# SYNTHESIS TOWARDS AN AROMATIC α-DIXYLOSIDE

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## Abstract

A proteoglycan is a protein with at least one glycosidic chain or glycosaminoglycan (GAG) covalently bound to it. Since a common linker between the core protein and the GAG chain has a xylose present, the biosynthesis of GAGs can be primed using xylosides containing hydrophobic aglycons providing that these xylosides are capable of penetrating the cell membrane.

2-naphthyl β-D-xylopyranoside primes synthesis of chondroitin sulfate/dermatan sulfate and heparan sulfate/heparin as showed by Mani et. al. and in addition 2-(6-hydroxynaphthyl) β-D-xylopyranoside also shows selective antiproliferative effect between normal cells and cancer cells. Also Bakker et. al recently showed that oligosaccharides containing dixylopyranosides linked α1-3 show biological activity in mammalian cells. These findings gave the idea that α–linked aromatic dixylopyranosides might prime GAG synthesis, if their polarity allows them to penetrate the cell membrane, and show antiproliferative effect.

In this report, a synthetic pathway towards an aromatic dixyloside 2-naphthyl α-D-xylopyranoside-(1-4)-1-O-α-D-xylopyranoside was attempted using α-selective O-glycosylation with thioxylosides on aromatic xylosides with suitable protective groups. An aromatic α-dixyloside was indeed produced in somewhat low yields, but the last deprotection step in the synthetic pathway tended to break the glycosidic bond between the xylosides.

## Abbreviations

Aglycon - A non-carbohydrate bound to a sugar (figure 2.5)

Anomer - Differs only in the conformation of the anomeric carbon.

BDA - Butane trans-diacetal (figure 2.4)

Epimer - Differs in one conformation of a stereocenter.

$Et_3N$ - Triethylamine (base)

EtOAc - Ethylacetate (polar solvent)

FGI - Functional Group Interconvertion

GAG - Glycosaminoglycan (figure 1.5)

Gal - Galactose

GalNAc - 2-deoxy-2-acetamido-α-D-galactopyranose

Glc - Glucose

GlcA - β-L-glucoronic acid

GlcNAc - 2-deoxy-2-acetamido-α-D-glucopyranose

Glycoside - A sugar bound to a non-carbohydrate (figure 2.5)

IdoA - α-L-iduronic acid

Isomer - Same molecular formula, different structural formula.

LDA- Lithium Diisopropyl Amine (strong, bulky base)

NaH - Sodium hydride (small, strong base)

PG - Protective group

$Ph_3CNa$ - Triphenylmethylsodium (very strong, bulky base)

t-BuOK - Potassium *tert*-butoxide (strong, bulky base)

TMB - 2,2,3,3-Tetramethoxybutane (figure 2.4)

Xyl - Xylose

XylNapOH - 2-(6-hydroxynaphthyl) β-D-xylopyranoside (**5**, figure 5.1)

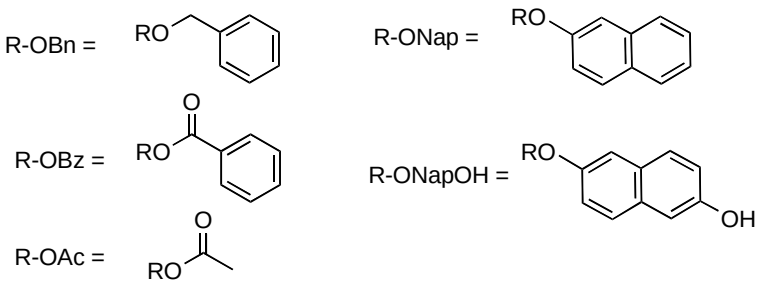


**Figure 0.1.** Common abbreviation used in this report.

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# 1 Carbohydrates

Carbohydrates, or saccharides, are one of the largest natural compound classes but to the average person the word carbohydrate may just be a synonym for semi-unhealthy ingredient in foodstuff that increases ones waistline should be avoided. Carbohydrates were long only considered as an energy component for the cell, but as cellular biology was explored, leading to more and more discoveries and advancements, it stood clear that the role of carbohydrates in biological systems is much more than just a simple energy storage. [1] Carbohydrates have the general formula $C_x(H_2O)_y$ with variations. In nature, they can be substituted with acyl, alkyl, phosphate, sulphate and other functional groups. [2] In mammalian organisms the carbohydrates are found in nearly all cellmembranes of living tissue. [1, 3] The immense diversity of the carbohydrates make them ideal as signaling substances and they play a vital role in biological recognition, bacterial and viral infection, fertility, among many other applications and processes. [1, 3, 4]

The chemistry of the carbohydrates was first extensively investigated in 1884 by the German chemist Hermann Emil Fischer (1852-1919) who among many things established the stereochemistry and isomerism of the common saccharides and also, starting from glycerol, synthesized the monosaccharides glucose, fructose and mannose. Fischer became a Nobel Prize winner in 1902 for his research on carbohydrates and purine. [1, 5]

## 1.1 Monosaccharides

The simplest of the carbohydrates are the monosaccharides which are the building blocks of the larger and more complex di-, oligo- and polysaccharides. In nature, a few hundred monosaccharides have been accounted for, of which approximately ten are found in the human body. [6] The simple monosaccharides are essential for the body as, for example, glucose is the fuel in the energy metabolism of the cell and the backbone of DNA and RNA is deoxyribose and ribose, respectively, linked with phosphate.

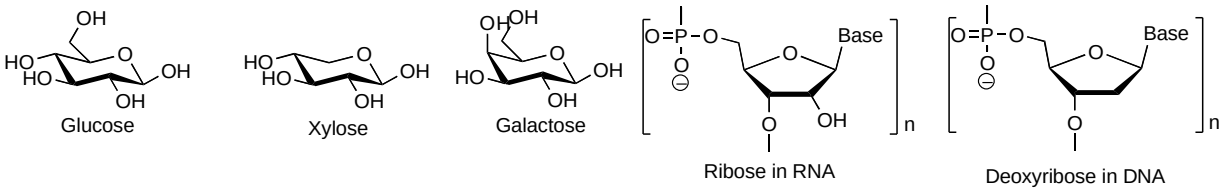



**Figure 1.1.** Examples of different monosaccharides.

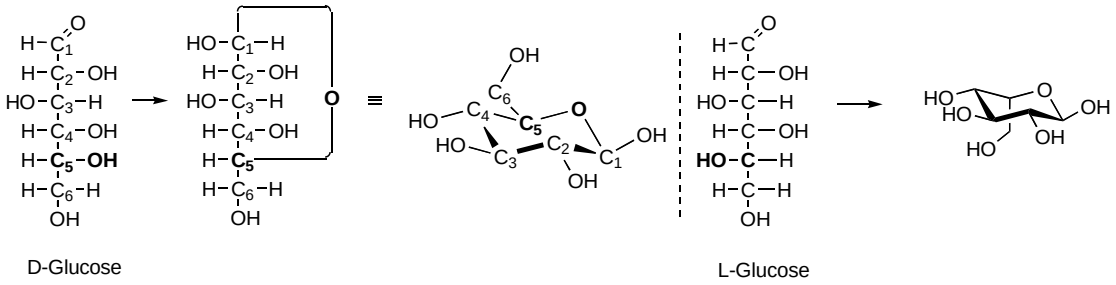



**Figure 1.2.** Numbering of carbons in a Fischer projection of glucose as well as D- and L-configuration.

A monosaccharide is identified by its number of carbons and the chiral configuration of these carbons. A five-carbon monosaccharide is called pentose, and a six-carbon monosaccharide is called hexose etc. The carbons in the monosaccharide are numbered $C_1$, $C_2$, and so on (figure 1.2). Furthermore, the hydroxyls are identified to as 2-OH, 3-OH etc. with reference to the carbon it is bound to. A monosaccharide is assigned D (lat. *Dexter* = right) or L (lat. *Laevus* = left) depending on the configuration of the highest numbered chiral carbon, also called the configurational carbon. In xylose, $C_4$ is the configurational carbon while in glucose it is $C_5$. If the hydroxyl is projected to the right in a Fischer projection, the sugar is assigned D whereas if projected to the left, the saccharide would be assigned L. [7]

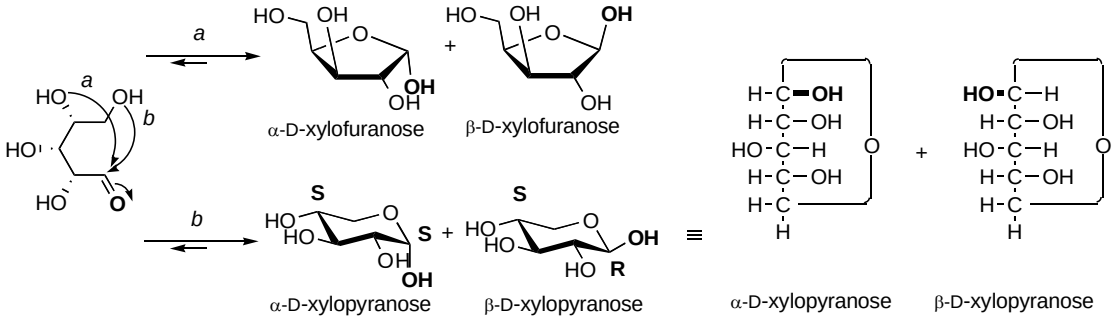



**Figure 1.3.** Ring closure of xylose via a nucleophilic attack on the carbonyl carbon $C_1$ by the oxygen positioned on $C_4$ (*a*) or $C_5$ (*b*) with formation of stereogenic center at $C_1$ (anomeric carbon).

