor at the anomeric carbon[28,33]. Since the C1 is sp^2 hybridized in the oxacarbenium species and a carbohydrate acceptor is often a weak nucleophile, the attack is generally S_N1-like and could occur from both faces. This leads to an α/β-mixture, which generally favours the thermodynamic α-product, because of electron density donation from the lone pair in the antibonding orbital of the C1-O1 bond: the endoanomeric effect[29]. Alternatively, the reaction could proceed also with a S_N2-like character (**Scheme 2**, red pathway): in this case, the acceptor directly attacks and substitutes the leaving group, thus without the formation of the typical oxacarbenium ion. Therefore, the inversion of configuration on the C1 occurs[33].



Scheme 2*:* General mechanism for the glycosylation reaction without a neighbouring participating group. The mechanism could show S_N1-like character, as in the blue pathway, or it could be S_N2-like, following the red one. PG: protecting group, LG: leaving group, Prom: promoter

Concerning the second possible mechanism (**Scheme 3**), the neighbouring participatory group on the 2-position can direct the stereochemical outcome, favouring the 1,2-*trans* (β) product. In fact, this second pathway proceeds through the formation of a bicyclic intermediate: when the activated donor bears a participatory group, such as a 2-O-acyl group at the C2, it could form the dioxacarbenium ion (also called acyloxonium) by intramolecular attack at the C1. This intermediate is much more stable than the oxacarbenium ion, thanks to the additional resonance stabilisation given by the two new heteroatoms[28]. Nevertheless, the dioxacerbenium ion is a short-lived intermediate[34] and it quickly gets attacked by the acceptor, generally leading selectively to the β-product[35,36] (**Scheme 3**, red pathway). However, a certain amount of the 1,2-*cis* product is often found (as represented from the blue pathway in **Scheme 3**), especially in presence of weak participatory groups and nucleophiles, or under acidic conditions[36].

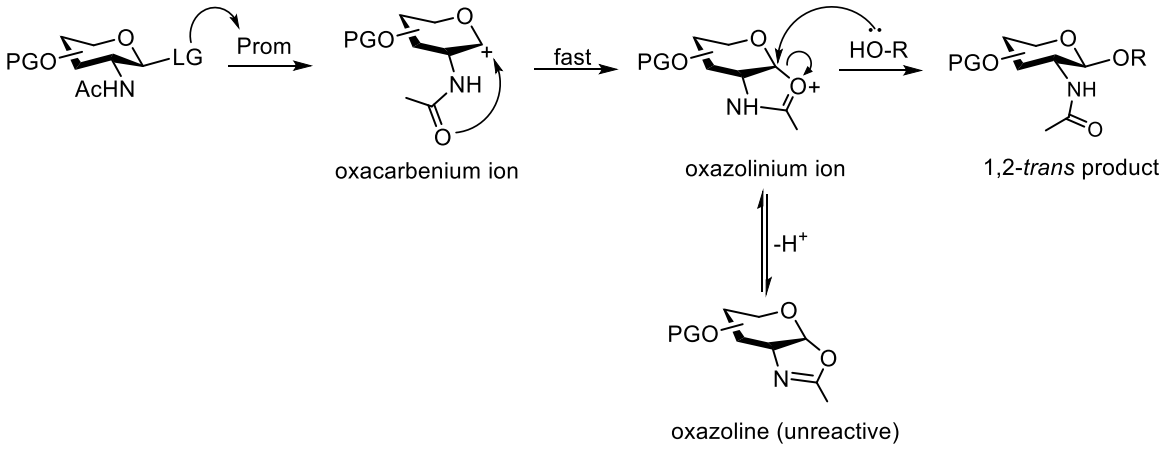
Glycosylation reactions are also much sensitive to reaction conditions. Indeed, varying solvent, temperature, protecting group, mode of activation or the donor-acceptor pair could highly influence the stereoselectivity and the reaction rate[28].



Scheme 3*:* General mechanism for the glycosylation reaction involving a participating group at the 2-position

1.4 Glycosylations with 2-acetamido bearing sugars: relevant issues

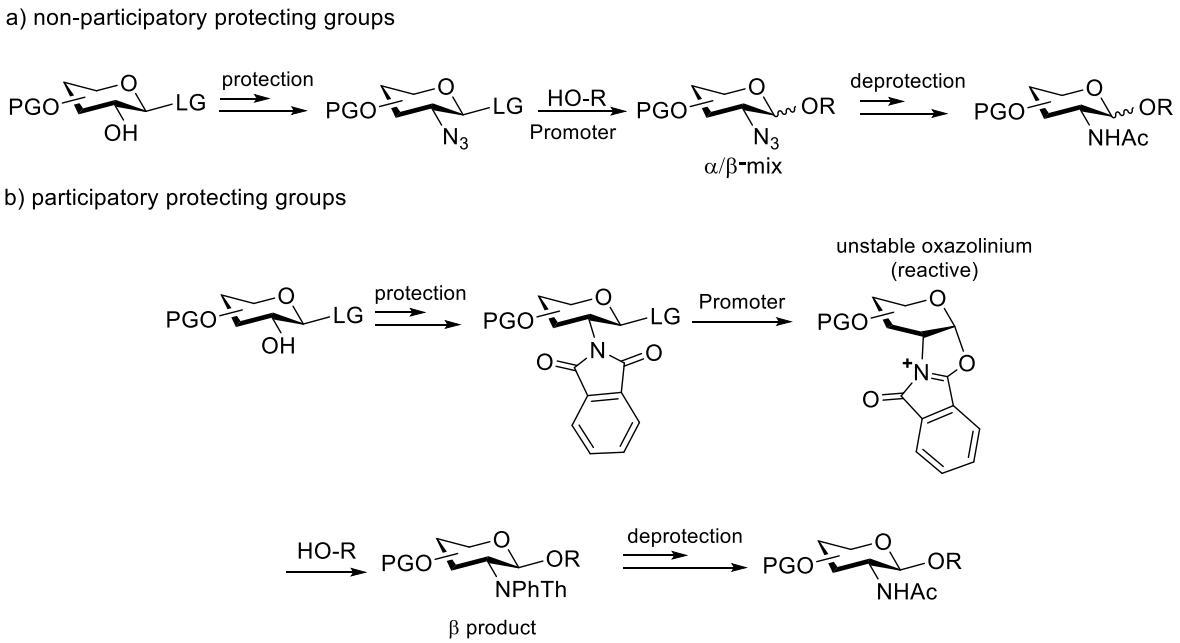
As previously mentioned, glycosylation reactions with GlcNAc are still very challenging. Their development is thus an urgent need in carbohydrate chemistry. It is widely accepted that GlcNAc-ylation is hampered by the formation of a 1,2-oxazoline intermediate (here represented as $^{4}H_{5}$ half-chair conformation)[37,38,39], which typically exhibits weak glycosyl-donor properties and reacts sluggishly to give the glycoside product[40,41]. The reason for this behaviour is given by the fact that the 2-acetamido functionality is a neighbouring participatory group, which leads to the formation of an oxazolinium ion[28], as represented in the mechanism below (**Scheme 4**): activation of the donor with a suitable promoter gives the anomeric oxacarbenium ion, which rearranges in a fast step to generate the more energetically favoured oxazolinium ion intermediate. This intermediate is different from the dioxacarbenium ion only in the substitution of an oxygen for a nitrogen. This small difference, however, seems to result in a significant reactivity difference in glycosylation. In contrast to the dioxacarbenium species, it can not only undergo the desired substitution to afford the β-glycoside, but also get deprotonated to afford the unreactive oxazoline side-product [29,42,43].



Scheme 4*:* A general mechanism for direct glycosylation with a 2-acetamido bearing donors

1.5 Typical GlcNAc-ylation approach: protection of the NHAc moiety

In addition to this obstacle to the synthesis, GlcNAc is known to be a tedious compound to deal with. For these reasons, traditionally, the chemical synthesis of the β-GlcNAc glycosidic linkage has been carried out using other masking groups than the biologically relevant N-acetyl. The protecting group strategy to avoid the acetamido functionality has been extensively investigated, by exploiting either non-participating protections, e.g. with azide ($-N_3$)[44] and N-2,4-dinitrophenyl (DNP) functionalities[15] (**Scheme 5a**); or participating N-protecting groups, such as trichloroethoxycarbonyl (-NTroc)[45], which gives an unstable, thus reactive, oxazoline, and especially the phthalimido group (N-Phth)[42,46], which oxazolinium cannot form the oxazoline. These latter protecting groups enable the selective formation of the 1,2-*trans* product (**Scheme 5b**) [15,40]. Although glycosylation generally functions well with these protected donors, the drawback is that extra synthetic steps are required for their introduction and later interconversion to the biologically-relevant 2-acetamido substituent[40]: this aspect obviously affects glycosylation outcome and decreases the overall yield and synthetic efficiency.



Scheme 5:Glycosylation mechanism in presence of a) non-participatory or b) participatory N-protecting groups of the 2-acetamido moiety

It would thus be desirable to find alternatives to such protecting group strategies. Obviously the best, yet most challenging, alternative would be the use of no N-protecting group at all, as extensively discussed in the following paragraph.

1.6 Unconventional GlcNAc-ylation approach: direct glycosylation without N-protecting groups

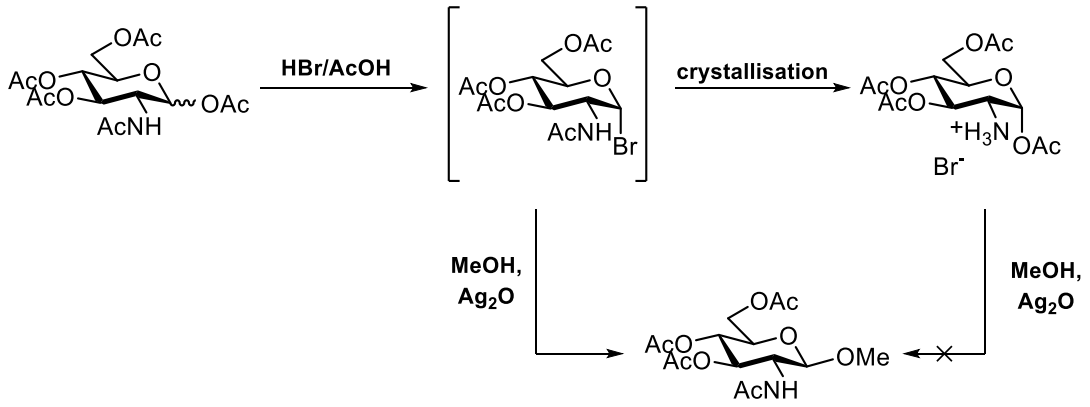
1.6.1 Direct glycosylation with 2-acetamido-2-deoxy halide donors

Glycosylation in the presence of the naturally occurring NHAc functionality either in the donor or in the acceptor has been known to be the cause of poor reaction outcomes[15]. In fact, the necessity for N-protecting groups in synthesis involving this monosaccharide has become such a well-established concept that many authors do not even comment on the approach in their publications about complex N-glycans synthesis[10,47,48].

Many historical examples associated with direct GlcNAc-ylation methodologies, which show how working in the presence of the 2-acetamido moiety could be challenging, are reported in the literature.

For instance, one of the oldest general glycosylation protocol, the Koenigs-Knorr reaction from 1901[30], in which glycosyl halides are activated using stoichiometric amount of silver(I) salts, has been tested for glycosylation with GlcNAc donors. In general, the Koenigs-Knorr reaction represents a popular choice for many general applications even today, being an effective 2-steps method to obtain 1,2-*trans* configured glycosides from ester protected donors.

Nevertheless, early puzzling results from 1938 showed that the Koenigs-Knorr protocol was not viable for 2-acetamido-2-deoxypyranosyl donors[49]: treatment of tetraacetyl-GlcNAc with a mixture of hydrobromic and acetic acid, followed by crystallisation and addition of silver oxide, failed to convert the glycosidic bromide in the desired methoxy glycoside (**Scheme 6**). The same negative result was obtained using the glycosyl chloride[50]. The first progress overcoming these initial discouraging initial studies, were shown only in 1956: by direct activation of the unstable bromide donor in the crude mixture with Ag_2O/methanol, it was possible to afford the desired methyl glycoside[51]. The methoxy glycoside was not obtained in the first attempt since crystallization of the bromide formed an undesired hydrobromide salt.



Scheme 6*:* the typical Koenigs-Knorr protocol is not viable for glycosylation with the peracetylated N-Acetyl glucosamine: the glycosyl bromide formed in the first step is unstable and rearranges during crystallisation to afford an hydrobromide salt. This compound will not react as a donor to afford the desired methoxy glycoside. However, direct activation with Ag_2O in the presence of methanol eventually affords methyl glycoside.

Even though glycosyl chloride donors are known to be less reactive than the bromide ones, these compounds have been investigated in more detail as GlcNAc derivative donors[50] during the years, giving better results for several glycosylation reactions. However, yields were at best mediocre, with both secondary carbohydrate acceptors[52] and primary

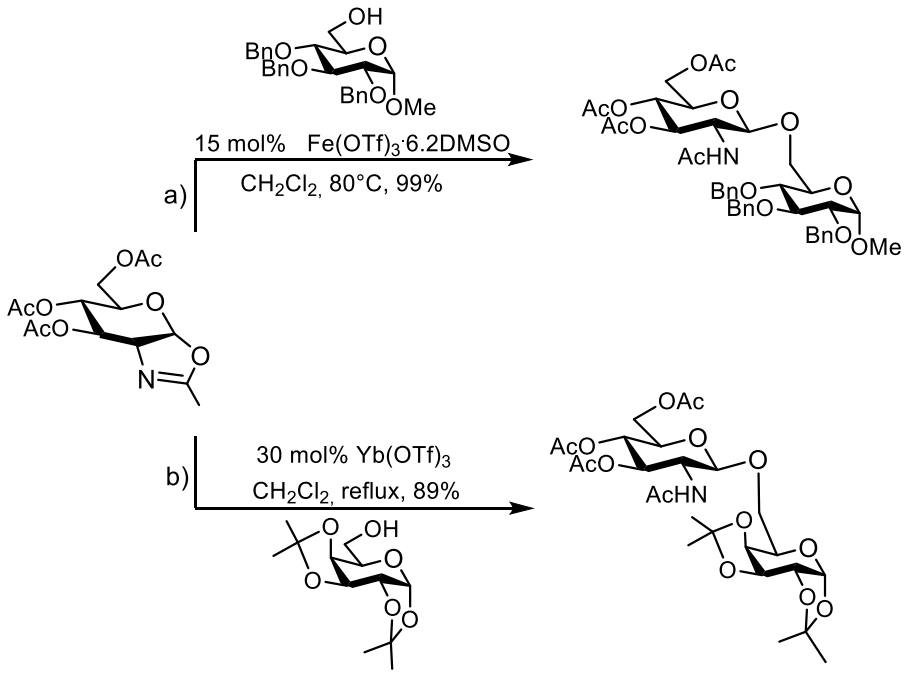
acceptors[53]. Moreover, these procedures require the use of stoichiometric amounts of heavy metal salts as promoters, even though they give access to simple glycosides in a short synthetic route from low cost starting materials.

1.6.2 Glycosylation with glucopyranosyl 1,2-oxazoline donors

Oxazolines are relatively stable compounds at neutral or basic conditions and can be isolated as pure compounds[54]. They have therefore been widely explored as potential glycosyl donors, in order to avoid all the protection and de-protection steps usually required with GlcNAc donors. Indeed, an oxazoline donor can in principle directly yield the N-acetyl function without any further manipulation[42,54].

To date, a wide range of Lewis acids such as $FeCl_3$[55], $AlCl_3$[56], TMSOTf[15] and $CuCl_2$[57] has been used as chemical promoters with some good results for oxazoline activation in GlcNAc-ylation.