In aqueous solution, the monosaccharaide is in equilibrium between open form and cyclic form, since the open monosaccharide can react with itself to form a cyclic hemiacetal (Figure 1.3). [1] The ring size will vary depending on which hydroxyl that performs the nucleophilic attack on the carbonyl carbon. Any of the hydroxyls on the chain may perform the ringclosure, however the two most stable cyclic forms are the six-membered pyranose and the five-membered furanose. Most preferred of these two cyclic forms is the pyranose due to the low conformational energy achieved in the so called chair conformation. [7] Closing of the ring make $C_1$ a stereogenic center, also called the anomeric center. The two conformations it can achieve are assigned α or β, depending on the

stereochemical configuration compared between the anomeric carbon and the conformational carbon. The α-anomer has opposite stereochemistry at the two carbons (S and R) and in the β-anomer both carbons share the stereochemical configuration (both R or S). In a Fischer projection the α-anomer has the hydroxyl group on the same side (*cis*) as the ring while the β-anomeric hydroxyl group is on the opposing side (*trans*) in comparison to the oxygen that has formed the ring.[7] Because of the equilibrium, a mixture of α- and β-D-xylose will be observed overtime in a solution of pure α-D-xylose. The process is catalyzed by weak acid or base and the phenomenon is called mutarotation.[7] The monosaccharide can be locked cyclic form by a Fischer glycosylation which is later described in section 2.1.1. [1]

The reactivity, or nucleophilicity, of the hydroxyl groups on a saccharide varies depending on what type of reaction it is and on which position the hydroxyl have on the saccharide. The most reactive hydroxyl group is found on $C_1$, due to the ability of a hemiacetal to react and form a more stable acetal. The second most reactive hydroxyl is a primary hydroxyl due to the lack of stabilizing electron donating groups, such as the hydroxyl on $C_6$ in glucose. The reactivity of the remaining secondary hydroxyls generally varies as the distance to the anomeric carbon increase, however, if the reactivity increases or decreases is dependent on the nature of the reaction as a combination of electron density, steric hindrance, etc. [1, 8]

## 1.2 Di-, Oligo-, and Polysaccharides

As mentioned before, the monosaccharides are the building blocks of larger saccharides. When a saccharide consists of two to ten monosaccharides it is called an oligosaccharide. Depending on how many saccharide units the oligosaccharide consists of it is referred to as a di-, tri-, tetra-, pentasaccharide and so on. [7] One of the most commonly known carbohydrate is the disaccharide, consisting of glucose and fructose (Figure 1.4), also recognized as sucrose or table sugar which can be found in close to every kitchen. Another common disaccharide is maltose, a product from enzymatic breakdown of starch, which is an important ingredient in the brewing of making beer.

When studying the linking possibilities of oligosaccharides, the diversity of the carbohydrates really begin to show. This can be explained by the many axial or equatorial hydroxyl groups of the monosaccharide, which are offering numerous possibilities. Assuming that a linear oligosaccharide may be constructed of up to five different D-pyranosides, the number of isomers of this pentasaccharide becomes just about 31 million. If the monosaccharides within the linear pentasaccharide can be reoccurring, the number of isomers becomes just about 819 million. If the pentasaccharide would not be linear but instead branched, the number of isomers becomes 2633 million. [2]

In polysaccharides the number of fused monosaccharides exceeds ten, and they often have a molecular weight which may exceed $10^8$ g·mol$^{-1}$. An excellent example of polysaccharide and carbohydrate diversity is when D-glucose is polymerized to form the base of four important polysaccharides; cellulose, chitin, starch and glycogen (Figure 1.4). The first two, cellulose and chitin, are very important structural components of living organisms. In the flora, linear repeats of β-D-glucose gives cellulose, whereas in the fauna, linear repeats of N-acetylated β-D-glucose gives the structural component chitin, which is found in the shells and exoskeletons of shellfish and insects. The latter two, starch and glycogen, function as energy storages. Starch is a lightly branched polysaccharide made of repeats of α-D-glucose and it acts as energy storage for plants. The animal equivalent is glycogen, a more branched polysaccharide with the protein glucogenin forming a core. The strong interaction between the β-D-glucoses in the cellulose chain is the reason why humans cannot digest and obtain the energy stored in plants like grass eaters can due to an enzyme in their GI-tract which we lack. However, the energy stored in starch we can account for since the linkage between the monosaccharides is different resulting in less interaction between the monosaccharides in the chain.

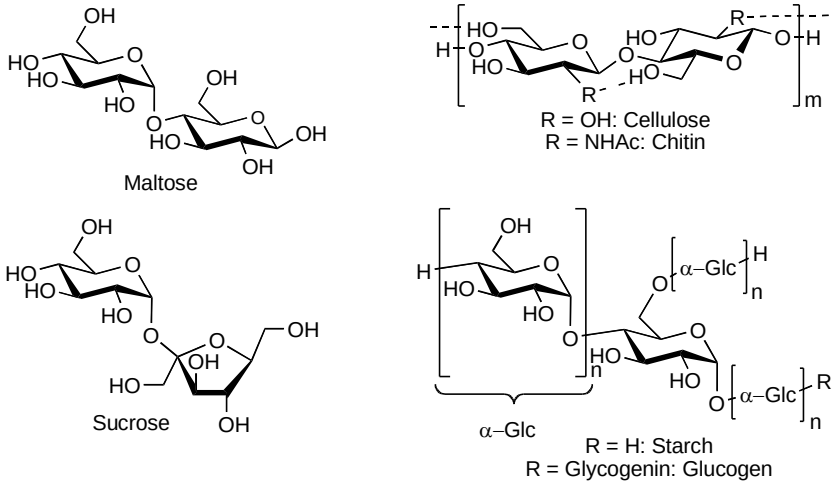



**Figure 1.4.** Examples of different oligosaccharides.

## 1.3 Proteoglycans, Glycosaminoglycans and Xylose

A Proteoglycan is a protein with at least one saccharide, or glycosaminoglycan (GAG), covalently bound to it, like the previously described glucogenin in section 1.2. The protein forms a core while the GAG chains are pointing outwards from it (figure 1.5). Proteoglycans are mostly found in the extracellular matrix and they are involved in regulation of cell-cell adhesion, cell growth, providing rigidness for the internal organs, and so on. They are also found in the smooth regions in the skeletal joints as a structural component and lubricant. [9, 10, 11]

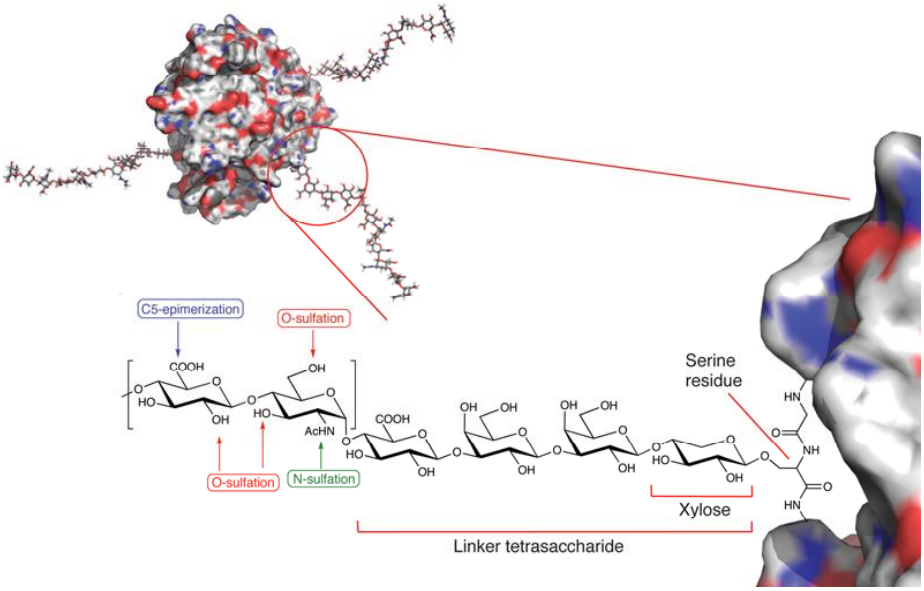



**Figure 1.5.** Proteoglycans and the common tetrasaccharide linker in some glycosaminoglycans [12]

GAGs are unbranched polysaccharides of repeating disaccharide units, which may be sulfated or N-acetylated. There are four different classes of GAG-chains; Heparan sulfate/heparin (HS), chondroitin sulfate/dermatan sulfate (CS/DS), hyaluronic acid and keratan sulfate. The difference between these GAGs is the repeating disaccharide unit of the polysaccharide, but they share a common tetrasaccharide which acts as a linker between the GAG chain and the protein, except hyaluronic acid which does not have a protein core and keratan sulfate which have a different linker.

The biosynthesis of GAGs starts when the hydroxyl on a serine residue in the proteoglycan core protein is xylosylated by xylotransferases XT-I or XT-II in the Golgi apparatus membrane. Three more monosaccharides are added to form the tetrasaccharide GlcA-β1–3-Gal-β1–3-Gal-β1–4-Xyl-β1–O-Ser. The following two monosaccharides added form a repeating disaccharide, which determine the identity of the GAG-chain, followed by selective N- or O-sulfation, acetylation, deacetylation of the GlcNAc monosaccharides as well as epimerization of $C_5$ turning certain GlcA into IdoA. For HS the repeating unit is [–4-GlcA-β1–4-GlcNAc-α1–] and for CS/DS it is [–4-GlcA-β1–3-GalNAc-β1–]. When the synthesis is complete, the proteoglycan is transported to extra cellular matrix of the cell via secretory vesicles. [9, 10, 11]

## 1.4 Xylosides as Primers of Glycosaminoglycan Synthesis

In mammalian cells, the monosaccharide xylose is currently only known to appear in two positions: It is found as the primary monosaccharide in the previously described tetrasaccharide linker between the core protein and the GAG chain in a proteoglycans and it is also found in the oligosaccharide Xyl-α1–3-Xyl-α1–3-Glc-β1–O-Ser, which is found on proteins involved in blood coagulation processes and Notch signaling. [9, 13]

Priming of GAG synthesis was investigated in 1968 by Helting and Rodén with various xylose derivatives, such as Xyl-β1–O-Ser and Gal-β1–4-Xyl-β1–O-Ser, which in their studies initiated addition of monosaccharides. Further investigations by Okayama et al. in 1973 with a *p*-nitrophenyl β-D-xylopyranoside followed by others investigations of GAG priming with various xylopyranosides. [10, 14] It was shown that β-D-xylosides containing hydrophobic aglycons can penetrate cell membranes and prime GAG biosynthesis, for example 2-naphthyl β-D-xylopyranoside (XylNap) primes HS synthesis. [15] In the 1990's, Mani et al. tested 2-(6-hydroxynaphthyl)-β-D-xylopyranoside (XylNapOH) for priming of GAG synthesis. In both *in vitro* and *in vivo* studies Mani showed that XylNapOH primed both HS and CS/DS biosynthesis and inhibited cell growth in several cell types. Furthermore, *in vivo* investigations showed reduction of tumor load in SCID mice by 70-97% with XylNapOH. [16] Recent *in vitro* investigations showed that XylNapOH primes GAG biosynthesis in both normal and cancer cells and the GAG chain are secreted into the extracellular matrix. The GAG chain originating from a cancer cell can enter both cancer cells and normal cells and induce an antiproliferative effect in the nuclei of the cell, followed by apoptosis. It was also shown that XylNapOH lowers level of histone H3 acetylation selectivity in bladder and breast carcinoma cells without affecting the expression of histone H3. This causes the DNA-string attached to the histone to not unwind, making transcription impossible and forces the cell into apoptosis. In contrast, the XylNapOH-primed GAG chains originating from normal cells do not enter neither cancer nor normal cells and neither does XylNap-primed GAG chains. [17]