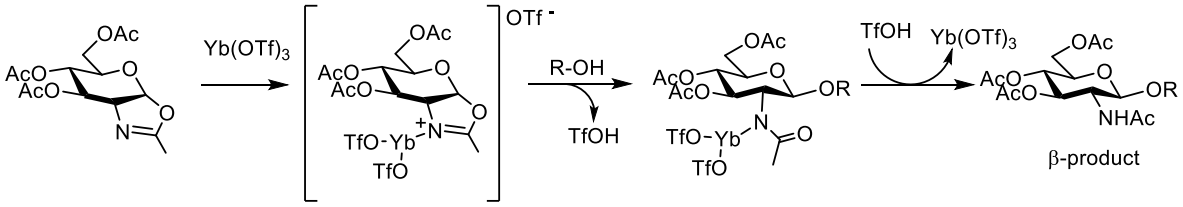
Lately, metal triflates were established as useful catalysts for the synthesis of simple glycosides of GlcNAc. Some remarkable recent studies using these Lewis acids as promoters with a peracetylated oxazoline donor are illustrated in **Scheme 7**.



Scheme 7*:* significant examples of glycosylation reactions using oxazoline as donor and metal triflates as promoters: a) study from Beau and co-workers involving iron(III)triflates promoters b) Crasto and Jones work reporting $Yb(OTf)_3$ as catalyst

A first important study was carried out by Beau and co-workers, using $Fe(OTf)_3 \cdot 6.2DMSO$ as promoter[58]. They showed that a 15 mol% loading of this inexpensive and environmentally friendly Lewis acid could efficiently promote glycosylation with oxazoline as donor, at least using primary acceptors: as shown in **Scheme 7a**, the peracetylated oxazoline afforded virtually quantitative yield in glycosylation with the 6-OH glucose primary acceptor, performed under microwave irradiation.

Another relevant result was published by Crasto and Jones group[59] (**Scheme 7b**), which developed a mild and efficient coupling strategy using $Yb(OTf)_3$, obtaining an excellent 89% yield in the glycosylation of oxazoline with the diisopropylidene protected galactose acceptor (**Scheme 8**). They found that rare earth metal triflates could catalytically activate the oxazoline donor by coordination, thus obtaining the anchimeric activation to suitable acceptors.

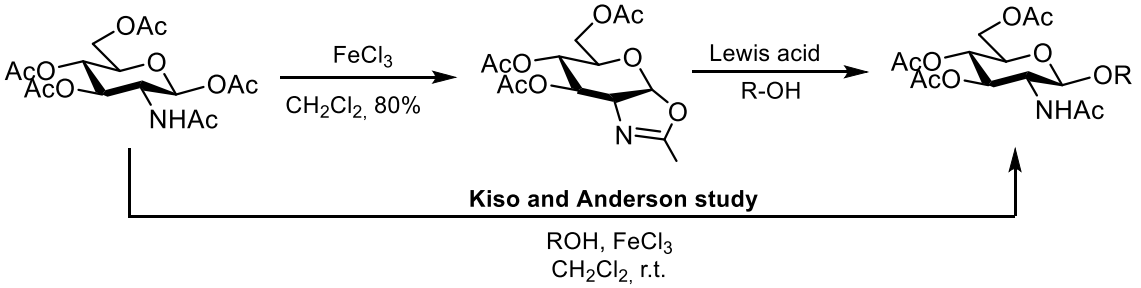


Scheme 8*:* Activation mechanism of oxazoline with lanthanide triflates proposed by Crasto and Jones

Although oxazoline can efficiently provide good β-selectivity in glycosylations with Lewis acids activators[54], it often reacts sluggishly with challenging acceptors. Furthermore, the harsh reaction conditions required for this conversion have precluded its wide application in the synthesis of complex oligosaccharides. Moreover, its use as donor is troublesome due to its tedious preparation and low shelf stability. Therefore, it would be advantageous to find a way to conduct chemical GlcNAc-ylation using another stable, storable glycosyl donor.

1.6.3 Direct glycosylation with 2-acetamido-2-deoxy-glucopyranosyl donors: recent advances in Jensen group

Since the oxazoline is often both prepared and activated under Lewis acidic condition, it would be reasonable to wonder if these two processes can take place in the same reaction vessel, using the same activator. Remarkably, in the late 1970s Kiso and Anderson had already reported that adding an acceptor alcohol in the procedure for oxazoline synthesis from esters-protected GlcNAc[60], it was possible to obtain directly the desired glycosidic final product (illustrated in **Scheme 9**). This was one of the first examples of direct chemical glycosylations of 2-acetamido-2-deoxypyranosyl donors, and thus stimulated many other works in the next years[58,61,62].



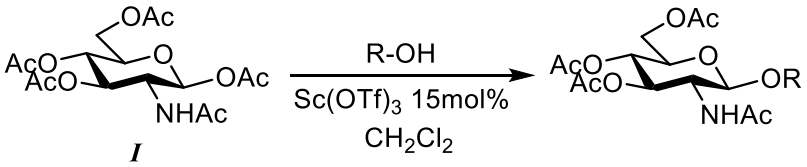
Scheme 9*:* Direct glycosylation reported from Kiso and Anderson using super stoichiometric $FeCl_3$, who modified the oxazoline protocol synthesis ideated by Matte and Bahl

As demonstrated in the previous results, rare Earth metal triflates have proven to be highly valuable in a wide range of Lewis acid mediated glycosylation reactions. The advantage of using these metals as catalysts is their high level of Lewis acidity in combination with their stability towards moisture.

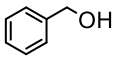
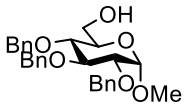
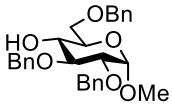
Inspired by these combined findings, Jensen and co-workers investigated and selected a promising range of metal triflates for the catalytical activation of direct GlcNAc-ylation[63]. Indeed, following Crasto and Jones work[59], they had realized that lanthanide triflates were able to catalyse both anomeric activation, facilitating the leaving group departure, and the later breakdown of oxazoline intermediate[64]. From this screening, they developed in 2008 a general protocol for the direct glycosylation of a tetraacetate GlcNAc donor using 15 mol% of metal triflate catalyst in dichloromethane (illustrated in **Table 1**)[63]. Among all the catalysts tested, $Sc(OTf)_3$ was found to be the most efficient (but also the most expensive), leading to significantly shorter times and higher yields, followed by $Cu(OTf)_2$

and $Yb(OTf)_3$. This procedure has proven to work well with both primary simple alcohols and carbohydrate acceptors, although it furnished barely decent results with more challenging acceptors. As shown in **Table 1**, in preliminary investigations with benzyl alcohol (entry 1), a temperature of 45°C was sufficient to obtain 81% yield in 4 hours with 3 equivalents of acceptor. With carbohydrate based acceptors instead (entry 2), increasing temperature to ensure acceptable reaction times was necessary. The desired disaccharides were obtained in a successful 85% yield for the primary glucose acceptor, while only in a 21% yield with the secondary and thus more challenging acceptor (entry 3).

Table 1: Relevant examples from the first direct GlcNAc-ylation protocol developed from Jensen group involving 15mol% $Sc(OTf)_3$ as promoter with a) simple alcohol acceptor, b) primary carbohydrate acceptor and c) secondary carbohydrate acceptor

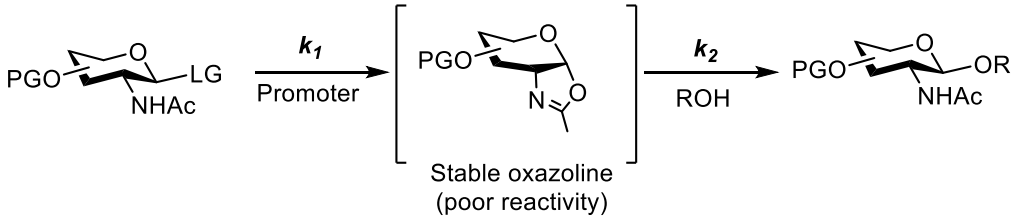


I

Entry	Acceptor	eq	Temperature	reaction time	yield
1		3	45 °C	4h	81%
2		0.5	80 °C	72h	85%
3		0.25	80 °C	72h	21%

After the first promising results, in the Jensen group new various donors have been investigated, such as the less conventional thioglycoside and pentenyl glycoside donors[41]: although they seemed to be quite more reactive than simple acetate donors, leading to a faster activation, they resulted in lower reaction yields.

Therefore, it was surmised that both the donor functionality and the acceptor reactivity could heavily affect the glycosylation outcome. Jensen and co-workers speculated that balancing the rate of the donor activation (k_1, **Scheme 10**) with that of the reaction between the oxazoline intermediate and the acceptor alcohol (k_2, **Scheme 10**) could lead to an improved overall reaction outcome [40]. In other words, with more active acceptors it is necessary to work under conditions favouring the formation of the oxazoline, while when less active acceptors are employed, conditions enhancing the reactivity of the oxazoline are required.

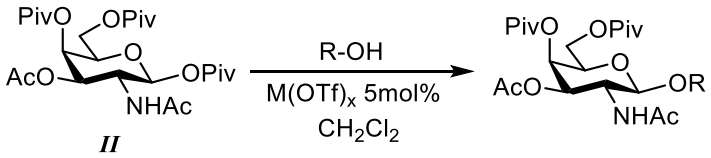


Scheme 10: Glycosylation with 2-acetamido donors is a two-step process involving activation (k_1) and glycosylation (k_2) and they have to be balanced to better the overall reaction outcome

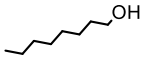
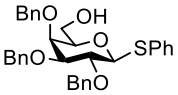
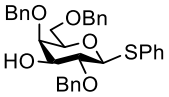
In the case of poorly reactive secondary carbohydrate based acceptor alcohols, this might be realised by keeping the activation slow of the anomeric leaving group (small k_1, *e.g.* with a 1-acetate donor), speeding up at the same time the glycosylation rate, (large k_2, for example using armed ether-protected donors) and thus maintaining the oxazoline concentration always low in solution. Moreover, a mild metal triflate as promoter, as well as a good match between donor and acceptor reactivity could ensure the optimization of the reaction rate[40,65].

Given these new important insights more suitable donors were synthesised.
Firstly, Rasmussen et al.[64] investigated the properties and reactivity of the 1-pivaloate donor 1-OPiv GalNAc derived from N-Acetyl-galactosamine ***(II)***, a less common monosaccharide in Nature than GlcNAc but equally biologically relevant and more reactive. The most representative examples of this work are reported in **Table 2**. Since similar pivaloates had been activated previously[66], they investigated if this 1-OPiv anomeric functionality could act as a nucleofuge in glycosylations upon activation in a similar fashion to the acetyl group. Although $Bi(OTf)_3$ in refluxing CH_2Cl_2 revealed to be the best conditions for β-selective glycosylations with simple alcohols (e.g. octanol gave 87% yield in 4 hours, entry 1), the reaction time increased considerably with more sterically demanding and/or electron-deficient acceptors (**Table 2**, entry 2 and 4). However, decreasing the catalyst loading to 2.5 mol% and using the milder Lewis acid $Yb(OTf)_3$ (entry 3 and 5), while increasing the temperature to 80°C provided significant better yields, especially for the less reactive acceptors (entry 5 vs entry 4, 59% yield instead of 15%)[64]. These results are in accordance with the hypothesis previously proposed by the Jensen group about the requirement of a suitable reaction fine-tuning by balancing activation and glycosylation rate.

Table 2: Some of the most significant examples obtained by Rasmussen et al. using GalNAc donor in the presence of metal triflates promoters

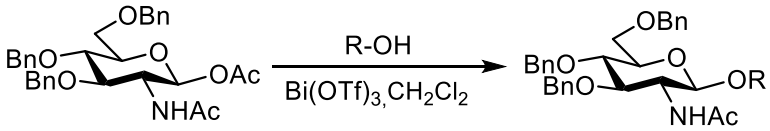


II

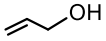
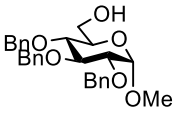
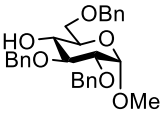
Entry	Acceptor	eq	Catalyst	Temperature	reaction time	yield
1		3	$Bi(OTf)_3$	40 °C	4h	87%
2		0.5	$Bi(OTf)_3$	40°C	23h	83%
3		0.7	$Yb(OTf)_3$	80 °C	2h	87%
4		0.5	$Bi(OTf)_3$	40 °C	72h	15%
5		0.7	$Yb(OTf)_3$	80 °C	2.5h	59%

Recently, Marqvorsen et al. published the results gained with benzyl protected 1-OAc GlcNAc ***(III)*** as donor in chemical direct glycosylation [40]. **Table 3** summaries some of the most relevant results. Also in this case the 2-acetamido donor performed good yields with simple alcohols (allyl alcohol, entry 1) and primary carbohydrate acceptors (entry 2 and 3), using $Bi(OTf)_3$ as catalyst in CH_2Cl_2 at 40°C. However, glycosylation with more hindered secondary acceptors gave as usual lower yields, even decreasing catalyst loading from 5 mol% to 2.5 mol% (entry 4). Moreover, the formation of by-products precluded the possibility of increasing the reaction temperature.

Table 3: Some of the glycosylation reaction tested by Jensen and co-workers with ether benzyl protected 1-acetyl GlcNAc as donor and $Bi(OTf)_3$ as catalyst

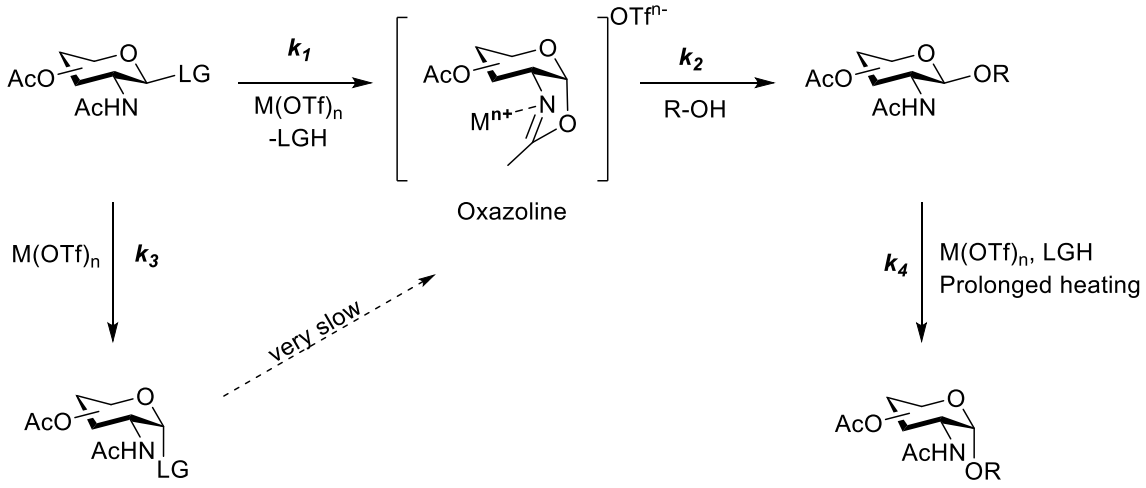


III

Entry	Acceptor	eq	Catalyst	Temperature	reaction time	yield
1		3	5 mol%	40 °C	40 min	90%
2		0.5	5 mol %	40 °C	48h	84%
3		0.5	2.5 mol %	80 °C	43h	98%
4		0.5	2.5 mol %	40 °C	6 days	29%

It should be pointed that this latter ether-protected acetate donor ***(III)*** gave however better yields and shorter reaction times than the other acylated 2-acetamido donors tested ***(I)*** and ***(II)***, also with milder conditions. Nevertheless, the synthesis of benzylated donors such as ***(III)*** is lengthier and more tedious than those of the acylated species; it has thus revealed to be in some way less practical.

On the basis of the above studies, a reaction model describing the key ideas evaluated by Jensen group was proposed. **Scheme 11** sketches indeed the possible activation pathway of the donor and subsequently of the oxazoline intermediate, by means of coordination by the metal triflate Lewis acid promoter.



Scheme 11: Proposed reaction pathway for direct glycosylation catalysed by $M(OTf)_n$ with 2-acetamido bearing donors[64]

Reactions proceed to afford high yields in the case of reactive alcohol acceptors such as n-octanol, allyl alcohol and benzyl alcohol[40,63,64] even with a high catalyst loading. Presumably, these acceptor substrates provide a high glycosylation rate (k_2), making variation in catalyst loading and thus donor activation rate insignificant (k_1). With slowly reacting acceptors, however, a lower catalyst loading and a milder Lewis acid catalyst has been shown to provide better yields as this seems to avoid anomerisation of both donor (k_3) and product (k_4). Although it has not been tested yet, anomerised α-donor (formed by k_3) is presumed to be a slowly activating donor under the reaction conditions employed, as shown previously with GalNAc[64]. Post-glycosylation *in situ* anomerisation is also possible upon a prolonged heating or a too high reaction temperature. Suitable reaction conditions must hence be chosen to ensure oxazoline formation, yet minimizing at the same time the side reactions.

To summarise all the results gained thus far by Jensen and co-workers, acetate function activation seems to be acceptor dependent, leading to reactions where donor activation and product formation occur in a synchronous manner with no accumulation of oxazoline or oxazolinium ion even with prolonged reaction times. A higher catalyst loading, however,

leads to higher steady state concentrations of oxazolinium ion. As demonstrated by Rasmussen et al.[64], prolonged heating, which is often necessary with poorly reactive acceptors, leads to the formation of the α-product or to degradation pathways[40]. However, a lower catalyst loading at high temperatures seems to restore reaction balancing, sometimes resulting in higher isolated yields of the desired glycosides[64,65].

Therefore, the nature of the donor functionality is clearly the dominating factor controlling the rate of activation of the donor and, thus far, acylated donors appear to be highly promising for this direct GlcNAc-ylation protocol. The Jensen group studies have led to important insights into the reason for the disappointing results obtained with conventional 2-acetamido donors used in the past. However, it is fundamental now to find new and more efficient donors for the glycosylation, which could improve the already surprising results gained thus far for the glycosylation of less reactive carbohydrate acceptors. Thus, the scope of this glycosylation reaction, as well as the synthesis and properties of the donors are still unexplored.

2 Aim of the Thesis

The necessity of finding a new synthetic route to easily access naturally occurring GlcNAc motifs has been widely underlined in the previous chapters. Indeed, GlcNAc containing oligosaccharides represent an important target in carbohydrate chemistry and biology, due to their essential functions and highly potential applications in human body. Nevertheless, these 2-acetamido bearing compounds are still very challenging to synthesise; in fact the conventional approach to GlcNAc glycosylation employs extra-synthetic steps for manipulation of the troublesome NHAc functionality to ensure acceptable yields. This strategy is generally required to avoid formation of the 1,2-oxazoline intermediate, which typically exhibits weak glycosyl-donor properties and reacts sluggishly to give the glycoside product.

Only recently, a new direct Lewis acid-catalysed GlcNAc-ylation protocol has been developed by Jensen group, which could promote direct glycosylation on 1-acyl GlcNAc donors even in the presence of slowly activating carbohydrates acceptor alcohols.

The aim of this thesis project is thus to synthesise a class of different and promising acylated GlcNAc donors by a novel diastereoselective 1-O-acylation strategy and to investigate their reactivity in a new direct glycosylation method catalysed by metal triflates.

The four different acylated GlcNAc target donors **A**, **B**, **C** and **D** (shown in **Figure 4**) will be then synthesised and the synthetic protocol will be optimized.

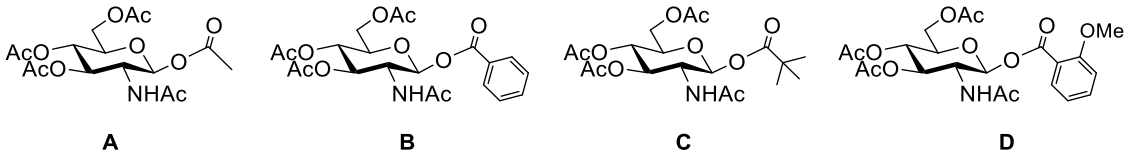
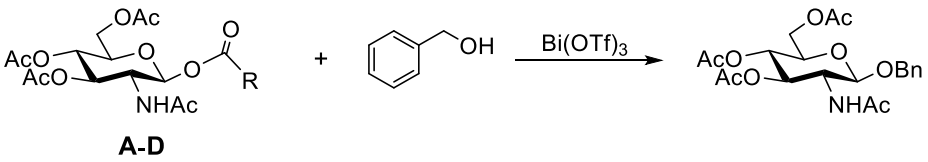


Figure 4: Target acylated GlcNAc donors

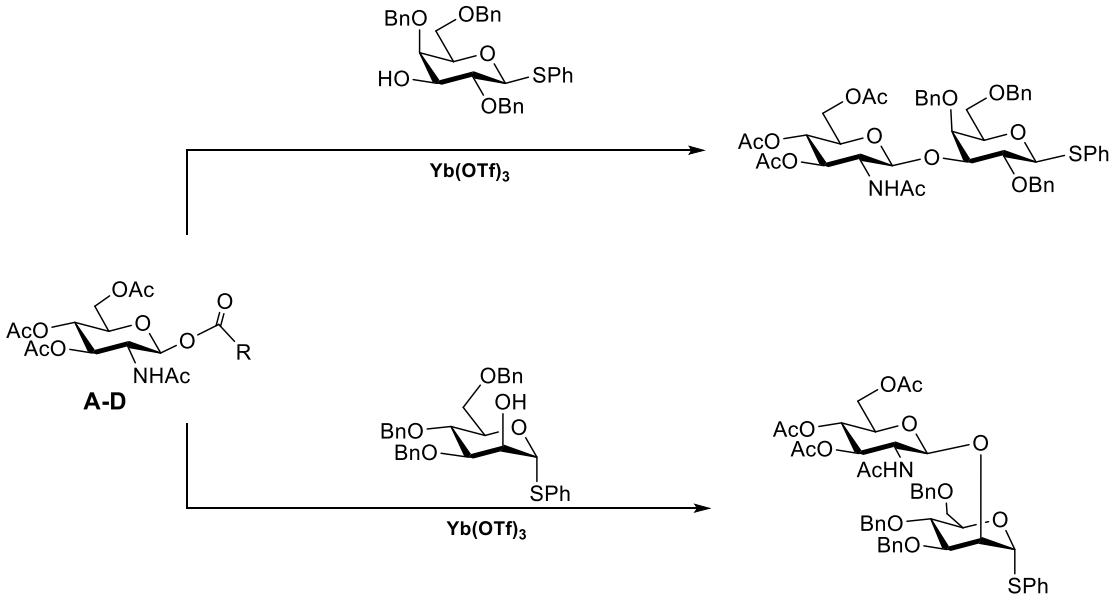
In order to ascertain how the fine tuning of the donor leaving group could improve direct glycosylation outcome, they will be investigated under the same glycosylation reaction conditions employing different acceptor alcohols.

Firstly, the potential of the method will be assessed, by testing the different acylated donors in the novel direct catalytic glycosylation protocol in presence of benzyl alcohol as a representative primary alcohol, employing suitable reaction conditions (**Scheme 12**).



Scheme 12: first glycosylation screening of the 1-acyl donors using benzyl alcohol as the acceptor

Considering some relevant naturally occurring N-glycans, two challenging carbohydrate acceptor alcohols will be successively synthesised and applied as acceptors for further glycosylation screenings of the acylated GlcNAc donors (**Scheme 13**).



Scheme 13: target glycosylation reactions for the screening of the acylated donors in the novel direct glycosylation protocol involving slowly activating carbohydrate acceptors

The results obtained from the target direct glycosylation reactions displayed above, could lead to further elucidation about the reactivity of the acyl donors and the importance of tuning the donor leaving group and the metal triflate catalyst. Indeed, the suitable matching between donor, acceptors and reaction conditions would achieve optimal results in direct glycosylation reactions.

An efficient direct glycosylation protocol with a suitable donor of the monosaccharide GlcNAc would provide a valuable tool for rapid access to naturally occurring N-glycan motifs, such as the human milk oligosaccharides (HMOs), complex sugars highly abundant in human milk only, which are still really hard to synthesize, due to their limited availability and their challenging glycosyl linkages, as the ones affordable in this project with GlcNAc donors.

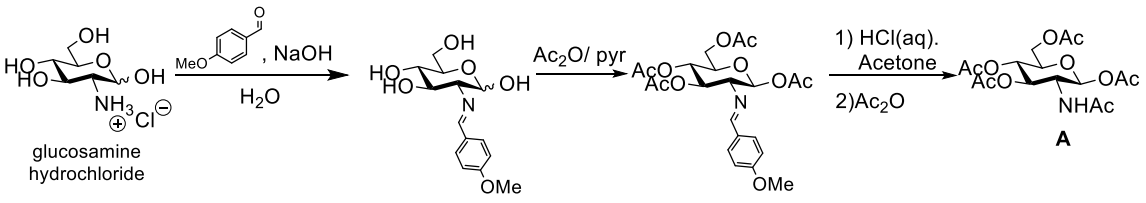
3 Results and Discussions

The previous mentioned studies and speculations in Jensen group led to some important working models which could be useful for employing the new direct chemical glycosylation methodology for the synthesis of glycans containing 2-acetamido sugar motifs. As acylated donors appeared to hold potential for high yielding glycosylation reactions with at least some secondary carbohydrate derived acceptor alcohols under optimized conditions, Jensen and co-workers decided to explore and broaden the scope of this protocol.

As mentioned above, the leaving group has been shown to have high impact on yields in such direct glycosylation. Indeed, employing a slowly activating 1-acyl donor combined with a mild Lewis acid catalyst could increase yields in presence of a poorly reactive carbohydrate acceptor. Therefore, the tetraacetyl-β-GlcNAc donor **A** was revisited.

However, the effect of substituting the anomeric acetate for other acylates with varying nucleofuge abilities had not been investigated yet in a comparative manner. In order to ascertain how the fine tuning of the leaving group could influence glycosylation outcome, an efficient diastereoselective (β-selective) 1-O-acylation strategy to access a diverse variety of acylated donors was thus needed.

The current generally employed protocol to access peracetylated β-GlcNAc is shown in **Scheme 14**.



Scheme 14: current method employed to synthesise tetraacetyl β-GlcNAc

As it could be noticed from the scheme above, this protocol starts from glucosamine hydrochloride, and proceeds through the typical protection step by formation of an imine function. Subsequently, the protected glucosamine could undergo selective acetylation in presence of acetic anhydride in pyridine. Hydrolysis of the imine followed by a second acetylation delivers the target compound.

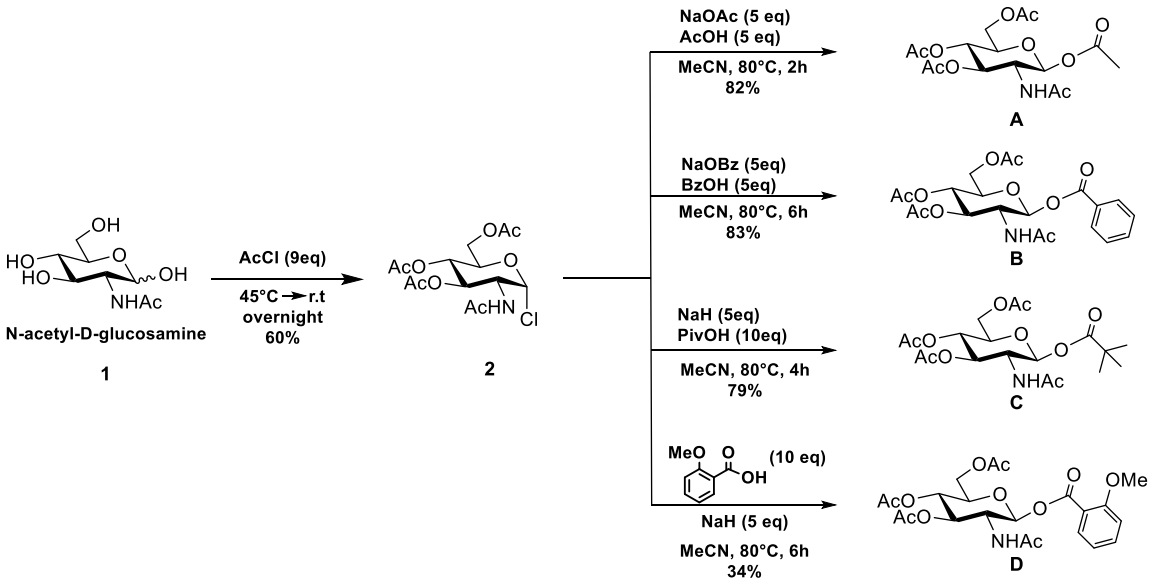
Although this method allows the use of the crude product from previous steps with no further purification and affords selectively tetreaacetyl β-GlcNAc, it requires extra-synthetic steps for manipulation of the -NHAc moiety, in addition to using some expensive and toxic chemicals.

Therefore, we proceeded to develop a more efficient and direct method to get access to the target 1-acyl β-GlcNAc donors.

3.1 Synthesis of Donors

Inspired by some previous works reported in literature [50,67,68], a new synthetic protocol of β-configured anomeric esters starting from the glycosyl chloride of GlcNAc has been developed (**Scheme 15**).

In addition to providing access to the desired donors, **B**, **C** and **D**, this simple catalyst-free procedure is also useful for the preparation of peracetylated donor **A** on a large scale via the glycosyl chloride ***(2)***, which could be easily synthesised from the commercially available N-acetyl glucosamine ***(1)***.



Scheme 15: Synthesis of donors **A**, **B**, **C** and **D** starting from the commercially available N-Acetyl glucosamine via the α-glycosyl chloride ***(2)***

The first step consists in a well-known solvent-free chlorination optimized by Jensen group and alternative to the Koenigs-Knorr procedure. Firstly, the excess of acetate affords the full acetyl protection of N-acetyl glucosamine, then the acidic conditions promote the

leaving of the anomeric acetate, leading to the expected 1,2-oxazoline, as explained in the general mechanism in chapter **1.4.** The opening of the poorly reactive oxazoline intermediate can thus be ensured by the great excess of HCl in solution. Initially, such opening should lead selectively to the β-chloride. However, presumably rapid equilibrium occurs under the acidic conditions, leading to the anomerisation to the α-product, which in fact is generally favoured as the sole reaction product in glycosyl halides formation. Indeed, the thermodynamically favoured α-chloride ***(2)*** was selectively obtained, due to the anomeric effect.

The α-chloride ***(2)*** was thus be subjected to nucleophilic substitution in presence of a 1:1 mixture of acetic acid and sodium acetate in acetonitrile at 80°C, yielding the 1-OAc donor **A**. The same procedure was followed for the 1-OBz donor **B**, while for **C** and **D** the carboxylate (respectively pivalate and ortho-methoxy benzoate ion) was formed *in situ* by reaction of NaH with the corresponding carboxylic acid. In preliminary experiments, reaction to **B**, **C** and **D** showed several problems in stirring, giving the low solubility of the reagents in acetonitrile and the formation of salts during the reaction. Therefore, some tests at different concentration in acetonitrile have been conducted, in order to find a compromise between reaction rate and good solubility. The final concentration adopted for donor **B** synthesis was 0,03 M (respect to the chloride) and the same optimization was realized for the other donor syntheses.