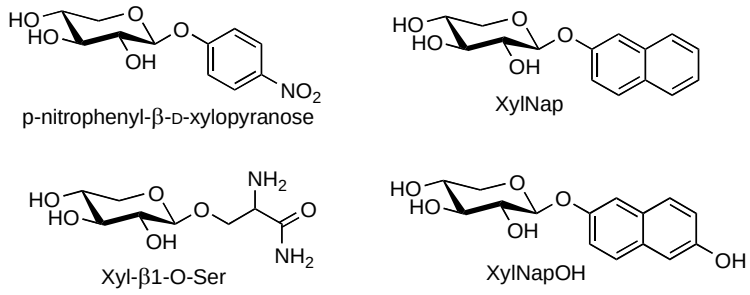



**Figure 1.6.** Molecules which primes GAG-synthesis.

# 2 Carbohydrate synthesis

Carbohydrate chemistry was founded by Fischer in the late 1800's and had a relatively late breakthrough compared to other fields in chemistry but has lately grown into quite a research field rapidly, mainly due to the rather unique biological applications. [1, 2, 3] Two important fields in carbohydrate chemistry are developing and investigations of protective group (PG) chemistry and glycosylation methods.

## 2.1 Protective Groups

The most important part of the synthetic path of larger saccharides is the protection and deprotection of functional groups, hydroxyls in general, which otherwise may interfere and cause undesirable side-reactions. When planning a carbohydrate synthesis and choosing between protective groups, several parameters must be considered such as which functional group that needs be protected, if there are any functional groups that should not be protected, what shall the functional group be protected from (acid, base, nucleophiles, etc.), their reactivity and position on the saccharide and so on. [1, 18]

### 2.1.1 The Anomeric Position

Protection of the anomeric carbon, $C_1$, is usually performed first due its enhanced ability to react, and also to lock the monosaccharide in its cyclic form in its α- or β-anomer, as mentioned in section 1.1. The primary way to lock the α-configuration is the Fischer glycosylation, in which the hemiacetal reacts with an aglycon, often methanol, under acidic conditions to form the acetal in its α-configuration since it is the thermodynamically stabile anomer (*a*, figure 2.1). When the anomeric hydroxyl of the pyranose gets converted, the saccharide is called a pyranoside. To cleave off the methyl ether, strong acids and heat are usually required. To protect the anomeric carbon and instead lock it in the β-configuration, ester or thiol protective groups are commonly employed in a similar fashion (*b*, figure 2.1). [1, 18]

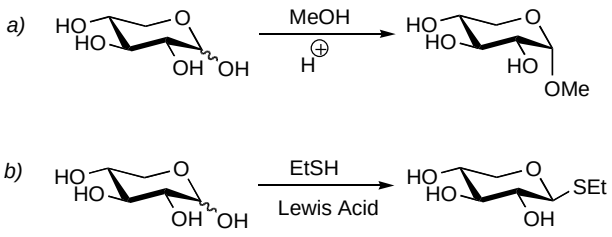



**Figure 2.1.** Protection of the anomeric position on the pyranose, yielding a stable pyranoside.

### 2.1.2 Ester Protective Groups

A widely used method for protecting a hydroxyl group is to use ester protective groups such as acetyl (Ac) or benzoyl (Bz) derivates (figure 2.2). These protective groups are commonly introduced to the saccharide using pyridine as a base. Selective protection of a primary hydroxyl of glucose with acetyl may also be achieved using lipase, an enzyme, which also is useful for selective acetyl deprotection of the same primary hydroxyl. [1, 18] The ester protective groups are relatively stable under acidic conditions and catalytic hydrogenation conditions. They can also be very helpful in the stereospecific glycosylation reaction later described in section 2.2. [1] The ester protective groups are removed under basic conditions. The acetyl is cleaved of at pH >8.5 and the benzoyl is somewhat more stable and is cleaved of at pH >10. [18]

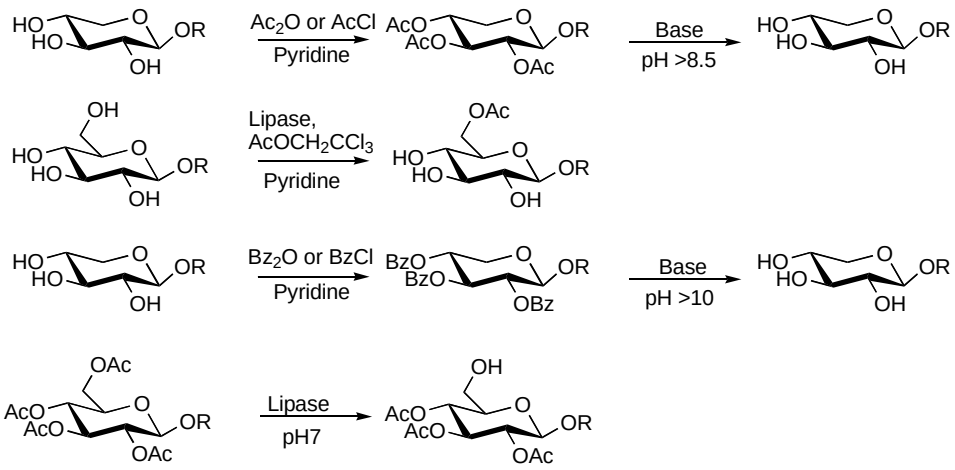



**Figure 2.2.** On/off for acetyl and benzoyl protective groups as well as selective protection with acetyls.

### 2.1.3 Ether Protective Groups

Ether protecting groups, such as benzyls (Bn), are also widely used in carbohydrate synthesis (Figure 2.3). The ether protective group is typically formed using a base together with the corresponding alkyl halide, but may also form under neutral and acidic conditions if necessary. They endure strong base as well as weak acidic conditions and are usually removed by catalytic hydrogenation. [8, 18]

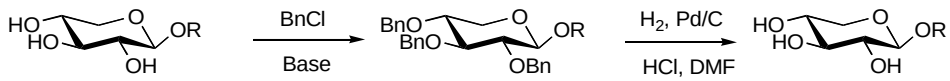



**Figure 2.3.** On/off for the benzyl protective group.

### 2.1.4 Cyclic Acetal Protective Groups

Protection of two neighboring or nearby hydroxyl groups in a monosaccharide can be achieved by forming cyclic acetals with benzaldehyde derivatives or 2,2,3,3-tetramethoxybutane (TMB) (Figure 2.4). The cyclic acetals are formed under acidic conditions and they are stable against nucleophiles and bases. Benzylidene acetals form stable six-membered rings when reacted with hexoses, such as D-glucose, and when removed there is an alternative of removing the whole benzylidene acetal or to cleave it selectively by the 4-O or the 6-O. [11] TMB can be used to protect two neighboring diequatorial hydroxyl groups on a saccharide and it forms a butane trans-diacetal (BDA). [22] When BDA is formed on xylosides, the 4-OH or the 2-OH is left unprotected. The cyclic acetal protective group is stable towards nucleophiles and bases and is cleaved of under acidic conditions. [8, 11, 18]

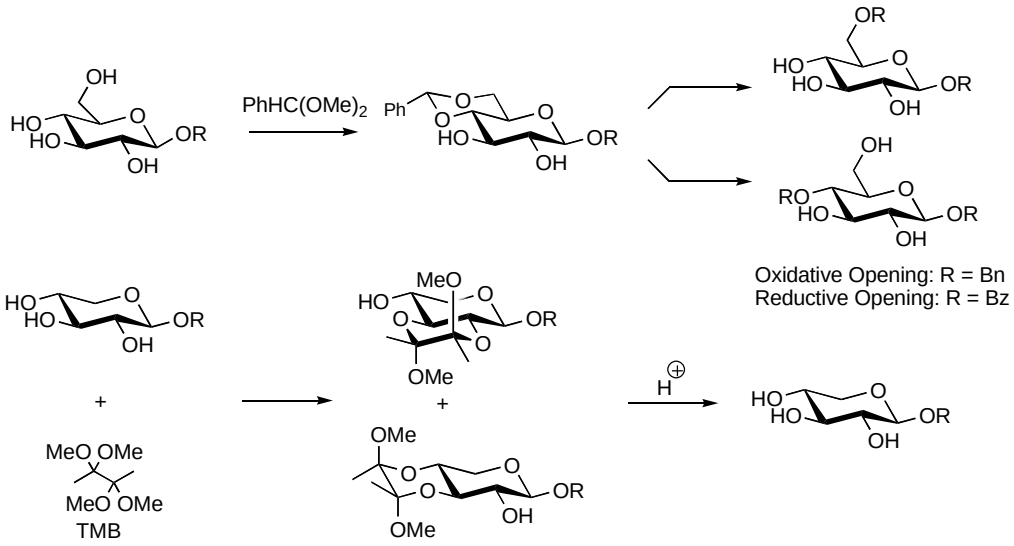



**Figure 2.4.** Cyclic acetals employed on monosaccharides.

## 2.2 *O*-Glycosylation

The reaction for fusing a saccharide and an aglycon or two saccharide derivatives to form a larger di-, oligo- or polysaccharide is called a glycosylation (Figure 2.5). In a classic glycosylation a glycosyl donor, the electrophile, is introduced to a glycosyl acceptor, the nucleophile, and they join in a covalent bond. [1] A promoter is usually required to initiate the reaction by activating the glycosyl donor. In the commonly used the Koenigs-Knorr glycosylation method (*a*, Figure 2.5), the donor is a glycosyl halide, formed by treating an acetylated saccharide with a halogen acid in acetic acid. [1] The donor is activated by heavy metal salts such as mercury oxide, silver triflate or silver carbonate and the formed electrophilic intermediate reacts as described before with the acceptor and yields the new saccharide. A major drawback is the instability of the glycosyl halide since they are sensitive to moisture and decompose during storage. The use of thio- or allylglycosides as donors is an alternative to the Koenings-Knorr method (*b* and *c*, Figure 2.5). These donor-glycosides have increased stability during storage then the glycosyl halide, making the saccharide available to additional modifications if so is required. Thioglycosides can be synthesized from acetylated saccharides and allylglycosides from unprotected saccharides. To perform the O-glycosylation the donor glycoside must first be activated by a promoter. [19]