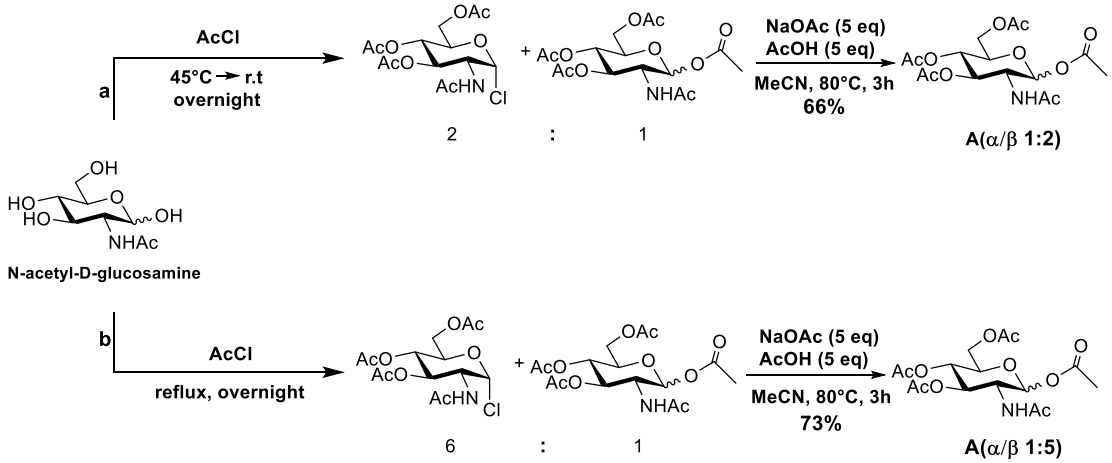
As shown above, 1-OAc, 1-OBz and 1-OPiv donors (respectively **A**, **B**, **C**) were obtained in very good yields via the nucleophilic substitution at the anomeric carbon. The same procedure with *ortho*-methoxybenzoyl donor **D** resulted instead in a less clean reaction, according to TLC and ^{1}H-NMR analysis, and complete purification of the product was troublesome, affording the desired β-donor **D** with a low 34% yield due to product losses during purification by chromatography on silica gel.

3.1.1 Further attempt of optimization of tetraacetyl β-GlcNAc synthesis protocol

The new 2-step synthetic protocol we developed is innovative and provides access to a wide range of β-configured acylated sugars, with respect to the current method (**Scheme 14**). Nevertheless, this method still requires purification by chromatography column at the end of both steps.

Therefore, different optimization attempts to this procedure were carried out, in order to find the easiest and most efficient strategy to afford peracetylated GlcNAc with at least a good β-selectivity, avoiding unnecessary steps.

As illustrated in **Scheme 16**, the first step of the chloride method affords a co-product, which should be the peracetylated GlcNAc, present as an α/β anomeric mixture. Since such co-product is the final product (**A**), it was thus decided to test whether using directly the crude product from the first step could ensure the same excellent outcome avoiding the purification after the first step. Nevertheless, since the peracetylated sugar from the first step should not undergo any reaction under the final *O*-glycosylation conditions, this would definitely lead to a certain amount of the corresponding α-donor in the final product.



Scheme 16: optimization tests of tetraacetyl GlcNAc

As displayed in **Scheme 16a**, when the mixture was heated at 45°C until dissolution and then stirred at room temperature overnight, the crude product showed the presence of the α-chloride along with a certain amount of the expected peracetylated GlcNAc (34 mol% with respect to the product). The following O-glycosylation with AcOH/NaOAc afforded the product **A** in a moderate 66% yield after chromatography column, given the difficulty in separating the hydrolysed sub-product from the co-polar anomeric mixture **A** (α/β 1:2). On the contrary, when the reaction was heated to reflux overnight (**Scheme 16b**), a lower amount of peracetylated co-product (13%, almost all α) was observed in the crude mixture, although with higher amount of impurities. The second step led to the desired product in a better yield (73%) than the first optimization attempt, along with a good 1:5 α/β ratio.

Since the two performed optimization tests led to lower yields than the one obtained in the first synthetic route adopted (**Scheme 15**), the latter is still preferred. Indeed, although it requires more purification steps, this method ensures a clean reaction together with a direct access to the desired β-product **A**, with no need of extra protection steps.

However, the attempted strategy displayed in **Scheme 16b** would provide an efficient method to easily access acetylated GlcNAc compounds if a high stereocontrol at the anomeric carbon is not required.

3.2 Synthesis of Acceptors

Once completed and optimized the synthesis of the desired acylated donors, it was necessary to synthesise suitable acceptors, in order to investigate how the donor leaving group could affect the glycosylation outcome with hindered and poorly reactive secondary carbohydrate acceptors. The aim was to reproduce the same kind of glycosyl linkages which are found in naturally occurring GlcNAc motifs. Since human milk oligosaccharides and naturally occurring N-glycans often carry GlcNAc β1→3-linked to galactose and β1→2 linked to mannose in their repetitive units[69], we have thus decided to synthesise the 3-OH galactose thioglycoside acceptor ***(3)*** and the 2-OH mannose thioglyocoside acceptor ***(4)*** shown in **Figure 5**. They could then be exploited for the further direct Lewis acid catalysed GlcNAc-ylation.

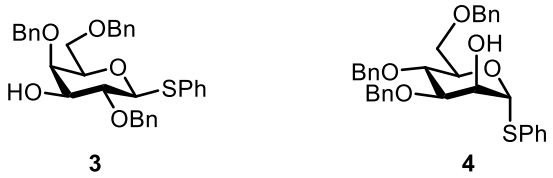
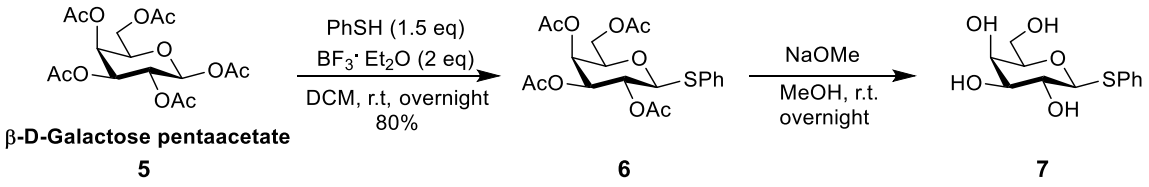


Figure 5: target acceptors phenyl 2,4,6-tri-*O*-benzyl-1-thio-β-D-galactoside ***(3)*** and phenyl 3,4,6-tri-*O*-benzyl-1-thio-α-D-mannoside ***(4)***

3.2.1 Synthesis of 3-OH galactose thioglycoside acceptor

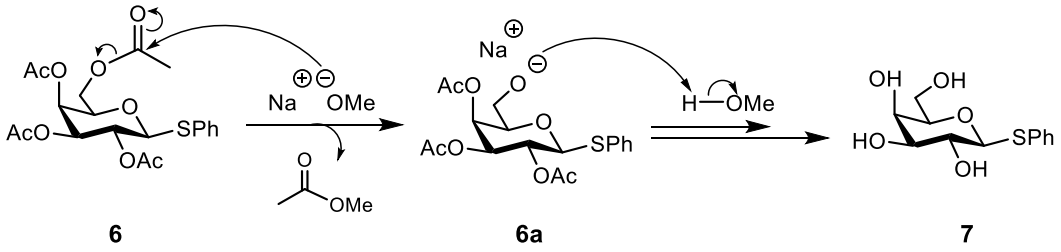
The desired galactose acceptor was obtained starting from the commercially available β-galactose pentaacetate, adapting a literature procedure[70]. For the achievement of the target acceptor ***(3)***, deprotection and substitution on the anomeric carbon with thiophenyl function was necessary, followed by selective protection of the alcohol at the C3. Successively, the benzyl protection and the final deprotection of the 3-OH led to the desired product. Due to the need of a suitable quantity of target acceptor for further glycosylations reactions and to improve step-economy, some of the intermediate products obtained in the multi-step synthesis were used as crude for the next steps.



Scheme 17: formation of the thioglicoside and following deacetylation starting from commercially available galactose pentaacetate

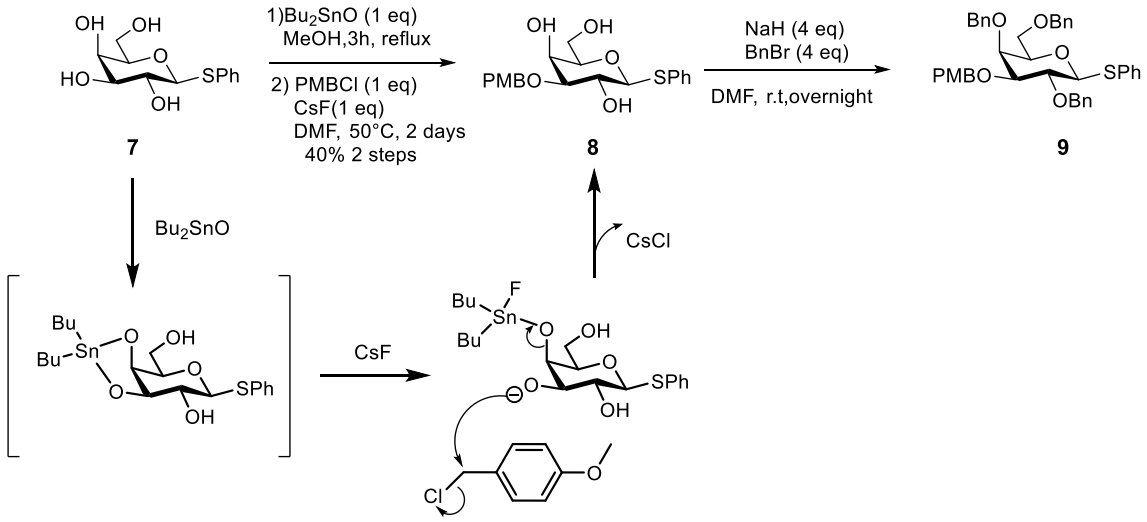
In the first step the galactose pentaacetate is converted into the corresponding phenyl thioglycoside (**Scheme 17**, first step). The Lewis acid boron trifluoride diethyl etherate ($BF_3{\cdot}Et_2O$) favours the leaving of the acetate at the C1, thus the thiophenol can undergo substitution on the anomeric carbon via dioxacarbenium ion intermediate formation. This kind of mechanism in presence of ester protecting group affords selectively the β-glycoside ***(6)***, due to neighbouring group participation, as as already established in previous chapters (**1.3**).

Thioglycoside ***(6)*** can now be subjected to the protection by Zemplén deacetylation (second step in **Scheme 17**), using catalytic amount of sodium methoxide. Compound ***(6)*** was dissolved in methanol and a sodium methoxide solution was added until an alkaline solution was obtained. The mechanism for this reaction is displayed below (**Scheme 18**).



Scheme 18: general mechanism of Zemplén reaction. Only deprotection of one acyl group is shown for clarity

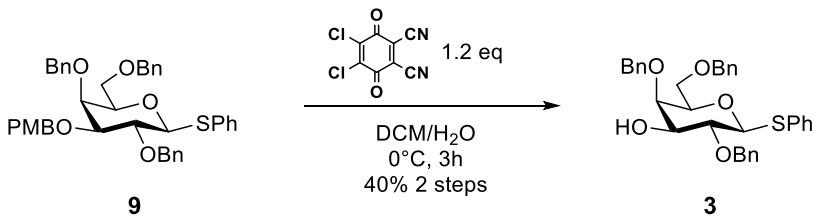
The methoxide nucleophile attacks the carbonyl carbon of the acetyl groups leading to formation of alkoxide ***(6a)*** upon departure of methyl acetate. The alkoxide abstracts a proton from the solvent methanol. This pathway is followed for all the protecting groups, thus the deprotected galactoside ***(7)*** is formed and the methoxide catalyst regenerated for each cycle. The scheme above shows the third and fourth step of the thiogalactoside synthesis. Firstly, a regioselective organotin-mediated protection was effectuated and the corresponding general-accepted mechanism is displayed in the first step in **Scheme 19**.



Scheme 19: regioselective dibutyltin oxide-mediated protection of 3-OH with *para*-methoxybenzyl chloride protecting agent, followed by benzylation of the remaining hydroxyl functionalities

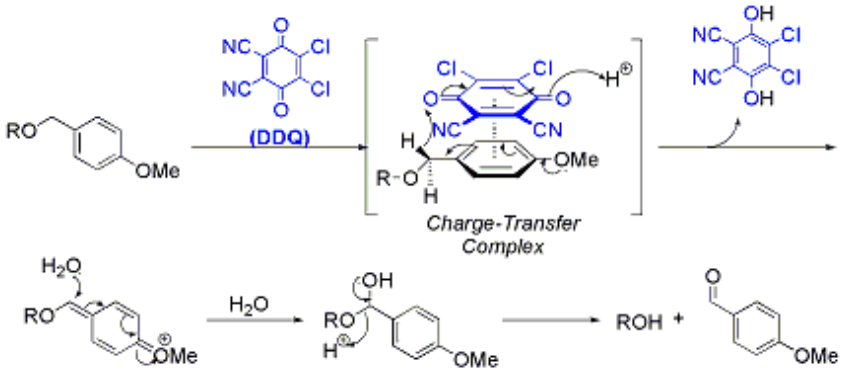
Treatment of the crude glycoside ***(7)*** with dibutyltin oxide (Bu_2SnO) leads selectively to the formation *in situ* of a tin acetal intermediate. Protection via dibutyltin acetals on cis-diols gives generally a selective protection of the equatorial hydroxyl group[71]. Indeed, subsequent treatment with a Lewis base such as CsF facilitates the ring-opening of the unstable intermediate and thus the attack by an electrophile on the equatorial C3 alcohol, due to steric hindrance of the tin complex. Therefore, *para*-methoxybenzyl chloride (PMBCl) approaches from the side rather than from the top or bottom side, affording selectively the C3-protected glycoside ***(8)***. The following step consists in a simple unselective benzylation under alkaline condition of the remaining free hydroxyl groups, affording the protected glycoside ***(9)***.

The final step toward obtaining galactose thioglycoside acceptor ***(3)*** is the deprotection of the C3 alcohol using the oxidising agent 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, **Scheme 20**).



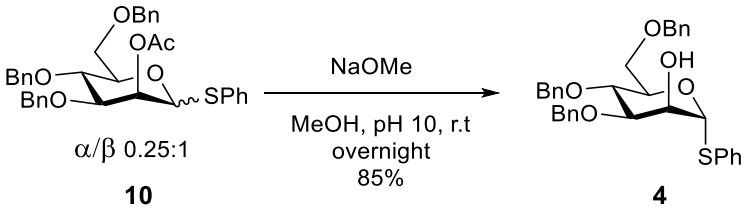
Scheme 20: Final deprotection to the target 3-OH thiogalactoside acceptor ***(3)*** using DDQ deprotecting agent

As shown in the proposed mechanism in **Scheme 21** , the treatment of a PMB-protected alcohol with DDQ in wet dichloromethane (DCM) promotes the oxidation of PMB through a charge transfer complex, due to conjugation between the two π-systems. This leads to oxidized DDQ departure, followed by hydrolysis of the PMB oxidized function. Therefore, the release of *para*-methoxy benzaldehyde affords the deprotected target 3-OH galactose acceptor ***(3)***.



Scheme 21: proposed general mechanism of DDQ-mediated alcohols deprotection in the presence of PMB protecting group

3.2.2 Synthesis of 2-OH mannose thioglycoside acceptor



Scheme 22: final deacetylation step of mannose acceptor ***(4)*** synthesis

The second target acceptor ***(4)*** was obtained starting from the intermediate 2-acetyl-protected mannopyranoside ***(10)*** already synthesised and available within the research group (**Scheme 22**). Therefore, compound ***(10)***, which was available as an α/β mixture 1:0.25, only needed to be subjected to Zemplén deacetylation to get the desired 2-OH acceptor (mechanism shown in **Scheme 18**). Release of the ester protecting group at the 2-position led to formation of the desired α-mannose thioglycoside acceptor ***(4)***, which was possible to separate from the β-anomer by flash column chromatography.

3.3 Screening of donors in direct catalytic glycosylation reaction

With the different acylated donors in hand direct glycosylation reactions could be performed. It could thus be investigated how the *fine tuning* of the match between the donor leaving group and the catalyst could improve the glycosylation yield under the newly optimized Lewis acidic conditions.

As already introduced, oxazoline formation is unavoidable in direct glycosylation with the free N-acetyl moiety. However, Jensen and co-workers have found out that the optimal reaction condition and reaction partners can favour its activation and following breakup, leading to better reaction outcome.

Therefore, with a slowly activating leaving group such as the acyl functionality, it is fundamental to choose the suitable catalyst, depending on the reactivity of the acceptor alcohol. When the acceptor is enough reactive, as in the case of a primary acceptors, a stronger Lewis acid catalyst is needed to speed up oxazoline activation and formation. On the contrary, a high demanding carbohydrate acceptor reacts slower. A milder promoter, which enables a better oxazoline activation rather than oxazoline formation is thus preferable, in order to minimise the side reactions.

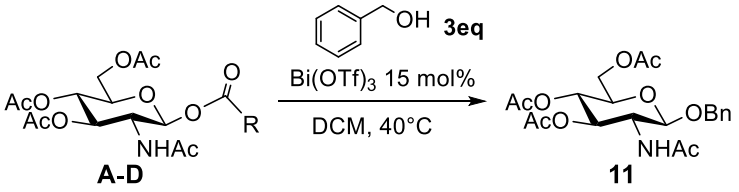
3.3.1 Direct glycosylation with primary simple alcohols

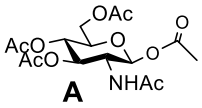
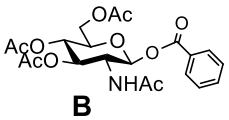
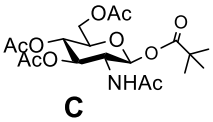
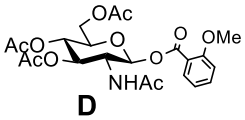
During the project, we have firstly decided to evaluate the nucleofuge ability of the different leaving groups and the potential of the method, by screening the synthesised acyl donors **A**-**D** in presence of the benzyl primary alcohol (**Table 4**).

Given the previous studies with other donors[40,64], glycosylation yields with simple alcohols were expected to lead to better results, with respect to glycosylations with carbohydrate acceptors.

According to the evaluation above, the catalyst chosen for this preliminary glycosylation was $Bi(OTf)_3$, which is one of the strongest Lewis acid within the already tested metal triflates.

Table 4: screening of synthesised acylated donor **A**-**D** in the novel direct Lewis acid catalysed glycosylation method in the presence of fast activating benzyl alcohol as acceptor. Suitable reaction conditions require a stronger Lewis acid along with a lower catalyst loading.



Entry[a]	Donor	Time[b]	Yeld[c]
1	 **A**	2 h	80%
2	 **B**	5 h	82%
3	 **C**	12 h	75 %
4	 **D**	4 h	78 %

[a]All reactions perfomed in CH_2Cl_2 at 40 °C with 3 equivalents of benzyl alcohol as the acceptor and 15 mol% of $Bi(OTf)_3$ as catalyst. [b] Time required for full conversion of donor as determined by TLC analysis. [c] Isolated yield after chromatography.

Previous studies in the group [40,63,64](Chapter **1.6.3**) reported better results using a high catalyst loading combined with an excess of acceptor, when simple acceptor alcohols were employed. Given these findings, a 15 mol% catalyst loading with a 1:3 ratio between donor and acceptor has been employed for each donor.

Glycosylations were performed in dichloromethane at 40°C in sealed tubes. Benzoylated donors **B** and **D**, reported quite good yields (respectively 82% and 78%, entry 2 and 4) combined with short reaction times (5h and 4h), thus indicating a good leaving group ability of the two benzoyl groups. 1-OPiv donor instead, seemed to be the slowest (12h, entry 3), as expected from the poor nucleofuge ability. On the contrary, 1-OAc donor **A** resulted to be the most reactive, with a good 80% yield after only 2 hours. Therefore, it

could be considered the quickest donor to get activated under these glycosylation conditions.

Given these preliminary results, we could evaluate the different reactivity of the four acyl donors and thus their activation rate by the Lewis acid promoter, displayed in **Figure 6**.

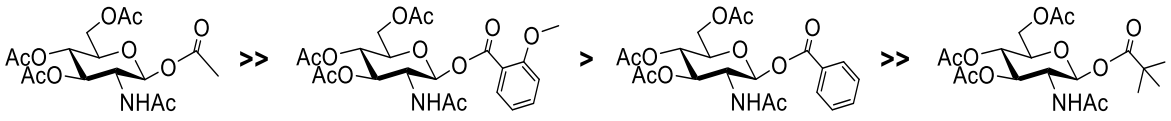


Figure 6: donor reactivity scale in order of decreasing activation rate under this reaction condition

These findings fit quite well with the expected, since it has been shown[31] that the electron density of the carbonyl group in Lewis-acid coordination is more important than the Brønsted-acid strength of the corresponding acid, which could be expected to correlate to nucleofugality. Nevertheless, *ortho*-methoxybenzoyl donor **D** was expected to be much more reactive than the others, since it was speculated that a chelate form between the *ortho*-OMe group and the ester carbonyl would be possible in that case, facilitating the departure of the leaving group during donor activation[31]. This was not the case.

The results obtained in this first glycosylation screening could also be useful to analyse and compare further glycosylations outcome.

3.3.2 Direct glycosylation with secondary carbohydrate acceptors

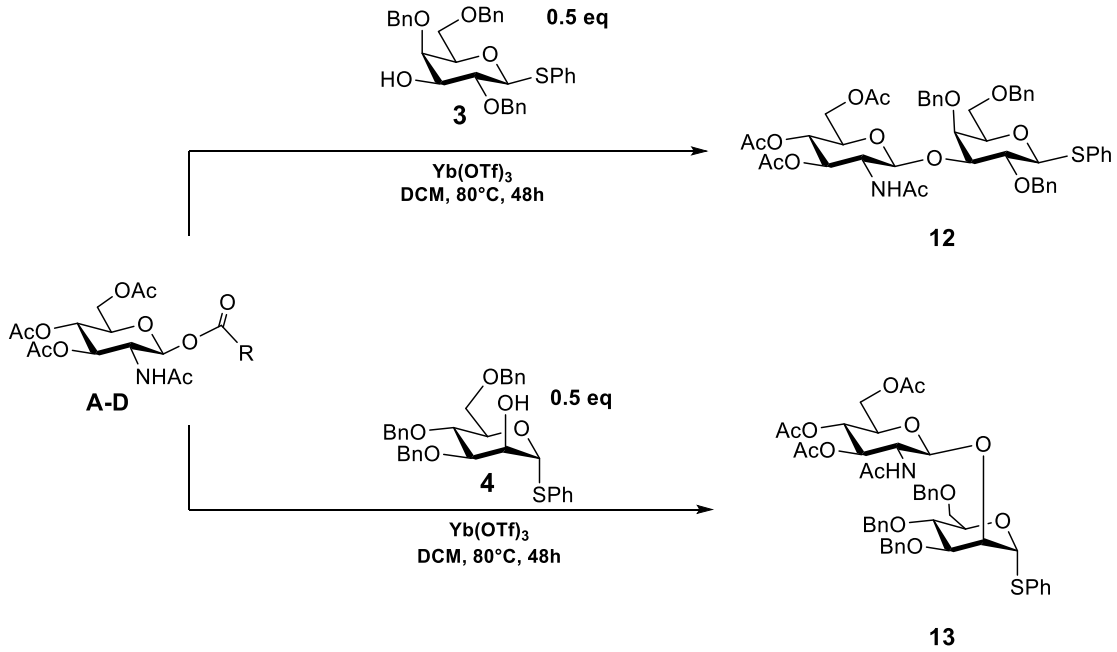
Since acetate function activation seems to be an acceptor-dependent process, investigation of the different acyl donors reactivity proceeded to testing higher demanding secondary carbohydrate acceptor alcohols. However, this required the use of suitable reaction conditions, which favour oxazoline activation rather than the possible side reactions.

Preliminary catalyst screenings carried out within the group[65] reported $Yb(OTf)_3$ as the most promising metal triflate catalyst for direct GlcNAc-ylation employing 2-acetamido acylated donors with the secondary galactoside acceptor ***(3)***.

Indeed, a milder Lewis acid as $Yb(OTf)_3$ could better promote the acyl leaving group departure and then oxazoline activation, favouring the following reaction with the acceptor.

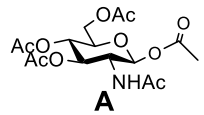
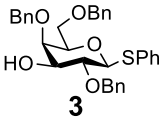
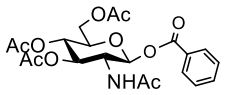
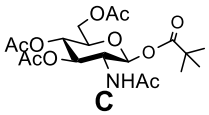
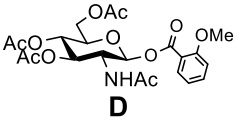
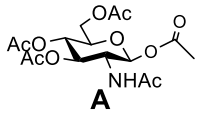
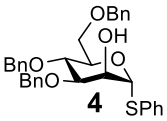
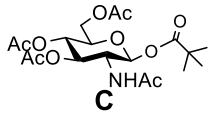
According to previous studies (see Chapter **1.6.3**), a higher temperature and a lower catalyst loading, as well as an excess of donor are required, in order to speed up reaction rate and improve yields, due to less side reactions.

Following this strategy, the synthesised acyl donors **A-D** were tested under identical glycosylation conditions with acceptor ***(3)*** and ***(4)***. The two series of glycosylation performed and the desired disaccharides from each reaction are displayed in **Scheme 23**.



Scheme 23: target glycosylation reactions for the screening of the synthesised acylated donors in the novel direct glycosylation protocol in presence of slowly activating carbohydrate acceptors ***(3)*** and ***(4)***. The expected disaccharide product are ***(12)*** and ***(13)***, depending on the match donor-acceptor

Table 5: screening of donors for direct acid Lewis catalysed glycosylation protocol in the presence of secondary carbohydrate acceptors ***(3)*** and ***(4)***. Suitable reaction conditions require a lower catalyst loading of a milder Lewis acid combined with a higher reaction temperature, as established in previous screenings within the group

Entry[a]	Donor	Acceptor	Cat%	Product	Yield[b]
1	 **A**	 **3**	2.5 mol%	**12**	56% (33%)
2			5 mol%	**12**	43%
3	 **B**	**3**	2.5 mol%	**12**	46%
4			5 mol%	**12**	43%
5	 **C**	**3**	2.5 mol%	**12**	63%[c] (32%)
6			5 mol%	**12**	57%[c] (38%)
7	 **D**	**3**	2.5 mol%	**12**	48% (45%)
8	 **A**	 **4**	2.5 mol%	**13**	-
9	 **C**	**4**	2.5 mol%	**13**	-

[a]All reactions perfomed in CH_2Cl_2 at 80 °C with 0.5 equivalents of acceptor and $Yb(OTf)_3$ as catalyst and proceeded for 48 hours. [b] Isolated yield after chromatography. Recovered acceptor in parenthesis.[c]Low purity

Table 5 illustrates the results achieved by the new direct GlcNAc-ylation method with the different acyl donors.

Firstly, every donor was subjected to glycosylation with compound ***(3)*** as the acceptor, using a 2.5 mol% or 5 mol% catalyst loading. As reported in entries 1-6, when 2.5 mol%

$Yb(OTf)_3$ was employed (entries 1, 3 and 5), better yields were always achieved, as well as cleaner products, according to ^{1}H-NMR analysis. However, it is apparent that the glycosylation yields follow the same trend under both the reaction conditions, leading to important conclusions about donors reactivity.

As expected from the findings of the first screening, benzoylated donors **B** and **D** did not result in best glycosylation yields (respectively 46% and 48% entry 3 and 7). Acetyl donor **A** reported a very good result affording the desired β-linked-disaccharide ***(12)*** in a 56% yield (entry 1). Pivaloyl donor **C** seemed initially to give the best result, with an excellent 63% yield (entry 5) after chromatography. Nevertheless, ^{1}H-NMR revealed the presence of an impurity which could not be identified (probably a pivalic compound formed during the reaction). Thus, the yield is overestimated.

All the glycosylation tested did not proceed to full conversion. However, in some cases the precious unreacted acceptor could be recovered after reaction by chromatography (entries 1, 5, 6 and 7 in parenthesis).

Therefore, while donor **A** yielded the cleanest product, which could be directly employed for further glycosylations, the disaccharide derived from donor **C** could be used only after further purification. Moreover, donor **B** and **C** did not perform a totally β-selective glycosylation, since ^{1}H-NMR analysis on disaccharides obtained from donor **B** and **D** showed traces of the α-linked product, although in very small amounts (less than 0.04:1) with respect to the desired product.

It can be thus concluded that acetyl donor **A** resulted as the best donor for this glycosylation method using galctoside ***(3)*** as acceptor, as it afforded a quite good yield and also provided a fully β-selective glycosylation (no α-linked disaccharide found, according to NMR analysis).

As shown in **Table 5**, donors **A** and **C** were also subjected to glycosylation in the presence of mannose acceptor ***(4)*** **(**entries 8 and 9), since they had been shown to be the best donors in glycosylation with the other acceptor. Unfortunately, both the reaction did not lead to a significant amount of product. According to NMR analysis, they resulted in a mix of disaccharides in very low yield, probably caused by the relatively high steric hindrance of the free hydroxyl group on mannose acceptor ***(4)***, which hindered the formation of the new glycosyl linkage. Since this thioglycoside could act also as donor and could be even too reactive for such a glycosylation method, it has been thus speculated that employing a different acceptor than a thioglycoside for this protocol would ensure better results. Future efforts will investigate this hypothesis.

Being the first attempt to such a direct glycosylation method and given the highly challenging factors related to work with GlcNAc, it should be pointed that the results achieved with this new direct catalytic GlcNAc-ylation protocol are highly promising. Work in the group to optimise the conditions are still ongoing.

4 Conclusions

At first, a novel and efficient diastereoselective 1-O-acylation protocol to easily access a diverse variety of β-configured acylated GlcNAc compounds has been developed.

We efficiently synthesised four different 1-acyl GlcNAc donors by this method, without the need of N-protecting groups for manipulation of the troublesome 2-acetamido moiety.

Therefore, we proceeded to test the synthesised acylated GlcNAc donors investigating how the *fine tuning* of the match between the donor leaving group and the catalyst could influence the glycosylation yield, in the presence of acceptors with different reactivity.

Firstly, the 1-acyl donors have been screened in direct glycosylation with benzyl alcohol as representative primary acceptor, in order to assess the potential of the method. Employing the strong Lewis acid $Bi(OTf)_3$ catalyst in presence of the reactive primary alcohol resulted in excellent yields, thus confirming the working hypothesis and providing information about the reactivity scale of the donors in this method.

Subsequently, two secondary carbohydrate acceptors have been synthesised and thus exploited for a further direct GlcNAc-ylation screening of the four promising acylated donors. In order to favour a good glycosylation outcome even with these poorly reactive carbohydrate acceptors, the milder Lewis acid $Yb(OTf)_3$ was employed at low loadings, combined with a higher reaction temperature. Under these conditions, the slowly activating acyl donors afforded the desired disaccharides with surprising good yields, with the 1-OAc donor **A** appearing as the most promising among the acyl-GlcNAc donors investigated.

Interestingly, the yields afforded by the different donors followed the same trend observed for the first screening, confirming the hypothesis that the donor leaving group has high impact on yields in such direct glycosylations. 1-OAc donor **A** has been shown as the best donor for this direct glycosylation method under both the reaction conditions investigated and will thus be subject of further research.

In conclusion, this project is the very first attempt in such a direct metal triflates-catalysed glycosylation reaction employing acylated GlcNAc donors.
Moreover, the challenging factors related to handle GlcNAc must be considered: this troublesome compound presents indeed a low solubility in most of the commonly used solvents and generally does not undergo even well-established carbohydrate chemistry reactions as efficiently as other sugars. Given the novelty and the challenging nature of the process, the results gained are thus quite remarkable and hold great promise for further development.