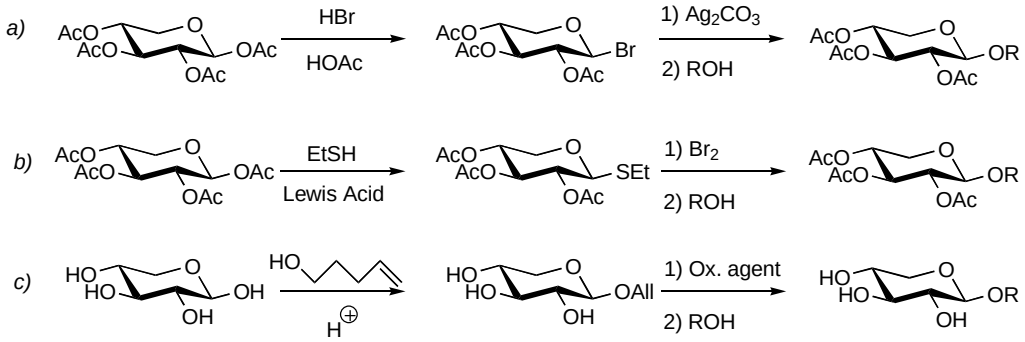



**Figure 2.5.** O-glycosylation with *a*) halideglycoside, *b*) thioglycosides and *c*) allylglycosides as glycosyl donors and ROH as a glycosyl acceptor.

The challenge concerning a glycosylation is the stereochemical outcome of the reaction, specifically if the α- or the β-anomer is the primary product of the reaction. It is possible to affect the stereochemical outcome of the glycosylation with the protective group on $C_2$. If an ester group is present at $C_2$ in the donor, the β-anomer is favored due to neighboring group participation (Figure 2.6, a). When activated, the donor is transformed to an oxocarbenium ion, which undergoes an intermolecular cyclisation with the neighboring ester protective group on $C_2$. The acceptor then attacks and cleaves the five-membered ring at $C_1$, with the ether group hindering nucleophilic attack from the axial-direction, resulting in the equatorial β-anomer as the product of the reaction. To obtain the corresponding axial α-anomer, the protecting group at $C_2$ preferably has to be a non-

participating group like an ether protective group. An ester protective group at $C_3$ may also help promote formation of the α-anomer (b, Figure 2.6). [1]

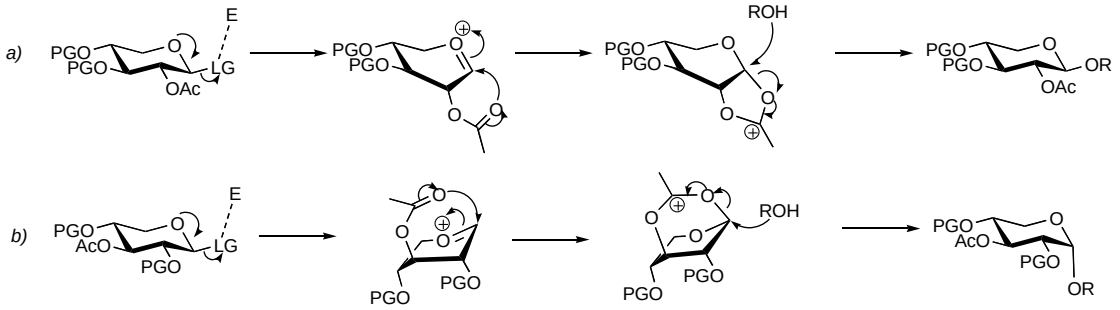



**Figure 2.6.** Neighboring group participation from ester protective groups on $C_2$ and $C_3$ on xylose.

It is also possible to yield the α-anomer despite the presence of β-directing protective groups. In general, the axial α-anomer is the thermodynamic product of an O-glycosylation while the equatorial β-anomer is the kinetic product. This means that the β-anomer is the first to be formed as illustrated in figure 2.7. But over time, with an excess of Lewis acid, the equilibrium will be pushed towards the α-anomer. [20] As well as increased reaction time, an increased temperature would also gain formation of the α-anomer.

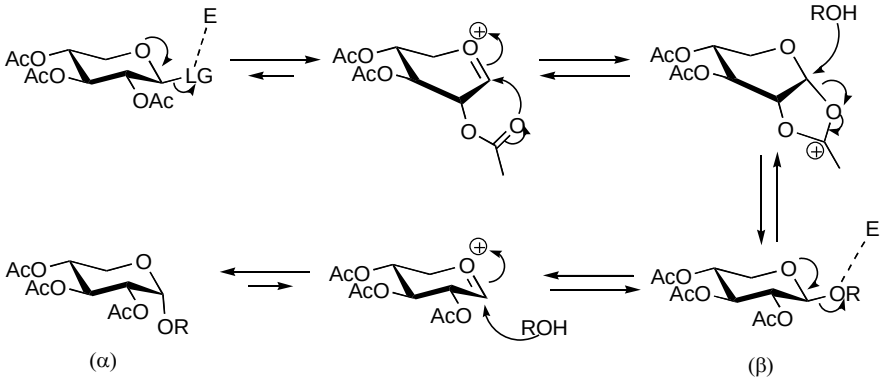



**Figure 2.7.** O-glycosylation on acetylated xylopyranoside with excess of Lewis acid (E) and glycosyl acceptor.

## 3 The Microrector System

In recent years the interest has increased for automated systems which makes scaleup and parameter modification easier. The so-called microreactor system is essentially a tubereactor in microscale, a T or Y shaped channel in a medium made of polymers, quartz, silicon glass or metal. The diameter of the channel is typically between 1 mm and 10 µm giving the volume of the microreactor down to 0.1 µL, and upwards only limited by the length of the reactor and residual tubing. A typical microreactor system consists of a control unit, pumps, tubing and the microreactor (Figure 3.1). With the microreactor system the difference between labscale and productionscale is just a matter of adding additional microreactors to the process and increase pumpflow accordingly to maintain reaction parameters and pumpflow per channel constant. There are many advantages with the microreactor, e.g. modification of the reaction parameters is easy. To investigate different molar ratios of the reactants the flow of the reactant solutions of respective pump is increased or decreased.

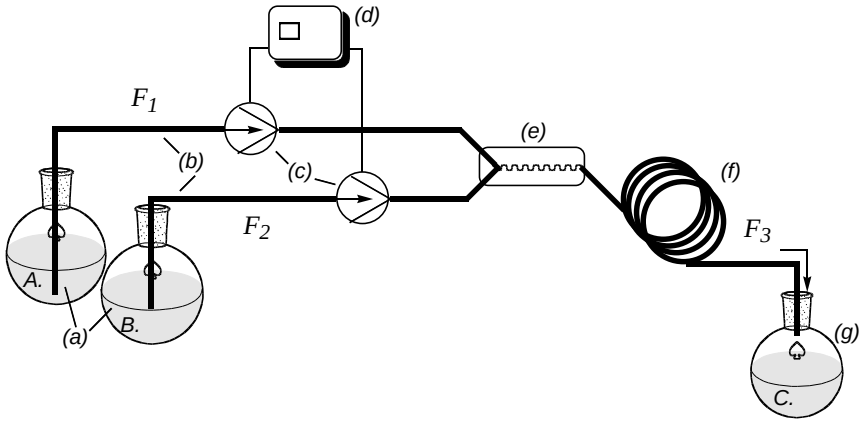



**Figure 3.1.** A typical microreactor system. *a*) reactant flasks, *b*) reactant channels, *c*) pumps, *d*) control unit, *e*) microreactor, *f*) residual tubing, *g*) collector flasks.

The microreactor has an improved concentration profile compared to the conventional batch reaction since the reagents are first mixed nearly instantly in a superturbolent mixing zone followed by the residual tubing where flow is laminar and mixing is driven by diffusion. Depending on the reaction mechanism, this may be an advantage or a disadvantage. In some cases higher yields and selectivity are observed with the microreactor in comparison to conventional batch systems. Also heating and cooling is much faster since removal of heat in a microreactor is more efficient due to up to 10-500 times larger surface to volume ratio and the small diameter in the microchannels. This gives a high heat exchange coefficient, 10 to 25 $kW \cdot m^{-2} \cdot K^{-1}$ compared to 1 $W \cdot m^{-2} \cdot K^{-1}$ of a 1L glass flask. These features minimize the risk of hotspots which increases safety since even highly exothermic reactions are less likely to run out of control.

However, since the reaction takes place in a channel and/or tube, there is a limitation of reaction time, due to the limitation of length of tube and the minimum pump flow. If a solid precipitate is formed during the reaction it may block the channels and when the pressure rises, tubes or joints may burst. Also formation of gas causes problems since the bubbles in the channels shortens the reaction time by reducing the reaction volume. Mechanical pumping using pistons or similar pumps causes pulsating flow, but can be evaded by instead using electroosmotic flow, but that requires charged or polar molecules in the solvent. [21]

The time it takes for a molecule to be transported through the system from the mixing point throughout the channels is called the retention time ($\tau_{Ret}$) and is calculated with equation 1. $\tau_{Ret}$ may also be reffered to as the reaction time. Steady state ($\tau_S$) is an estimate of when the system has reached equilibrium after a change of the reaction parameters and it is estimated according to equation 2. It is also important to take into account that the mass balances change in the mixing zone according to equation 3.