A first aim of future studies, will be to improve the glycosylation yields in presence of secondary carbohydrate acceptors by varying the reaction conditions and/or the catalyst employed. Different carbohydrate acceptors other than the thioglycosides will be investigated, such as 1-methoxy glycosides, which hopefully will not compete as donor in the glycosylation reaction resulting in better efficiency.

5 Experimental section

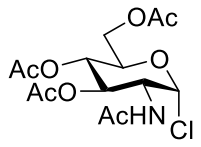
5.1 General methods and materials

Commercially available chemicals were used without further purification unless otherwise stated. All reactions involving air and moisture sensitive compounds were conducted in oven or flame dried glassware under inert atmosphere. Solvents were dried over aluminium oxide via a Braun solvent purification system or over molecular sieves (4 Å). DMF was purchased as dry. Evaporation of solvents was performed *in vacuo* at 45 °C or 60°C. TLC analysis was carried out on silica coated aluminium foil plates (Merck Kieselgel 60 F_{254}). The TLC plates were visualized using UV light (254 nm) and/or by staining with 10 % sulphuric acid (aq) solution. Flash column chromatography was performed with a Merck silica gel (230-400 Mesh) as the stationary phase.

NMR spectra were recorded on a Bruker 400 spectrometer at 400 MHz for ^{1}H-, gCOSY- and gHMQC-NMR spectra and at 100 MHz for DEPT-135- and ^{13}C-NMR spectra. The chemical shifts (δ) are reported in ppm downfield to TMS (δ = 0) and referenced using either the residual $CHCl_3$ resonance (δ = 7.26) for ^{1}H NMR and the central $CDCl_3$ resonance (δ = 77.16) for ^{13}C NMR. For ^{1}H NMR spectra the coupling constants (*J*) are given in Hertz (Hz). ^{1}H and ^{13}C NMR spectra were interpreted on the basis of gCOSY, gHMQC and DEPT-135 techniques.
High resolution MS (HRMS) were recorded using a Micromass LC-TOF spectrometer utilizing positive electrospray ionization. Masses of analytes are calculated and reported in Daltons for charged species. Optical rotations were measured on a Perkin-Elmer 241 polarimeter and concentrations are reported in g/100 mL. Melting points were measured on a Büchi B-540 instrument.

5.2 Synthesis of Donors

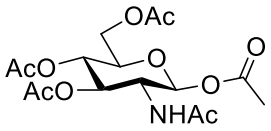
5.2.1 2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride *(2)*



Acetyl chloride (60 mL, 0.0812 mol, 9 equiv) was added to *N*-acetyl-D-glucosamine (20 g, 0.0904 mol, 1 equiv) and the mixture was heated to reflux under an atmosphere of N_2 until dissolution (1h). The reaction was stirred at room temperature overnight, affording an orange solution/suspension. TLC analysis (EtOAc-Pentane 2:1) indicated complete conversion of the starting material. The crude mixture was diluted with CH_2Cl_2 and poured into ice/water. The mixture was washed with saturated $NaHCO_3$ aq (x4) and extracted with CH_2Cl_2 (x1). The combined organic layers were dried over $MgSO_4$, filtered and evaporated *in vacuo*. The residue was purified by flash column chromatography (40% Pentane in EtOAc) to give the desired α-chloride ***(1)*** as white solid (19.8 g, 0.0514 mol, 60 %).

R_f 0.43 (EtOAc-pentane 2:1). $[\alpha]_D^{297K}$125.6 (*c*1, $CHCl_3$). (T_{degrad}) 119.2 °C ($CHCl_3$). **^{1}H-NMR (400 MHz, $CDCl_3$)**: δ_H (ppm) 6.17 (d, $J_{1,2}$ 3.6 Hz, 1H, H-1), 5.89 (d, $J_{NH,2}$ 8.3 Hz, 1H, N*H*), 5.31 (t, $J_{3,4=2,3}$ 10.4 Hz, 1H, H-3), 5.21 (t, 1H, H-4), 4.53 (ddd, 1H, H-2), 4.25 (m, 2H, H-6a, H-5), 4.12 (dd, 1H, H-6b), 2.09 (s, 3H, 2x C(O)C*H*$_3$), 2.04 (s, 6H, C(O)C*H*$_3$), 1.98 (s, 3H, NHC(O)C*H*$_3$). **^{13}C-NMR (100 MHz, $CDCl_3$)**: δ_C (ppm) 171.5, 170.6, 170.1, 169.2, (4xC=O), 93.6 (C1), 72.9 (C5), 70.1 (C3), 66.9 (C4), 61.1 (C6), 53.5 (C2), 23.1 (NHC(O)*CH*$_3$), 20.6, 20.7 (OC(O)*C*H$_3$). **HRMS(ES+)**: Calcd. for $C_{14}H_{20}ClNO_8H^+$ m/z 366.0956; found m/z 366.0960.

5.2.2 2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-glucopyranose *A*

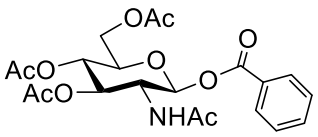


Chloride ***(2)*** (500 mg, 1.37 mmol) was dissolved in dry CH_3CN (5 mL). Sodium Acetate (560 mg, 6.84 mmol, 5 equiv) and acetic acid (390 µL, 6.84 mmol, 5 equiv) were added and the mixture was stirred under N_2 at 80 °C. After 2 hours TLC analysis (EtOAc) indicated full conversion of the chloride. The mixture was diluted with $CHCl_3$ and co-evaporated with Toluene (x3). The residue was washed with sat. $NaHCO_3$ aq (x5). The aqueous phase was extracted with $CHCl_3$ (x3) and the organic phase was dried over $MgSO_4$ and filtered. The crude product

was evaporated onto Celite and subjected to flash column chromatography (CH_2Cl_2-EtOAc 1:3), affording the desired β-product as white solid (0.436 g, 1.12 mmol, 82 %).

***R*$_f$** 0.39 (EtOAc-CH_2Cl_2 3:1). $[\alpha]_D^{297K}$ 2.2 (*c*1, $CHCl_3$). Mp 188.7-189.1 °C ($CHCl_3$). **^{1}H-NMR (400 MHz, $CDCl_3$)**: δ_H (ppm) 5.77 (d, $J_{NH,2}$ 9.3 Hz, 1H, NH), 5.69 (d, $J_{1,2}$ 8.7 Hz, 1H, H-1), 5.14 (m, 2H, H-3, H-4), 4.29 (m, 1H, H-2), 4.28-4.23 (dd, $J_{6a,6b}$ 12.4 Hz, $J_{5,6a}$ 4.5 Hz, 1H, H-6a), 4.14-4.09 (dd, $J_{6b,6a}$ 12.4, $J_{5,6b}$ 2.2, 1H, H-6b), 3.81 (ddd, 1H, H-5), 2.12-2.01 (3s, 12H, C(O)C*H*$_3$), 1.92 (s, 3H, NHC(O)C*H*$_3$). **^{13}C-NMR (100 MHz, $CDCl_3$)**: δ_C (ppm) 171.3, 170.7, 170.2, 169.6, 169.3 (5xC=O), 92.6 (C1), 72.8 (C5), 72.6, 67.8 (C4, C3) , 62.2 (C6), 52.9 (C2), 23.2 (NHC(O)CH3), 20.9-20.6 (OC(O)*C*H$_3$). **HRMS(ES+)**: Calcd. for $C_{16}H_{23}NO_{10}Na^+$ m/z 412.122; found m/z 412.121.

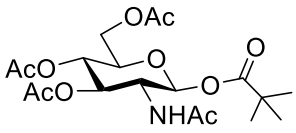
5.2.3 2-Acetamido-1-*O*-benzoyl-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose *B*



Chloride ***(2)*** (200 mg, 0.550 mmol, 1 equiv) was dissolved in dry CH_3CN (20 mL). Sodium benzoate (634 mg, 4.4 mmol, 8 equiv) and benzoic acid (336 mg, 2.75 mmol, 5 equiv) were added and the mixture was stirred under N_2 at 80°C. After 5 hours TLC analysis (pentane-EtOAc 2:1) indicated full conversion of the chloride. The resulting mixture was diluted with EtOAc, washed with sat. $NaHCO_3$ aq and back-extracted two times with EtOAc. The combined organics were dried over $MgSO_4$, filtered, and evaporated in *vacuo* to give a crude which was subjected to flash column chromatography (15% → 25% EtOAc in CH_2Cl_2), affording the desired β-product ***(4)*** (0.206 mg, 0.456 mmol, 83%) as white crystals.

***R*$_f$** 0.25 (15% EtOAc in CH_2Cl_2). $[\alpha]_D^{297K}$ -42.4 (*c*1, $CHCl_3$). Mp 154.4-155.6 °C ($CHCl_3$). **^{1}H-NMR (400 MHz, $CDCl_3$)**: δ_H 8.06 (ppm) (d, J_{orto} 8.2 Hz, 2H, ArH-1a, ArH-1b) 7.59 (t, J_{orto} 7.3 Hz,1H, Ar-H3), 7.45 (t, J_{orto} 8.2 Hz, 2H, Ar-H2a, Ar-H2b) 5.86 (d, $J_{1,2}$ 8.5 Hz, 1H, H-1), 5.67 (d, $J_{NH,2}$ 9.3 Hz, 1H, NH), 5.25-5.15 (m, 2H, H-3, H-4), 4.58-4.49 (q, 1H, H-2), 4.30 (dd, $J_{6a,6b}$ 12.4 Hz, $J_{5,6a}$ 2.4 Hz, 1H, H-6a), 4.14 (dd, $J_{6b,6a}$ 12.4 Hz, 1H, H-6b), 3.90 (s, 1H, H-5), 2.07 (m, 9H, C(O)C*H*$_3$), 1.88 (s,3H, NHC(O)C*H*$_3$). **^{13}C-NMR (100 MHz, $CDCl_3$)**: δ_C (ppm) 171.4, 170.7, 170.1, 169.3, 165.2 (5xC=O), 133.9, 130.3, 128.6, 128.4 (4xAr), 93.3 (C1), 72.9 (C5), 72.6, 67.7 (C4, C3) , 61.6 (C6), 52.8 (C2), 23.2 (NHC(O)*C*H$_3$), 20.8-20.6 (OC(O)*C*H$_3$). **HRMS(ES+)**: Calcd. for $C_{21}H_{25}NO_{10}Na^+$ m/z 474.138; found m/z 474.137.

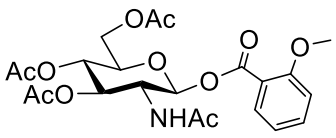
5.2.4 2-Acetamido-1-*O*-pivaolyl-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose *C*



NaH (60% in mineral oil, 273 mg, 6.84 mmol, 5 equiv) was slowly added to a solution of trimethylacetic acid ($(CH_3)_3CCO_2H$) (1.39 mg, 13.7 mmol, 10 equiv) in dry CH_3CN (27 mL). When gas evolution ceased, chloride ***(2)*** (500 mg, 1.37 mmol) was added and mixture was stirred under N_2 at 80 °C. After 3 hours TLC analysis (pentane-EtOAc 2:1) indicated full conversion of the chloride. The mixture was diluted in EtOAc before washing with satd. $NaHCO_3$ aq (x5) and extracting with EtOAc (x2). The residue was dried over $MgSO_4$, filtered and concentrated under reduced pressure. Flash column chromatography on silica gel (25% EtOAc in CH_2Cl_2) afforded the desired β-product as a white solid (0.466 g, 1.08 mmol, 79 %).

R_f 0.21 (25% EtOAc in CH_2Cl_2). $[\alpha]_D^{297K}$ -1.2 (*c*1, $CHCl_3$). Mp 63.8-66.3 °C ($CHCl_3$). **^{1}H-NMR (400 MHz, $CDCl_3$)**: δ_H (ppm) 5.68 (d, $J_{NH,2}$ 9.9 Hz, 1H, NH), 5.62 (d, $J_{1,2}$ 8.9 Hz, 1H, H-1), 5.13 (m, 2H, H-3, H-4), 4.36 (m, 1H, H-2), 4.30-4.24 (dd, $J_{6a,6b}$ 12.4 Hz, 1H, H-6a), 4.13-4.08 (dd, $J_{6b,6a}$ 12.4 Hz, $J_{5,6b}$ 2.4 Hz, 1H, H-6b), 3.80 (m, 1H, H-5), 2.09 (4 s, 12H, C(O)CH_3), 2.03 (d, 6H, C(O)CH_3), 1.90 (s,3H, NHC(O)CH_3), 1.19 (s, 9H, C(O)C(CH_3)$_3$) **^{13}C-NMR (100 MHz, $CDCl_3$)**: δ_C (ppm) 177.2, 171.3, 170.8, 169.8, 169.3 (5xC=O), 92.6 (C1), 72.9 (C5), 72.6, 67.9 (C4, C3), 61.7 (C6), 52.7 (C2),38,8 (OC(O)*C*$(CH_3)_3$), 26.8 (OC(O)C(*C*H_3)$_3$), 23.2 (NHC(O)*C*H_3), 20.8-20.6 (OC(O)*C*H_3). **HRMS(ES+)**: Calcd. for $C_{19}H_{29}NO_{10}Na^+$ m/z 454.168 ; found m/z 454.168.

5.2.5 2-Acetamido-1-*O*-(2-methoxybenzoyl)-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose *D*



NaH (60% in mineral oil, 273 mg, 6.84 mmol, 5 equiv) was slowly added to a solution of 2-methoxybenzoic acid (2.08 g, 13.7 mmol, 10 equiv) in 27 mL dry CH_3CN. When gas evolution ceased, chloride ***(1)*** (500 mg, 1.37 mmol, 1equiv) was added and the mixture was stirred under N_2 at 80°C. After 6 hours TLC analysis (pentane-EtOAc 2:1) indicated full conversion of the chloride. The mixture was diluted with EtOAc, before washing with satd. $NaHCO_3$ aq (x5) and extracting two times with EtOAc. The residue was dried on $MgSO_4$ and filtered. The crude product was concentrated onto Celite and subjected to flash

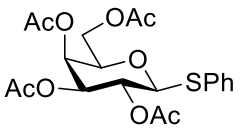
column chromatography (15%→20% EtOAc in CH_2Cl_2) afforded the desired β-product as a white solid (224 mg, 0.466 mmol, 34 %).

***R**f* 0.42 (EtOAc). $[\alpha]_D^{298K}$ -5 (*c*1, $CHCl_3$). Mp 66.2-67.4 °C ($CHCl_3$). **^{1}H-NMR (400 MHz, $CDCl_3$)**: δ_H (ppm) 7.90 (dd, J_{orto} 7.8 Hz, J_{meta}1.8 Hz, 1H, Ar-H), 7.51 (ddd, J_{orto} 8.3 Hz, J_{orto}7.4 Hz, J_{meta} 1.8 Hz, 1H, Ar-H), 7.04 – 6.93 (m, 2H, Ar-H), 5.89 (d, $J_{1,2}$ 8.8 Hz, 1H, H-1), 5.69 (d, $J_{NH,2}$ 9.7 Hz, 1H, NH), 5.25 – 5.13 (m, 2H, H-3, H-4), 4.53 (m, 1H, H-2), 4.29 (dd, $J_{6a,6b}$ 12.5 Hz, $J_{6a,5}$ 4.5 Hz, 1H, H-6a), 4.13 (dd, $J_{5,6b}$ 4.5 Hz, $J_{6b,6a}$ 12.5 Hz, 1H, H-6b), 3.90 (m, 4H, H-5, OC*H*$_3$), 2.16 – 2.00 (m, 9H, C(O)C*H*$_3$), 1.89 (s,3H, NHC(O)C*H*$_3$). **^{13}C-NMR (100 MHz, $CDCl_3$)**: δ_C (ppm) 171.3,170.7, 170.1, 169.3, 163.7, (5xC=O), 160.2 (*C*OCH_3),134.9, 132.7, 120.4, 117.5 (4xAr), 92.7 (C1), 72.9 (C5), 72.8, 67.8 (C4, C3) , 61.7 (C6), 55.9 (O*CH*$_3$), 52.8 (C2), 23.2 (NHC(O)*C*H_3), 20.8-20.7 (OC(O)*C*H_3). **HRMS(ES+)**: Calcd. for $C_{22}H_{28}NO_{11}$+ m/z 482.1657; found m/z 474.1657.