*Equation 1:*

$$\tau_{Ret} = \frac{V}{F_3} = \frac{V_{Reactor} + V_{Tube}}{F_1 + F_2}$$

$V = Total\ Volume\ [mL]$
$F_3 = Total\ Flow\ [mL \cdot min^{-1}]$

*Equation 2:*

$$\tau_S = 1.5 \cdot \tau_{Ret}$$

*Equations 3:*

$$\left.\begin{array}{c} C_A \cdot F_1 + C_B \cdot F_2 = C_C \cdot F_3 \\ F_1 + F_2 = F_3 \\ C_B = 0 \end{array}\right\} \frac{C_A \cdot F_1}{F_1 + F_2} = C_C$$

$C = Concentration\ [mg \cdot mL^{-1}]$
$F = Flow\ [mL \cdot min^{-1}]$

## 4 Aim of the Project

2-naphthyl β-D-xylopyranoside primes synthesis of CS/DS and HS as showed by Mani et. al. and XylNapOH also shows selective antiproliferative effect between normal cells and cancer cells. [16, 17] Also Bakker et. al recently showed that oligosaccharides containing dixylopyranosides linked α1-3 show biological activity in mammalian cells. [13] These findings gave the idea that α–linked aromatic dixylospyranosides might prime GAG synthesis, if their polarity allows them to penetrate the cellmembrane, and show antiproliferative effect.

The aim of this report is to test and assess a synthetic pathway towards 2-naphthyl α-D-xylopyranoside-(1-4)-1-O-α-D-xylopyranoside (**17**) starting from 1,2,3,4-tetra acetylated xylopyranoside. If the synthetic pathway works, and **17** is indeed yielded, it shall later be investigated to determine if it primes CD/DS and HS biosynthesis.

# 5 Retrosynthesis and Synthetic Planning

## 5.1 Retrosynthesis

Retrosynthetic analysis of the target molecule resulted in several synthetic pathways, of which the one presented below was attempted. Alternative pathways are presented in section 8.

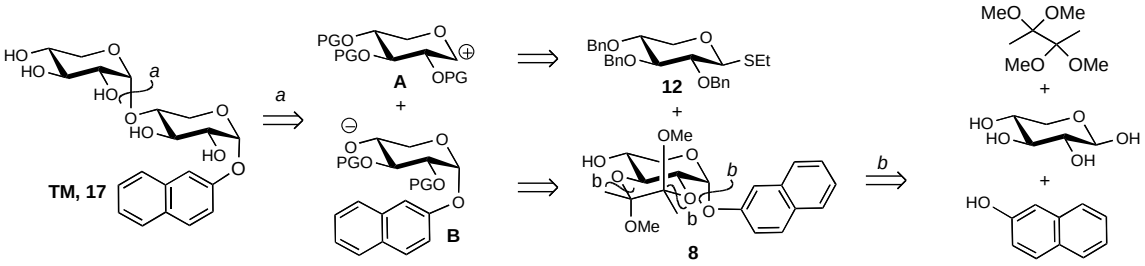



**Figure 5.1.** Retrosynthesis of the desired aromatic α-dixylopyranoside **17**.

Starting the retrosynthetic pathway with the breaking of the bond, *a*, between the two saccharides yields the two fragments A and B (Figure 5.1). Fragment A is found as glycosyl donor **12** with benzyl protective groups and an ethylthiol in the anomeric position promoting the formation of an α-anomer during an O-glycosylation. Fragment B fits perfectly as the aromatic glycosyl acceptor **8**, which may be prepared from TMB, 2-naphthol (HONAP), and xylose. The two neighboring hydroxyls on $C_2$ and $C_3$ make TMB a preferred protective group. The α-naphtoxyloside is promoted with an excess of Lewis acid.

## 5.2 Synthetic planning

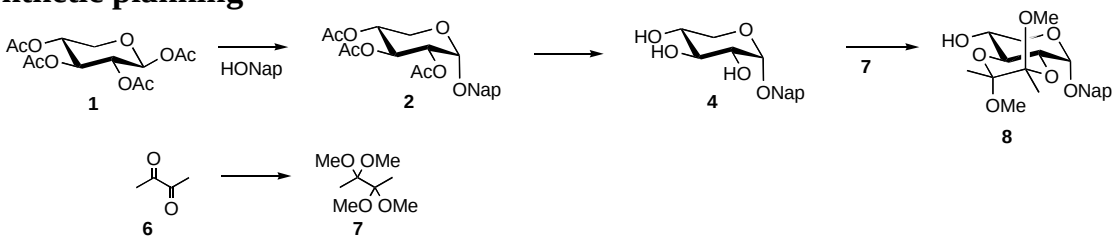



**Figure 5.2.** Synthetic path towards the glycosyl acceptor **8**.

In the synthetic path towards the glycosyl acceptor (**8**), **1** is initially fused with a 2-naphthol via an aromatic O-glycosylation to form **2** which is deacetylated to form **4**. 2,2,3,3-tetramethoxybutane (TMB, **7**) was chosen to protect the hydroxyls on $C_2$ and $C_3$ of **4** to form **8**. **7** is commercially available but was synthesized from cheaper 2,3-butadione (**6**).

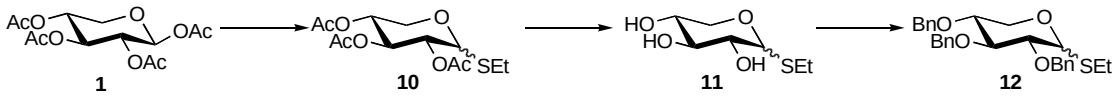



**Figure 5.3.** Synthetic path towards the glycosyl donor (**12**).

In the synthetic path of the glycosyl donor (**12**) the same starting material is used as in the synthesis towards the glycosyl acceptor. **1** is first fused with etylthiol to form the thioglycoside **10.** To promote the formation of a α-O-glucosidic bond, as described in section 2.4, the acetyl protective groups are removed (**11**) and replaced with benzyl protective groups (**12**).

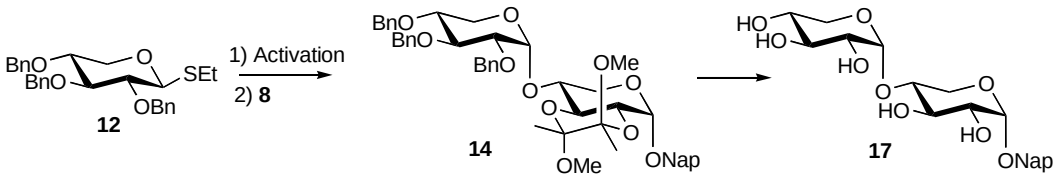
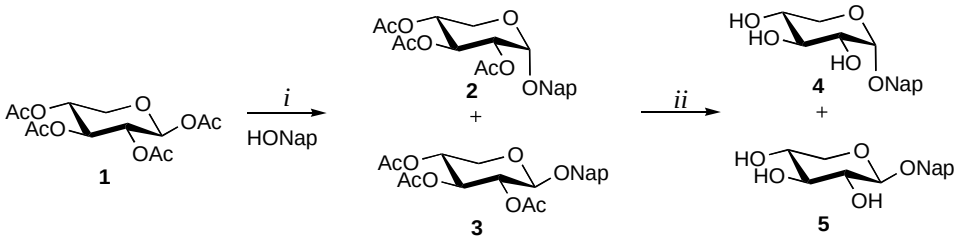



**Figure 5.4.** α-selective O-glycosylation and deprotection to yield 2-naphthyl α-D-xylopyranoside-(1-4)-1-O-α-D-xylopyranoside **(17**).

The O-glycosylation is initiated by priming the donor **12** and introducing it to the acceptor **8**, which gives the aromatic α-dixyloside, **14**, via a stereoselective O-glycosylation step**,** which is then deprotected to give 2-naphthyl α-D-xylopyranoside-(1-4)-1-O-α-D-xylopyranoside (**17**).

# 6 Result and Discussion

## 6.1 Synthesis of Glycosyl Acceptor using Conventional Batch Reactor




**Scheme 6.1.** Synthesis towards the glycosyl acceptor. i) HONap (5.7 eq.), $BF_3{\cdot}OEt_2$ (5.2 eq.), MeCN, 1h, **2** (45%), **3** (14%); ii) NaOMe/MeOH (1M) (cat), MeOH, 20 min, **4** (98%).

The first step in the synthesis of the glycosyl acceptor was to perform an aromatic O-glycosylation of the anomeric carbon, $C_1$, in **1** with 2-naphthol. This reaction was first performed as a batch reaction and later performed on a larger scale with a microreactor. In batch conditions, 1,2,3,4-tetra-acetyl-α-D-xylopyranoside (**1**) was stirred with an excess of 2-naphthol and $BF_3{\cdot}OEt_2$ as Lewis acid in dry acetonitrile. The reaction was quenched with $Et_3N$ and washed with saturated aqueous $Na_2CO_3$ to yield a mixture of the 2-naphthyl 2,3,4-tri-acetyl-1-O-α-D-xylopyranoside (**2**) and the stereoisomer 2-naphthyl 2,3,4-tri-acetyl-1-O-β-D-xylopyranoside (**2**), formed as described in section 2.2. The two isomers were separated and isolated with column chromatography. **2** was obtained as a pinkish solid in 45% yield and **3** was formed in 14% yield as a white solid.

The de-O-acetylation of **2** was performed with a strong base in methanol. **2** was first stirred in methanol to which 1M NaOMe/MeOH was added to the mixture. Then, after a short stir, Amberlite IR-120 $H^+$ which was added until pH-paper showed neutrality. Filtration and concentration gave the desired 2-naphthyl-α-D-xylopyranoside (**4**) as a white solid in 98% yield.

## 6.2 Synthesis of Glycosyl Acceptor using Micro Reactor

When performing the same aromatic O-glycosylation in a microreactor, setup as illustrated in figure 3.1, solution A was prepared by dissolving **1** and 2-naphthol in 750mL dry acetonitrile. Solution B was prepared by dissolving 65 mL $BF_3{\cdot}OEt_2$ in 750 mL dry acetonitrile and both solutions were put under a $N_2$ atmosphere. The microreactor used in this project has a reactor volume of 0.66 mL and a tube volume of 19.6 mL. A flow of 0.2 $mL{\cdot}min^{-1}{\cdot}pump^{-1}$ gave a retention time of 51 minutes and steady state is reached after approximately 75 minutes, according to equation 1 (section 3). After mixing, the reaction solution was led via the residual tubes to a vigorously stirred round bottle flask containing saturated aqueous $NaHCO_3$ which quenched the reaction.