5.3 Synthesis of Acceptors

5.3.1 Synthesis of phenyl 2,4,6-tri-O-benzyl-1-thio-β-D-galactopyranoside *(3)*

5.3.1.1 Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranoside *(6)*

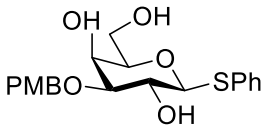


β-D-galactopyranose pentaacetate (20 g, 51.2 mmol, 1 equiv) was dissolved in dry CH_2Cl_2 (40 mL). PhSH (7.8 mL, 76.9 mmol, 1.5 equiv) and $BF_3{\cdot}Et_2O$ (12.7 mL, 0.102 mmol, 2 equiv) were added. The yellow clear solution was left stirring under nitrogen atmosphere at room temperature overnight. TLC analysis (EtOAc-pentane 1:1) revealed full conversion of the starting material. The mixture was diluted with CH_2Cl_2, washed several times with satd. $NaHCO_3$ aq. The organic phase was extracted with CH_2Cl_2. Combined organic layers were dried on $MgSO_4$, filtered and concentrated *in vacuo*, giving a yellow oil. Crystallisation of the resulting residue from Et_2O/pentane yielded the product (19.2 g, 43.5 mmol, 85%) as a white flocculent solid.

***R**f* 0.64 (40% EtOAc in pentane). $[\alpha]_D^{297K}$ +5 (*c*1, $CHCl_3$). **^{1}H-NMR (400 MHz, $CDCl_3$)**: δ_H (ppm) 7.54-7.48 (m,2H, Ar-H) 7.34-7.28 (m, 3H, Ar-H), 5.41(dd, 1H, $J_{4,3}$ 3.4 Hz, $J_{4,5}$ 1.1 Hz, H-4), 5.24 (t, 1H, $J_{2,3=2,1}$ 10.0 Hz, H-2), 5.04 (dd, $J_{3,4}$ 3.3 Hz, 1H, H-3), 4.71 (d, $J_{1,2}$

10.0 Hz, 1H, H-1), 4.21-4.07 (m, 2H, H-6a, H-6b), 3.94 (ddd, 1H, H-5), 1.98, 2.05, 2.10, 2.12 (4s, 12H, 4 × C(O)*CH*$_3$); **^{13}C-NMR (100 MHz, CDCl$_3$)**: δ_C (ppm) 170.4, 170.2, 170.1, 169.5 (4xC=O) , 132.5, 132.5, 128.9, 128.2 (Ar), 86.6 (C1), 74.4 (C5), 71.9 (C3), 67.2 , 67.2 (C2,C4), 61.6, (C6) 20.9, 20.7, 20.7, 20.6 (4xOC(O)*C*H3).
HRMS(ES+): $C_{20}H_{24}NaO_9S$+ calcd m/z 463.104, found m/z 463.103

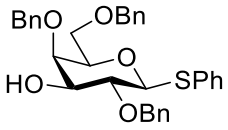
5.3.1.2 Phenyl 3-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside *(7)*



Sodium methoxide solution (25 wt % in MeOH) was added to a suspension of Phenyl 2,3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside ***(6)***, (16.9 g, 0.0384 mol) in MeOH (100 mL), until a pH-value of approximately 10 was reached. The reaction mixture was stirred under a nitrogen atmosphere overnight. The crude deprotected thioglycoside was evaporated to dryness and used for the next step without further purification.
The crude product was dissolved in dry methanol (150 mL) and dibutyltin oxide ((Bu)$_2$SnO, 9.75g, 0.0384 mol) was added, obtaining a white solution. The reaction mixture was heated to reflux under a nitrogen atmosphere. After 3 hours, the yellow solution was concentrated to dryness and redissolved in dry DMF (100 mL). PMBCl (4-Methoxybenzyl chloride, 5.21 mL, 0.038 mol) and CsF (5.83 g, 0.0384 mol) were added and the resulting suspension was stirred under N_2 at 50°C. After 2 days TLC analysis (EtOAc-pentane 1:1) showed no further reaction development. The crude mixture was concentrated onto Celite and subjected to flash column chromatography (EtOAc-pentane 1:1→2:3→1:2), affording the desired product (6.02 g, 15.4 mmol) as a yellow oil in 40% yield over 2 steps.

R$_f$ 0.6 (EtOAc). **^{1}H-NMR (400 MHz, CD$_3$OD)**: δ_H (ppm), 7.59-7.51 (m,2H, Ar-H) 7.40-7.18 (m, 5H, Ar-H), 6.92-6.84 (m, 2H, Ar-H), 4.70 (d, J_{gem} 10.4 Hz, 1H, CH*H*PhOCH$_3$), 4.62 (d, J_{gem} 10.4 Hz ,1H, C*H*HPhOCH$_3$),4.60 (d, $J_{1,2}$ 9.7 Hz, 1H, H-1), 4.05 (d, $J_{3,4}$ 3.2 Hz, 1H, H-4), 3.76 (s, 3H, OC*H*$_3$), 3.75-3.65 (m, 3H, H-6a, H-6b, H-2), 3.51 (dd, 1H, H-5), 3.39 (dd, $J_{3,2}$ 9.1 Hz, $J_{3,4}$ 3.2 Hz, 1H, H-3).
The NMR-data are in accordance with those previously reported in literature [70] .

5.3.1.3 Phenyl 2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside *(3)*

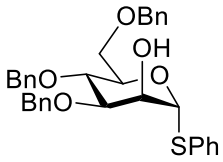


NaH (60% in mineral oil, 2.43g, 60.6 mmol, 4 equiv) was added to a solution of Phenyl 3-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside ***(7)*** (5.95 g, 15.2 mmol, 1 equiv) in dry DMF (100 mL) at 0° C. When gas evolution was ceased, benzyl bromide (7.21 mL, 60.6 mmol, 4 equiv) was added and the reaction mixture was stirred under Ar atmosphere, at room temperature overnight. TLC analysis indicated full conversion of the glycoside. The crude mixture was diluted with Et_2O and washed with satd. NaCl aq (x2) and water (x2). The aqueous phases were extracted with Et_2O. The combined organic phases were dried on $MgSO_4$, filtered and solvents were evaporated to dryness.

Crude phenyl 2,4,6-tri-*O*-benzyl-3-*O*-(4-methoxyphenyl)-1-thio-β-D-galactopyranoside (10.0g) was dissolved in a mixture of CH_2Cl_2/H_2O (100 mL/5.2 mL), and the solution was cooled to 0 °C. 2,3-Dichloro 5,6-dicyano-1,4-benzoquinone (DDQ, 4.13 g, 18.2 mmol, 1.2 equiv) was added, and the mixture was stirred at room temperature. After 3 hours TLC analysis (20% EtOAc in pentane) indicated not full conversion of the starting material. Subsequently, additional 0.5 equivalent of DDQ (1.7 g, 7.56 mmol) was added and the mixture was stirred for 30 minutes, until TLC indicated full conversion. The brown/red turbid suspension was filtered and washed with CH_2Cl_2. The organic phase was washed with H_2O until the solution became yellow and clear, dried over $MgSO_4$ and filtered. Flash column chromatography (15% EtOAc in pentane) and subsequent crystallisation of the mixed fractions in Et_2O/Heptane afforded the desired β-product as a white solid (3.31 g, 6.07 mmol) in 40% yield over 2 steps.

R_f 0.37 (20% EtOAc in pentane). $[\alpha]_D^{297K}$ 6.6 (*c*1, $CHCl_3$). Mp 92.4-93.9 °C (CH_2Cl_2).
^{1}H-NMR (400 MHz, $CDCl_3$): δ_H (ppm)7.58 – 7.47 (m,2H, Ar-H), 7.36 – 7.23 (m, 15H. Ar-H), 7.20-7.12 (m, 3H, Ar-H), 4.85 (d, *J* 11.2 Hz, 1H, OC*H*HPh), 4.70 (d, *J* 11.6 Hz, 1H, OCH*H*Ph), 4.63- 4.55 (m, 3H, OC*H*$_2$Ph, H-1), 4.63-4.55 (m, 3H, OC*H*$_2$Ph, H-1), 4.53 – 4.35 (m, 2H, OC*H*$_2$Ph), 3.87 (d, $J_{4,3}$ 2.2 Hz, 1H, H-4), 3.71 – 3.59 (m, 5H, H-2, H-3, H-5, H-6a, H-6b), 2.11 (br s, 1H, OH); **^{13}C-NMR (100 MHz, $CDCl_3$)**: δ_C (ppm) 138.5, 138.1, 137.8 (3x Aromatic C-C), 134.2 (Aromatic C-S), 131.3, 128.9, 128.7, 128.6, 128.5, 128.4, 128.4, 128.3, 128.1, 127.9, 127.9, 127.9, 127.8, 127.5, 127.2 (Aromatic C-H), 87.4 (C1) , 78.3, 77,4, 76.1, 75.7 (C2, C3, C4, C5), 75.3, 75.0, 73.6 (3x O*C*H$_2$Ph) , 68.6 (C6). **HRMS(ES+)**: $C_{33}H_{34}NaO_5S$+ calcd m/z 565.202, found m/z 565.202.

5.3.2 Synthesis of phenyl 3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside acceptor *(4)*

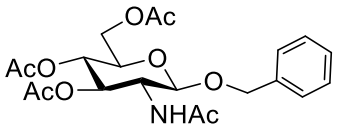


Phenyl 2-*O*-acetyl-3,4,6-tri-O-benzyl-1-thio-D-mannopyranoside (α/β 0.25:1, 1.86 g, 3.27 mmol) was dissolved in MeOH (20 mL). A sodium methoxide solution (25 wt % in MeOH) was added until a pH-value of approximately 10 was reached. The reaction mixture was stirred under a nitrogen atmosphere overnight. The crude 3,4,6-tri-*O*-benzyl thioglycoside was subjected to flash column chromatography (10%→20% EtOAc in pentane) yielding 1.46 g of the desired α-thioglycoside (2.77 mmol, 85%) as a clear oil.

R_f 0.29 (20% EtOAc in pentane). **^{1}H-NMR (400 MHz, $CDCl_3$)**: δ_H (ppm) 7.63 – 7.50 (m, 2H, Ar-H), 7.50 – 7.24 (m, 18H, Ar-H), 5,72 (d, $J_{1,2}$ 1.6 Hz, 1H, H-1), 4.94 (d, J_{gem} 10.8 Hz, 1H, OC*H*$_2$Ph), 4.83- 4.73 (m, 2H, OC*H*$_2$Ph), 4.70 (d, 1H, J_{gem} 10.8 Hz OC*H*$_2$Ph), 4.60 (d, J_{gem} 12.0 Hz, 1H, OC*H*$_2$Ph), 4.52 (d, J_{gem} 12 Hz, 1H, OC*H*$_2$Ph), 4.41 (ddd, $J_{5,4}$ 9.5 Hz, $J_{5,6a}$ 4.5 Hz, $J_{5,6b}$ 2.0 Hz, 1H, H-5), 4.33 (dd, $J_{2,3}$ 3.1 Hz, $J_{2,1}$ 1.6 Hz, 1H, H-2), 4.06 (t, *J* 9.5 Hz, 1H, H-4), 3.96 (dd, $J_{3,4}$ 9.5 Hz , $J_{3,2}$ 3.1 Hz, 1H, H-3), 3.90 (dd, $J_{6a,6b}$ 10.8 Hz, $J_{6a,5}$ 4.5 Hz, 1H, H-6a), 3.76 (dd, $J_{6a,\,6b}$ 10.8 Hz, $J_{6b,5}$ 2.0 Hz, 1H, H-6b), 3.12 (s, 1H, OH); **^{13}C-NMR (100 MHz, $CDCl_3$)**: δ_C (ppm) 138.2, 137.6, 133.8, 131.6 (4x Ar C-C), 129.1-127.2,(Ar C-H), 87.3 (C1), 80.3 (C3), 75.2 (O*C*H$_2$Ph), 74.4 (C4), 73.4 (O*C*H$_2$Ph) , 72.2(C5), 72.1 (O*C*H$_2$Ph), 69.9 (C2), 68.8 (C6). **HRMS(ES+)**: $C_{33}H_{34}NaO_5S$+ calcd m/z 565.2025; found m/z 565.2024.

The NMR-data are in accordance with those previously reported in literature[72].

5.4 General procedure for direct glycosylation using $Bi(OTf)_3$ as catalyst with various donors



2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose donor (1 equiv) and benzyl alcohol (3 equiv) were dissolved in dry CH_2Cl_2 to a donor concentration of 0.20 M. Lewis acid $Bi(OTf)_3$ (15 mol % with respect to donor) was added and the reaction was stirred at 40 °C. The reaction was monitored by TLC analysis until full conversion of donor. After reaction completion, the crude mixture was directly purified by flash column chromatography (CH_2Cl_2-EtOAc 5:1 →1:1), which afforded the desired glycoside product

(11). For glycosylation with Donor ***B*** and ***D*** further purification was needed, by washing the product with saturated $NaHCO_3$ aq.

5.4.1 Synthesis of benzyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside *(11)* from Donor *A*

The general glycosylation procedure was followed using Donor ***A*** (200 mg, 0.514 mmol), $Bi(OTf)_3$ (50.6 mg, 0.0771 mmol), and benzyl alcohol (160 µL, 1.54 mmol). TLC analysis indicated reaction cessation after 2 h. Flash column chromatography afforded the desired product ***(11)*** (0.180 mg, 0.411 mmol, 80%) as a white solid.

R_f 0.58 (EtOAc). $[\alpha]_D^{298K}$ -61.2 (*c*1, $CHCl_3$). Mp 161.9-162.8°C (CH_2Cl_2). **^{1}H-NMR (400 MHz, $CDCl_3$)**: δ_H (ppm)7.33-7.21 (m, 5H, ArH), 5.53 (d, $J_{2,NH}$ 8.9 Hz, 1H, NH), 5.17 (t, $J_{3,4}$ 9.8 Hz, 1H, H-3), 5.05 (t, $J_{4,5}$ 9.6 Hz, 1H, H-4), , 4.85 (d, J_{gem} 12.2 Hz, 1H, OC*H*HPh), 4.63 (d, $J_{1,2}$ 7.95 Hz, 1H, H-1), 4.56 (d, 1H, OCH*H*Ph), 4.23 (dd, $J_{6a,6b}$ 12.4 Hz, $J_{5,6a}$ 4.6 Hz, 1H, H-6a), 4.12 (dd, $J_{6b,6a}$ 12.4 Hz, $J_{5,6b}$ 2.6 Hz, 1H, H-6b), 3.94 (m, 1H, H-2), 3.64 (ddd, 1H, H-5), 2.06 (s, 3H, C(O)C*H*$_3$), 1.97 (s, 6H, C(O)C*H*$_3$), 1.86 (s,3H, NHC(O)C*H*$_3$). **^{13}C-NMR (100 MHz, $CDCl_3$)**: δ_C (ppm) 170.9, 170.8, 170.2 (3xC=O), 169.4 (NHC=O), 136.9 (*ipso*-Ar*C*),128.6-127.8 (ArC), 99.5 (C1), 72.4 (C3), 71.8 (C5), 70.7 (O*C*H_2Ph), 68.7 (C4), 62.2 (C6), 54.4 (C2), 23.3 (NHC(O)*C*H_3), 20.9-20.6 (OC(O)*C*H_3). **HRMS(ES+)**: $C_{21}H_{27}NO_9Na$+ calcd m/z 460.1584; found m/z 460.1587.

5.4.2 Synthesis of benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside *(11)* from Donor *B*

The general glycosylation procedure was followed using Donor ***B*** (200 mg, 0.443 mmol), $Bi(OTf)_3$ (43.6 mg, 0.0664 mmol) and benzyl alcohol (138 µL, 1.33 mmol). TLC analysis indicated reaction cessation after 5 h. The crude mixture was purified with column chromatography following the general procedure, then washed with satd $NaHCO_3$ (aq) and extracted with CH_2Cl_2.The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure, to afford the desired glycoside product (as a mixture of anomers (α 3.85%mol), 0.159 mg, 0.363 mmol, 82% yield).

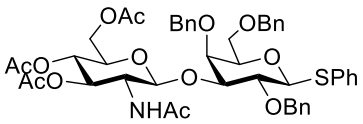
5.4.3 Synthesis of benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside *(11)* from Donor *C*

The general glycosylation procedure was followed using Donor ***C*** (200 mg, 0.464 mmol), $Bi(OTf)_3$ (45.7 mg, 0.0696 mmol), and benzyl alcohol (145 µL, 1.39 mmol). After 12 hours TLC analysis indicated reaction completion. The crude mixture was purified with column chromatography following the general procedure, then washed with sat. $NaHCO_3$ aq and extracted with CH_2Cl_2.The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure, to afford the glycoside product as a white solid (0.348 mmol, 152 mg, 75% yield).

5.4.4 Synthesis of benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside *(11)* from Donor *D*

The general glycosylation procedure was followed using Donor ***B*** (44.4 mg, 0.092 mmol), $Bi(OTf)_3$ (9.08 mg, 0.0138 mmol), and benzyl alcohol (28.8 µL, 0.277 mmol). TLC analysis indicated reaction cessation after 5 h. The crude mixture was purified with column chromatography following the general procedure, then washed with satd $NaHCO_3$ (aq) and extracted with CH_2Cl_2.The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure, to afford the desired glycoside product as a white solid (0.0718 mmol, 31.4 mg 78% yield)

5.5 General procedure for direct glycosylation using $Yb(OTf)_3$ as catalyst with various donors



2-Acetamido-2-deoxy-β-D-glucopyranose donor (2 equiv) and acceptor (1 equiv) were dissolved in dry CH_2Cl_2 to a donor concentration of 0.15 M. Lewis acid $Yb(OTf)_3$ (2.5 or 5 mol % with respect to donor) was added and the reaction was stirred at 80 °C for 48h. The crude mixture was directly purified using flash column chromatography (5% →15%→20% EtOAc in CH_2Cl_2), which afforded the desired glycoside products. The unreacted acceptor was re-isolated for every glycosylation reaction by flash column chromatography. For glycosylations with Donor ***B***, and ***D*** further purification was needed:

the residue was washed with saturated $NaHCO_3$ aq and extracted with CH_2Cl_2, dried on $MgSO_4$, filtered and concentrated in *vacuo*.

(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside *(12)*: ***R*$_f$** 0.62 (EtOAc-pentane 2:1). $[\alpha]_D^{298K}$ -14.6 (*c*1, $CHCl_3$). Mp 152.8-154.1°C (CH_2Cl_2). δ_H (ppm) 7.63 – 7.56 (m, 2H, Ar-H), 7.51 – 7.31 (m, 15H, Ar-H), 7.29 – 7.21 (m, 3H, Ar-H), 5.18 – 4.99 (m, 5H, H-3, H-5', OC*H*$_2$Ph), 4.91 (d, $J_{1,2}$ 8.3 Hz, 1H, H-1), 4.69 (d, $J_{1',2'}$ 9.5 Hz, 1H, H-1'), 4.63 (dd, 2H, H-6a', H-6b'), 4.56 – 4.43 (q, 2H, OC*H*$_2$Ph), 4.30 (dd, $J_{6a,6b}$ 12.3, $J_{6a,5}$ 5.2 Hz, 1H, H-6a), 4.22 (dd, $J_{6b,5}$ 2.5 Hz, 1H, H-6b), 4.15 – 4.05 (m, 2H, H-2, H-4'), 3.97 (t, $J_{2',1'=2',3'}$ 9.5 Hz, 1H, H-2'), 3.86 (dd, $J_{3',2'}$ 9.4 Hz, $J_{3',4'}$ 2.8 Hz, 1H, H-3'), 3.73-3.60 (m, 4H, H-5, OC*H*$_2$Ph), 2.12 – 2.02 (m, 9H, C(O)C*H*$_3$), 1.59 (s, 3H, NHC(O)C*H*$_3$).

^{13}C-NMR (100 MHz, $CDCl_3$): δ_C (ppm)170.9, 170.6, 169.9, 169.4 (C=O), 138.8, 138.7, 137.9, 134.1 (Aromatic C-C), 131.2-126.7(Aromatic C-H), 101.9 (C1), 87.7 (C1'), 83.7 (C3'), 77.5, 77.3 (C3'), 74.9 (C6'), 74.5, 73.5 (2xO*C*H$_2$Ph), 72.7, 71.8, 68.9 (O*C*H$_2$Ph), 68.4, 62.1 (C6), 54.6, 22.9(NHC(O)CH3), 20.7-20.6 (OC(O)*C*H$_3$). **HRMS(ES+)**: Calcd. for $C_{47}H_{53}NNaO_{13}S^+$ m/z 894.314; found m/z 894.314.

6 Bibliography

(1) Werz, D. B.; Ranzinger, R.; Herget, S.; Adibekian, A.; von der Lieth, C.-W.; Seeberger, P. H. *ACS Chem. Biol.* **2007**, *2* (10), 685–691.

(2) Taylor, M. E.; Drickamer, K. *Introduction to glycobiology*, 3rd ed.; Oxford University Press, **2011**.

(3) Berg, J. M. Jeremy M.; Tymoczko, J. L.; Stryer, L.; Stryer, L. *Biochemistry*, 5th ed.; W.H. Freeman, **2002**.

(4) Ernst, B.; Magnani, J. L. *Nat. Rev. Drug Discov.* **2009**, *8* (8), 661–677.

(5) Turnbull, J. E. *Science (80-.).* **2011**, *334* (6055), 462–463.

(6) Caruthers, M. H. *Science* **1985**, *230* (4723), 281–285.

(7) Grant, G. A.; Crankshaw, M. W.; Gorka, J. 1997; pp 395–419.

(8) Geyer, H.; Geyer, R. *Biochim. Biophys. Acta - Proteins Proteomics* **2006**, *1764* (12), 1853–1869.

(9) Plante, O. J. *Science (80-.).* **2001**, *291* (5508), 1523–1527.

(10) Wang, Z.; Chinoy, Z. S.; Ambre, S. G.; Peng, W.; McBride, R.; de Vries, R. P.; Glushka, J.; Paulson, J. C.; Boons, G.-J. *Science* **2013**, *341* (6144), 379–383.

(11) Thomas J. Boltje, Therese Buskas, G.-J. B. *Nat Chem* **2009**, *1* (8), 611–622.

(12) Wang, L. X.; Davis, B. G. *Chem. Sci.* **2013**, *4* (2012), 3381–3394.

(13) Seeberger, P. H.; Werz, D. B. *Nature* **2007**, *446* (7139), 1046–1051.

(14) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683–720.

(15) Bongat, A. F. G.; Demchenko, A. V. *Carbohydr. Res.* **2007**, *342* (3–4), 374–406.

(16) Liao, L.; Auzanneau, F. I. *J. Org. Chem.* **2005**, *70* (16), 6265–6273.

(17) Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2001**, *123* (28), 6819–6825.

(18) Bode, L. *Glycobiology* **2012**, *22* (9), 1147–1162.

(19) Zivkovic, A. M.; German, J. B.; Lebrilla, C. B.; Mills, D. A.; Klaenhammer, T. R. *Pnas* **2011**, *108*, 4653–4658.

(20) Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, Trikalinos T, L. J. *Breastfeeding and Maternal and Infant Health Outcomes In Developed Countries*; **2007**.

(21) Bode, L. *Nutr. Rev.* **2009**, *67* (SUPPL. 2), 183–191.

(22) Newburg, D. S.; Grave, G. *Pediatr. Res.* **2014**, *75* (5), 675–679.

(23) Bode, L.; Contractor, N.; Barile, D.; Pohl, N.; Prudden, A. R.; Boons, G. J.; Jin, Y. S.; Jennewein, S. *Nutr. Rev.* **2016**, *74* (10), 635–644.

(24) Kobata, A. *Proc. Jpn. Acad. Ser. B. Phys. Biol. Sci.* **2010**, *86* (7), 731–747.

(25) Urashima, T.; Saito, T.; Nakamura, T.; Messer, M. *Glycoconj. J.* **2001**, *18*, 357–371.

(26) Nyffenegger, C.; Nordvang, R. T.; Zeuner, B.; Łężyk, M.; Difilippo, E.; Logtenberg, M. J.; Schols, H. A.; Meyer, A. S.; Mikkelsen, J. D. *Appl. Microbiol. Biotechnol.* **2015**, *99* (19), 7997–8009.

(27) Bridiau, N.; Issaoui, N.; Maugard, T. *Biotechnol. Prog.* **2010**, *26* (5), 1278–1289.

(28) Boons, G.-J.; Hale, K. *Organic synthesis with carbohydrates*; Wiley, Ed.; Sheffield Academic Press, **2000**.

(29) Demchenko, A. V. *Handbook of chemical glycosylation : advances in stereoselectivity and therapeutic relevance*; Wiley, **2008**.

(30) Koenigs, W.; Knorr, E. *Berichte der Dtsch. Chem. Gesellschaft* **1901**, *34* (1), 957–981.

(31) Kristensen, S. K.; Salamone, S.; Rasmussen, M. R.; Marqvorsen, M. H. S.; Jensen, H. H. *European J. Org. Chem.* **2016**, *2016* (32), 5365–5376.

(32) Zhu, X.; Schmidt, R. R. *Angew. Chemie - Int. Ed.* **2009**, *48* (11), 1900–1934.

(33) Mydock, L. K.; Demchenko, A. V. *Org. Biomol. Chem.* **2010**, *8* (3), 497–510.

(34) Amyes, T. L.; Jencks, W. P. *J. Am. Chem. Soc.* **1989**, *111* (20), 7888–7900.

(35) Nukada, T.; Berces, A.; Zgierski, M. Z.; Whitfield, D. M. **1998**.

(36) Bérces, A.; Enright, G.; Nukada, T.; Whitfield, D. M. *J. Am. Chem. Soc.* **2001**, *123* (23), 5460–5464.

(37) Srivastava, V. K. *Carbohydr. Res.* **1982**, *103* (2), 286–292.

(38) Pertel, S. S.; Kononov, L. O.; Zinin, A. I.; Chirva, V. J.; Kakayan, E. S. *Carbohydr. Res.* **2012**, *356*, 172–179.

(39) Sinnott, M. *Carbohydrate chemistry and biochemistry : structure and mechanism*; RSC Publishing, **2007**.

(40) Marqvorsen, M. H. S.; Pedersen, M. J.; Rasmussen, M. R.; Kristensen, S. K.; Dahl-Lassen, R.; Jensen, H. H. *J. Org. Chem.* **2017**, *82* (1), 143–156.

(41) Krag, J.; Christiansen, M. S.; Petersen, J. G.; Jensen, H. H. *Carbohydr. Res.* **2010**, *345* (7), 872–879.

(42) Banoub, J.; Boullanger, P.; Lafont, D. *Chem. Rev.* **1992**, *92*, 1167–1195.

(43) Arihara, R.; Nakamura, S.; Hashimoto, S. *Angew. Chemie - Int. Ed.* **2005**, *44* (15), 2245–2249.

(44) Tsuda, T.; Nakamura, S.; Hashimoto, S. *Tetrahedron* **2004**, *60* (47), 10711–10737.

(45) Ellervik, U.; Magnusson, G. *Carbohydr. Res.* **1996**, *280* (2), 251–260.

(46) R. U. Lamieux , T. Takeda, B. Y. C. *Synthetic Methods for Carbohydrates*; El Khadem, H. S., Ed.; ACS Symposium Series; American Chemical Society:,**1977**; Vol. 39.

(47) Unverzagt, C. *Angew. Chemie Int. Ed. English* **1994**, *33* (10), 1102–1104.

(48) Dan, A.; Lergenmüller, M.; Amano, M.; Nakahara, Y.; Ogawa, T.; Ito, Y. *Chem. - A Eur. J.* **1998**, *4* (11), 2182–2190.

(49) Micheel, F.; Kamp, F.-P. Van De; Wulff, H. *Chem. Ber.* **1955**, *88* (12), 2011–2019.

(50) Leaback, D.; Walker, P. *J. Chem. Soc.* **1957**, No. 4754, 4754–4760.

(51) Inouye, Y.; Onodera, K.; Kitaoka, S.; Ochiai, H. *J. Am. Chem. Soc.* **1957**, No. 79, 4218.

(52) Sarkar, A. K.; Brown, J. R.; Esko, J. D. *Carbohydr. Res.* **2000**, *329* (2), 287–300.

(53) Carvalho, I.; Scheuerl, S. L.; Ravindranathan Kartha, K. .; Field, R. A. *Carbohydr. Res.* **2003**, *338* (10), 1039–1043.

(54) Stöckl, W. P.; Weidmann, H. *J. Carbohydr. Chem.* **1989**, *8* (2), 169–198.

(55) Kiso, M.; Anderson, L. *Carbohydr. Res.* **1979**, *72*, 12–14.

(56) Osawa, T. *Chem. Pharm. Bull. (Tokyo).* **1960**, *8* (7), 597–610.

(57) Wittmann, V.; Lennartz, D. *European J. Org. Chem.* **2002**, *2002* (8), 1363.

(58) Stévenin, A.; Boyer, F. D.; Beau, J. M. *European J. Org. Chem.* **2012**, *1* (9), 1699–1702.

(59) Crasto, C. F.; Jones, G. B. *Tetrahedron Lett.* **2004**, *45* (25), 4891–4894.

(60) Matta, K. L.; Bahl, O. P. *Carbohydr. Res.* **1972**, *21* (3), 460–464.

(61) Mandai, S.; Sharma, N.; Mukhopadhyay, B. *Synlett* **2009**, No. 19, 3111–3114.

(62) Sowell, C. G.; Livesay, M. T.; Johnson, D. A. *Tetrahedron Lett.* **1996**, *37* (5), 609–610.

(63) Christensen, H.; Christiansen, M. S.; Petersen, J.; Jensen, H. H. *Org. Biomol. Chem.* **2008**, *6* (18), 3276–3283.

(64) Rasmussen, M. R.; Marqvorsen, M. H. S.; Kristensen, S. K.; Jensen, H. H. *J. Org. Chem.* **2014**, *79* (22), 11011–11019.

(65) Marqvorsen, M. *Improved Methodology for the Direct Chemical Glycosylation with 2-Acetamido-2-deoxy-glycopyranosyl Donors And Iminosugars from L-Rhamnose for Biological Testing*, Aarhus University, **2016**.

(66) Pukin, A.; Zuilhof, H. *Synlett* **2009**, *2009* (20), 3267–3270.

(67) Haddoub, R.; Laurent, N.; Meloni, M.; Flitsch, S. *Synlett* **2009**, *2009* (20), 3328–3332.

(68) Macdonald, J. M.; Tarling, C. A.; Taylor, E. J.; Dennis, R. J.; Myers, D. S.; Knapp, S.; Davies, G. J.; Withers, S. G. *Angew. Chemie - Int. Ed.* **2010**, *49* (14), 2599–2602.

(69) Fukuda, M.; Hindsgaul, O. *Molecular and cellular glycobiology*; Oxford University Press, **2000**.

(70) Wang, Z.; Zhou, L.; El-Boubbou, K.; Ye, X.-S.; Huang, X. *J. Org. Chem.* **2007**, *72* (17), 6409–6420.

(71) Jäger, M.; Minnaard, A. J. *Chem. Commun.* **2016**, *52* (4), 656–664.

(72) Sanapala, S. R.; Kulkarni, S. S. *Chem. - A Eur. J.* **2014**, *20* (13), 3578–3583.