The yield of the α-anomer, **2,** and β-anomer, **3**, was observed to vary with temperature and flow in microreactor and is presented in table 6.1 and 6.2 respectively. Yields were determined by isolating and quenching 10 mL of reaction crude when system was stable after the parameter changes. The crude was then purified via column chromatography. The ratio of the reactants was constant at all time. An increased temperature results in increased formation of **2**, however the overall yields dropped at higher temperatures, until no reaction took place and also no starting material **1** could be retrieved. Also bubbles were observed to appear in the tubes at 50 °C and above.

| Temp. | Yield |
|---|---|
| 0 °C | 14% α, 25% β |
| r.t. | 53% α, 12% β |
| 40 °C | 59% α, 6% β |
| 50 °C | 20% α, 3% β |
| 60 °C | 0% α, 0% β |

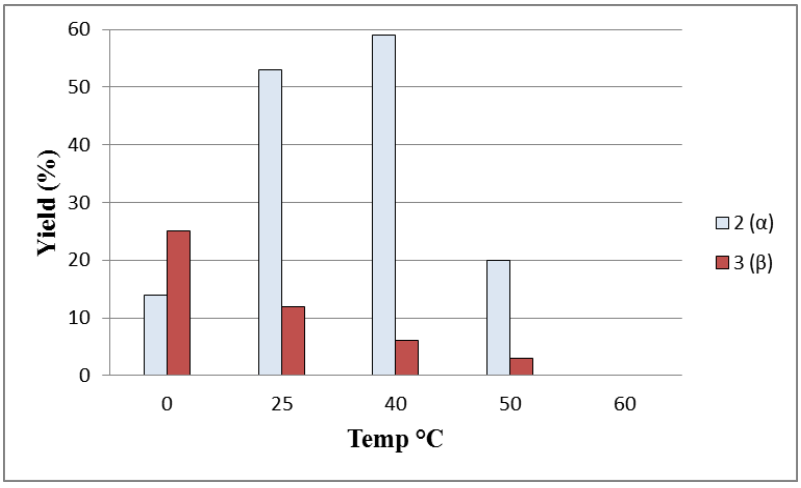



**Table and Graph 6.1.** Temperature affecting α/β yields. Flow: 0.2 $mL{\cdot}min^{-1}{\cdot}pump^{-1}$, Reaction Time: 50.65 min

Reduced reaction time by increased pump flow resulted in decreased yields of **2** and increased yields of **3**, however the overall conversion dropped as a consequence of the shortened reaction time. The change in yields may be explained by that **3** is the kinetic product whereas the **2**, is the thermodynamic product of an O-glycosylation at the anomeric carbon, as mentioned in section 2.2.

| $\tau_{Ret}$ [min], (Flow [ml·min$^{-1}$]) | Yield |
|---|---|
| 50.65, (0.4) | 53% α, 12% β |
| 5.06, (4.0) | 33% α, 20% β |
| 1.13, (18.0) | 22% α, 27% β |

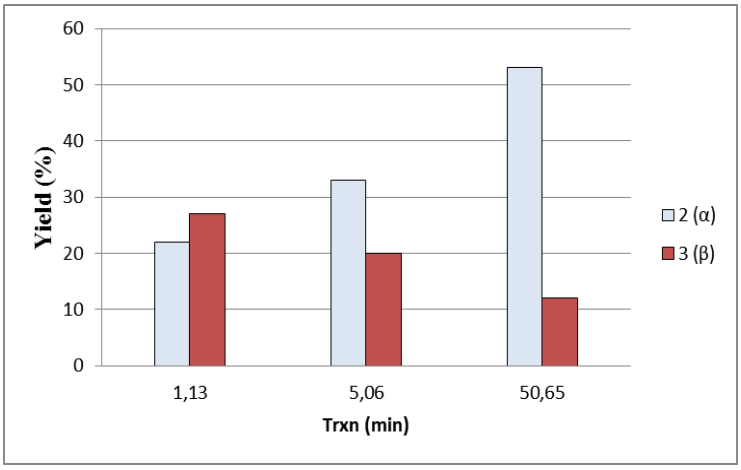



**Table and Graph 6.2.** Reaction time affecting α/β yield. All runs are at r.t.

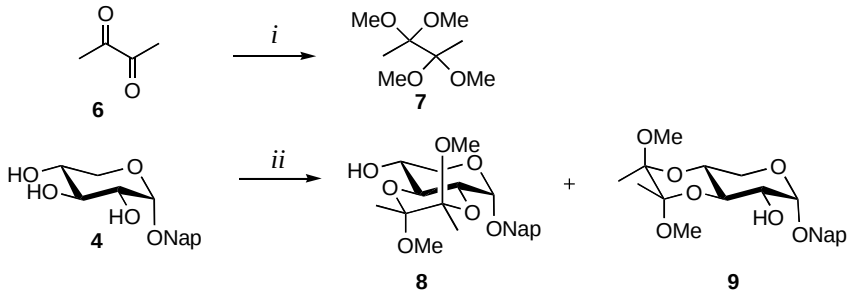
Separation and isolation of the two anomers **2** and **3** with column chromatography proved to be a problem as the anomers, and also 2-naphthol, tended to have very similar or identical $R_f$-values during TLC in a variety of solvents. Also, above all, the large amount of crude produced with the microreactor further complicated the purification. Although a large amount of 2-naphtol got removed from the organic phase by the $NaHCO_3$ saturated aquatic phase which was used to quench the reaction, a considerable amount was still present in the crude. To reduce this amount of 2-naphthol from the crude, three different methods of work-up procedures were tested as presented in table 6.3. The general thought was that the 2-naphthol would become more polar by losing its proton and become more soluble in the aqueous phase. **2** and **3** would remain unpolar and stay in the organic phase. In workup system 1, the crude was first shook in dilute NaOMe/MeOH with dichloromethane for a brief moment in a separation funnel. More dichloromethane was added to dissolve **2** and **3** and $H_2O$ was added to dissolve the 2-naphthol and washed with EtOAc. The method did not work well since **2** and **3** were deprotected, turned polar along with the 2-naphthol and was dissolved in the aqueous phase which could be detected with TCL after separation and neutralization of both phases with amberlite. In workup system 2, the reaction crude was simply shook in a separation funnel with EtOAc as organic phase and saturated aqueous NaOH as polar phase. Some 2-naphthol had transferred to the aqueous phase, but a large quantity was still present in the organic phase. In the final workup system 3, similar to system 2 except that the organic phase was dichloromethane, despite the change of polarity of the organic phase yielded similar results as the previous wash system. Furthermore, recrystallization of the crude from warm EtOH was time-consuming and only gave a small amount of pure **3** after several recrystallizations but no pure **2**. Recrystallization of crude from EtOAc with addition of toluene was also attempted. This proved to be a faster process then using warm EtOH with the crude and yielded **2** and **3** with greatly reduced impurities of HONap. However, when workup was done according to system 1, the two xylosides were de-acetylated to form **4** and **5**. As a consequence 2-naphthol and the two aromatic saccharides

could easily be separated and isolated using gradient column chromatography to remove 2-napthtol as well as recrystallization from warm EtOH to separate the two anomers **4** and **5**.

| Workup system | Recovered α, β | Remaining HONap |
|---|---|---|
| **1)** | 53%, 57% | 35% |
| **2)** | 93%, 94% | 63% |
| **3)** | 95%, 96% | 61% |

**Table 6.3.** Different workup systems tested with 3 × 500 mg crude to reduce amount of HONap: **1)** NaOMe/MeOH (1M, 1 mL) in $CH_2Cl_2$ (20 mL)/$H_2O$ (30 mL) + EtOAc (3×20 mL), **2)** NaOH (2M aq., 20 mL)/EtOAc (20 mL), **3)** NaOH (2M aq., 20 mL)/ $CH_2Cl_2$ (20 mL). Org. phases were concentrated and purified with column chromatography.

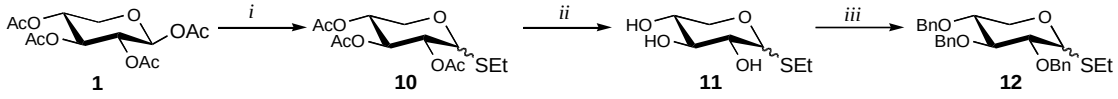
In the synthesis towards the glycosyl donor using the microreactor, the total yield over the two steps was 48% for the α-anomer, **4**, and 7% for the β anomer, **5**. Assuming a 95% yield in the O-deacetylation reaction, the lowest yield observed during the smaller runs, gives the calculated yield for the aromatic O-glycosylation using the microreactor as 51% for **2** and 8% for **3**, a yield similar to the yield from the isolated 10 mL reaction crudes during the runs in tables 6.1 and 6.2.




**Scheme 6.2.** Synthesis of the glycosyl acceptor **8**. i) $HC(OCH_3)_3$ (2.4 eq.), $H_2SO_4$ (cat), 2h, **7** (80%); ii) **7** (2 eq.), $BF_3{\cdot}OEt_2$ (cat), $CH_3CN$, o.n, **8** (30%), **9** (51%).

To yield the desired glycosyl acceptor **4** was protected on the $C_2$ and $C_3$ hydroxyls to yield a free 4-OH. This was achieved using TMB (**7**), which was prepared by dissolving trimethyl orthoformate in methanol followed by 2,3-butadione and $H_2SO_4$. The reaction was put to boil at 66 °C with reflux. The resulting orange liquid was distilled at 40 °C with reduced pressure to 6 mbar to give 2,2,3,3-tetramethoxybutane, **7**, as a colorless liquid in 80% yield. **7** was added to a solution of **4** in acetonitrile. The reaction was initiated by addition of Lewis acid $BF_3{\cdot}OEt_2$, which turned the color of the solution dark brown. The reaction was left overnight and was then quenched by addition of $Et_3N$ which turned. Concentration and purification yielded the desired glycosyl acceptor **8** and also the structural isomer with free 2-OH (**9**) with yields of, 30% and 51% respectively.

## 6.3 Synthesis of Glycosyl Donor



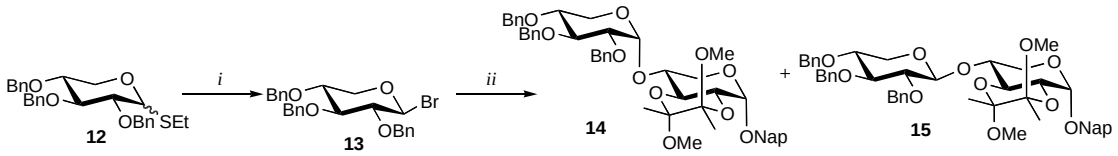

**Scheme 6.3.** Synthesis of the glycosyl donor **12**. i) EtSH (1.46 eq.), $BF_3{\cdot}OEt_2$ (1.33 eq.), $CH_2Cl_2$, 0°C, 30 min, **10** (63%); ii) NaOMe/MeOH (1M) (cat), MeOH, 20 min, **11** (94%); iii) NaH (4.75 eq.), BnBr (4.2 eq.), DMF, 0 °C to r.t, o.n, **12** (92%).

1,2,3,4-tetra-acetyl-β-D-xylopyranisode, **1**, was dissolved in dichloromethane at 0°C, to which ethanethiol and Lewis acid $BF_3{\cdot}OEt_2$ was added to the cool solution. Workup of the reaction crude gave Ethyl 2,3,4-tri-acetyl-1-thio-D-xylopyranoside (**10**) in 63% yield as a 0.08:1 mixture of α- and β-anomer, which did not need to be separated due to outcome of bromination prior to the O-glycosylation step.

Identical to previous de-*O*-acetylation of compound **2**, the same reaction conditions was employed without complications on **10** to give Ethyl 1-thio-D-xylopyranoside (**11**) as a yellow oil in a yield of 94%.

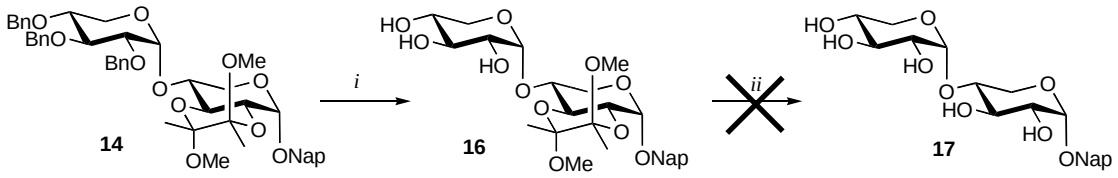
**11** was dissolved in freshly distilled DMF and was stirred at 0 °C until the solution had cooled. NaH was added swiftly, but carefully, as the reaction proved to be very sensitive to $H_2O$ exposure. A thick white foam was observed on top of the liquid in the reaction vessel after addition of NaH, a foam which very slowly disappeared. The reaction was kept at 0 °C when adding benzylbromide to the reaction which was then left to run overnight and was then quenched with cold methanol. Workup and purification gave Ethyl 2,3,4-tri-benzyl-1-thio-D-xylopyranoside (**12**) as a yellow oil with a yield of 92%.

## 6.4 Stereoselective O-Glycosylation and Deprotection




**Scheme 6.4.** Synthesis of the aromatic α-dixylopyranoside **14** via α-selective O-glycosylation and deprotection. i) $Br_2$ (0.05 eq.), $CH_2Cl_2$, cyclohexene, 15 min, **13** (quant.); ii) **x7** (0.5 eq.), $Bu_4NBr$ (0.9 eq.), MS 4Å, $CH_2Cl_2$/DMF 5:3, 60h, **14** (31%), **15** (5%).

To activate the glycosyl donor, Ethyl 2,3,4-tri-benzyl-1-thio-D-xylopyranoside (**12**), was first dissolved in dry dichloromethane to which bromine in a dichloromethane solution was gently added which turned the solution dark. After a short stir, cyclohexene was added until the distinct color of bromide disappeared. The crude was quickly concentrated and dissolved in dry dichloromethane. This donor-solution was added to a mixture of the glycosyl acceptor **8**, 4Å molecular sieves and tetra-*n*-butylammoniumbromine in a 5:3 dichloromethane/DMF solution. The reaction was left for 2.5 days after which it was quenched with pyridine. Purification gave the desired product **14** in a yield of 20%. The reaction was performed both with the glycosyl donor **12** as pure β-anomer and also as a α/β 1:1 mixture and this did not affect the yield of compound **14.** However when the reaction was repeated in a larger scale, the yield of the desired product **14** increased to 31% and also the undesired β-anomer, **15**, was also isolated in a yield 5%.




**Scheme 6.5.** Deprotection of the disaccharide. i) 10% Pd/C, HCl (21.7eq.), DMF, $H_2$, 2h, **16** (68%); ii) 95% TFA/$CH_2Cl_2$ 1:1 - 1:100, 0 °C - r.t, 5-20 min.

Deprotection of **14** towards the desired **17** was initiated by removing the O-benzyl protective groups. This was done as described in section 2.1.3 by catalytic hydration. **14** dissolved in DMF was added to a DMF mixture of Pd/C and HCl which then was stirred under hydrogen at atmospheric pressure. After quenching with $Et_3N$ and a thorough workup, making sure that no product was left in the aqueous phase, **16** was yielded in 68%.

To remove the BDA protective group, **16** was dissolved in dichloromethane to which trifluoroacetic acid in dichloromethane was added and the solution was stirred after which the solution was

concentrated via evaporation but with no yielded product **17**. Different ratios between the acid and solvent were tested and also cooling the reaction to 0 °C but without result. The deprotection step turned out to be problematic since the glycosidic bond between the two saccharides tended to break if too acidic conditions were applied, or simply just not react at all if too weak acidic conditions were applied.

# 7 Conclusions

The synthesis of 2-naphthyl α-D-xylopyranoside-(1-4)-1-O-α-D-xylopyranoside was attempted, via a α-selective O-glycosylation with a thioxylopyranoside as donor and an aromatic xylopyranoside as acceptor, both of these synthesized from 1,2,3,4-tetra-acetyl-β-D-xylopyranisode.

An aromatic α-dixyloside was indeed produced in somewhat low yields, but the deprotection step of the butane trans-diacetal protective group, used to yield a free hydroxyl on the acceptor seemed to break the glycosidic bond between the two xylosides as well as remove the protective group.

A large quantity of 2-naphthyl 2,3,4-tri-acetyl-1-O-α-D-xylopyranoside was produced using a microreactor system. Modifications in temperature and pump flow showed that the formation of 2-naphthyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside, the thermodynamic product, is favored by increased temperature as well as by a decreased pump flow. The anomer 2-naphthyl 2,3,4-tri-O-acetyl-β-D-xylopyranoside, the kinetic product, is favored by decreased temperature as well as an increased pump flow. However increasing the pump flow or the temperature too much resulted in significantly dropped yields of the desired product.

The simplest way to purify the large amount of crude produced with the microreactor was to deacetylate the anomers to increase their polarity and then use gradient chromatography to separate 2-naphtol from the two anomers. Recrystallization from warm ethanol then separated the two deprotected anomers.

# 8 Future Perspectives

## 8.1 Improvements on the Attempted Synthetic Path

If the objective is to produce large amounts of 2-naphthol xylopyranoside, it may be worthwhile to evaluate the effect on the α/β-ratio in the microreactor if the ratio between the reactants is changed. Also further extension of the tested parameters to give a better relationship between yield and temp, flow and flow ratio, finding the optimal reactions conditions to yield α- resp. β-anomer of 2-naphthol xylopyranoside.

Since the attempted synthetic route failed, an alternative synthetic route seems preferable. A possible approach may be to performing the two last deprotection steps in the reversed order, i.e. removal of the BDA protective group under acidic conditions prior to the removal of the O-benzyl protective groups with catalytic hydration. Also using a different acid then TFA might work as intended or instead of the BDA protective group use a protective group that would be removed during the catalytic hydrogenation, perhaps benzyl protective groups or similar.

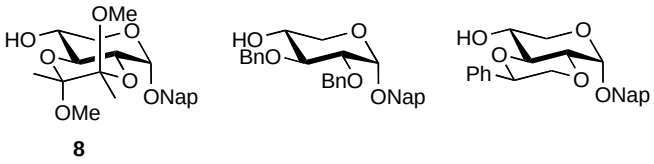



**Figure 8.1.** Alternatives to the glycosyl acceptor **8**.

## 8.2 Two Alternative Synthetic Pathways

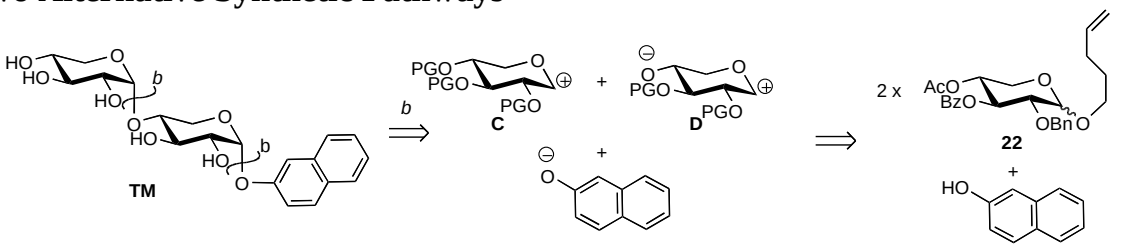



**Figure 8.2.** The alternative retro synthesis using α-yielding xylopyranoside building blocks.

An alternative retro synthetic route was based on the idea that the target molecule could be made out of xylopyranoside "building blocks" **22** (figure 8.2). The building blocks should act as either a glycosyl donor if the allyl is primed or as an acceptor of the acetyl is removed to yield a hydroxyl. In the retrosynthesic pathway, breaking of the two bonds *b* yields fragment C, D and 2-naphthol. The two fragments C and D could originate from compound **22** if primed individually. The compound **22** would be prepared from xylose.

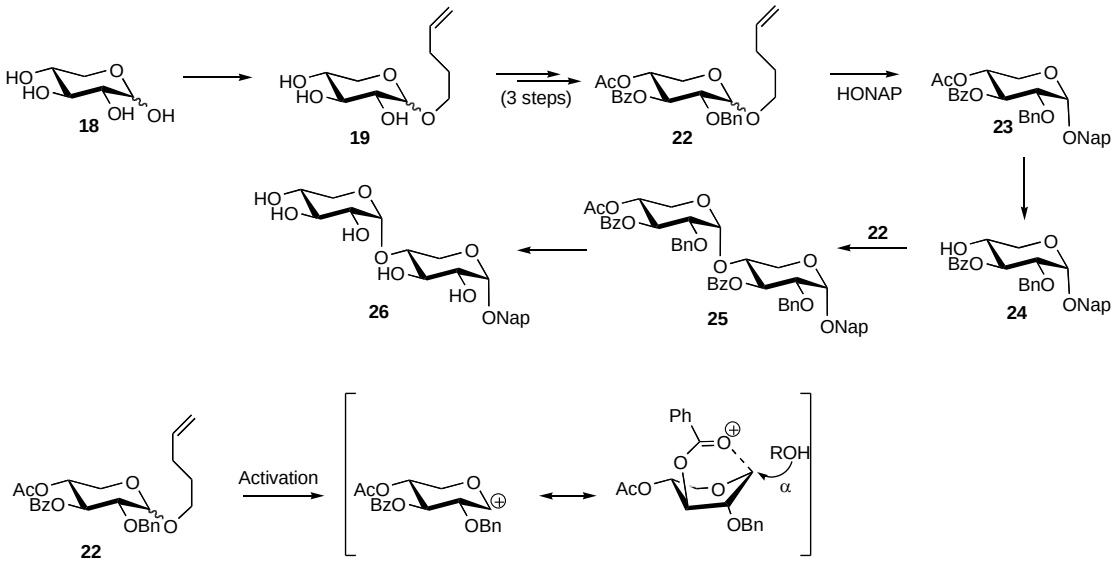



**Figure 8.3.** Synthetic route to the target molecule starting from xylose, towards the disaccharide via an α-yielding monomer: Also illustrating activation of the donor **22**.

Xylopyranose (**18**, Figure 8.3) is first glycosylated with 1-penten-5-ol to form n-pentenyl D-xylose, **19**. Stepwise protection of the hydroxyls using their difference in reactivity (benzylation of 2-OH, benzoylation of 3-OH and acetylation of 4-OH) would yield **22**, an α-yielding monosaccharides which functions, with respective priming method, as both donor and acceptor. The acceptor is prepared by glycosylation with 2-naphthol and deprotection of the acetyl with weak base (pH 8.5-9), or large, bulky bases such as LDA, $Ph_3CNa$ or t-BuOK, to form **24**. The O-allyl of **22** is then activated and introduced to the acceptor, **y7**, and the aromatic α-dixyloside **25** is yielded and then deprotected via catalytic hydrogenation and then basic conditions to yield the target molecule **26**.

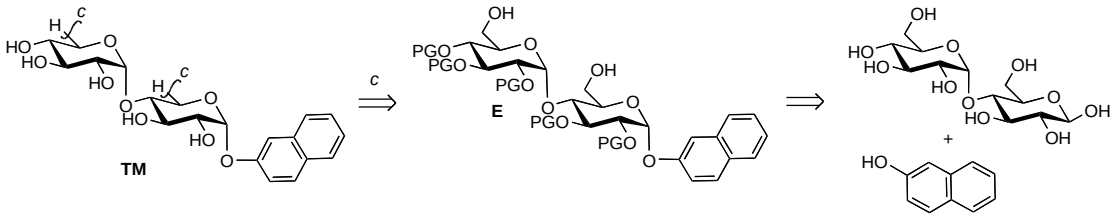



**Figure 8.4.** The last alternative retro synthesis. FGI of *c* yields a maltose compound

The last retrosynthesis is based on the observation that the target molecule resembles much of the previously mentioned (section 1.2) disaccharide maltose with its α1-4 linkage (FGI, *c*, Figure 8.4). If a maltose disaccharide were to be fused with a 2-naphthol and then transform the two primary alcohols into suitable leaving groups and replace them with two hydrogens, the target molecule could be yielded.

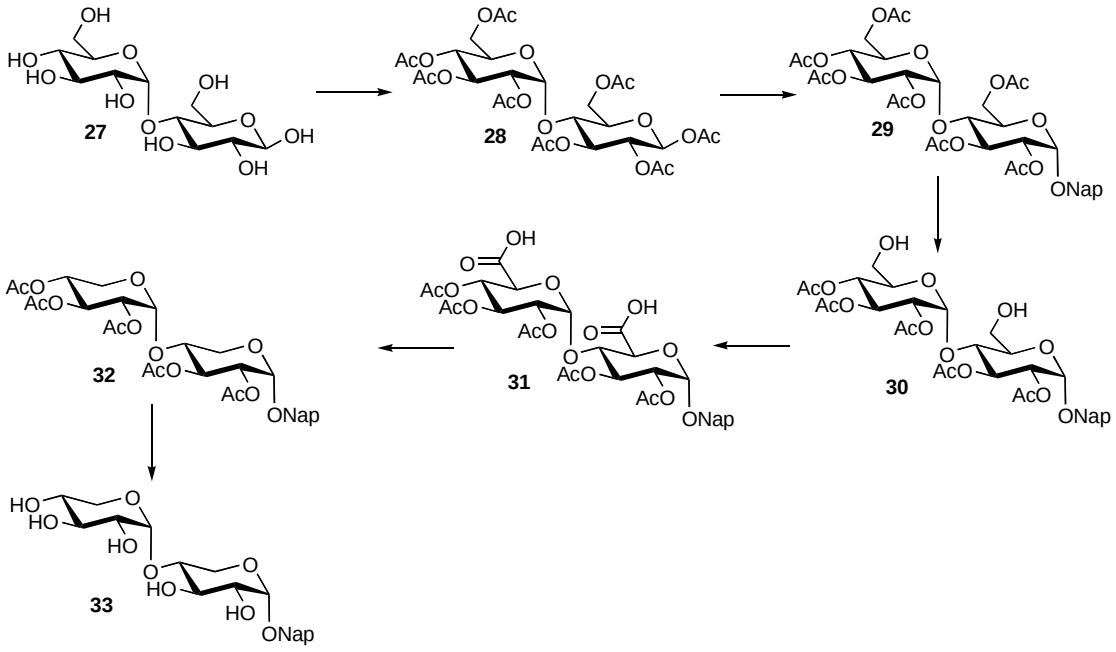



**Figure 8.5.** Synthetic route to the target molecule starting from maltose

The synthetic route starts from D-maltose (**27**, Figure 8.5), which is first O-acetylated on every hydroxyl to form **28**. α-selective O-glycosylation with 2-naphthol yields **29**, which is deprotected from the two acetyl protective groups yielding two primary hydroxyls by employing lipase at pH 7 to form **30**. [18] The two primary hydroxyls are oxidized with $CrO_3$ to form the di-acid **31** which is decarboxylated, via a Barton-decarboxylation, to form **32**. Deprotection under basic conditions yields the aromatic α-dixylopyranoside, **33**.

## 8.3 If the Aromatic Dixyloside is yielded.

If a synthetic route is found that yield the desired target molecule, the next step would be to investigate if the molecule primes CD/DS and HS biosynthesis and compare the priming ability with that of the α- resp. β-anomer of 2-naphthol xylopyranoside. Also an attempt with a slightly modified route where the aromatic aglycon 2-naphthol have been replaced with a 2,6-naphthol, yielding a 2-(6-hydroxynaphthyl) α-D-xylopyranoside-(1-4)-1-O-α-D-xylopyranoside, and investigate and compare its GAG-priming abilities. Also it might be interesting to compare GAG-priming abilities between the α-dixyloside and the β-dixyloside, 2-naphthyl β-D-xylopyranoside-(1-4)-1-O-α-D-xylopyranoside, which may be yielded after deprotection of the β anomer, **15**, that was formed in 5% yield in the O-glycosylation step.

## 9 Acknowledgements

Thanks to Prof. Ulf Ellervik for letting me work in the group and mess up his lab (again) and to Anna Siegbahn, my supervisor, for letting me realize that I do have some chemistry skills after all. Also a special thanks to Markus Ohlin, Agata Ochocinska, Ulrika Aili, Johanna Löfgren, as well as the rest of the department for all useful tips/tricks, crash courses on the microreactor, Pd hydrogenation, HPLC, etc. etc. etc.

## 10 Experimental Procedures

All reactions were performed under gaseous $N_2$ at room temperature unless other temperatures are specified. All solvents used in the synthesis were of HPLC-analysis grade and dry solvents were dried on $Al_2O_3$. Purchased reagents were used without purification. $^1$H-NMR and $^{13}$C-NMR spectras were recorded with a Bruker Advance 400 operating at 294 K. $^1$H-NMR spectras were assigned using COSY. Chemical shifts are given in ppm downfield from $SiMe_4$, with reference to respective solvent. Coupling constants values are given in Hz. Microreactor equipment was Sigma-Aldrich Microreactor Explorer Kit 19979 with a borosilicate glass reactor (Borofloat 33 Duran ®) with a channel diameter of 0.1 mm and total reaction volume of 20.26 mL. Thin layer chromatography was performed on Merck C60 F24x with detection by UV light and anisaldehyde charring solution. HPLC was performed on a WATERS 600 with a C60 reversed phase Column. $BF_3{\cdot}Et_2O$ is 95%. NaH is 55% in oil.

**2-naphthyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside (2) - Batch reaction**

**1** (78.9 mg, 0.248 mmol) and 2-naphthol (201.8 mg, 1.4 mmol) were dissolved in dry acetonitrile (3.7 mL). $BF_3{\cdot}Et_2O$ (0.16 mL, 1.28 mmol) was added and the solution was stirred for 30 min, after which it was diluted with EtOAc (20mL) washed with $NaHCO_3$ (sat. aq., 3 x 20 mL). The organic phase was dried on $MgSO_4$(s), filtered and co-concentrated with toluene. The crude was then column chromatographed ($SiO_2$, Toluene/EtOAc 8:1) which gave **2** and **3** as a colorless solid. Yield: 45.1 mg (45 %) of α (**2**) and 14mg (14%) of β (**3**); $R_{f,(\alpha)} = 0.40$, $R_{f,(\beta)} = 0.32$ (Toluene/EtOAc 8:1)

**2-naphthyl 2,3,4-tri-O-acetyl-α-D-xylopyranoside (2) - Micro reactor**

**1** (31522.5 mg, 99.04 mmol) and 2-Naphthol (81388.5 mg, 56.46 mmol) were dissolved in dry acetonitrile (750 mL, solution A). $BF_3{\cdot}Et_2O$ (65.26 mL, 410.5 mmol) was dissolved in dry acetonitrile (750 mL, solution B). Solutions A and B were run through and mixed in a microreactor ($F = 0.2$ mL·min$^{-1}$·pump$^{-1}$, $\tau_{Ret} = 50.65$ min) at 40°C into a solution of $NaHCO_3$ (sat. aq.) and stirred vigorously. The organic phase was co-concentration with toluene and the crude was then column chromatographed ($SiO_2$, Toluene/EtOAc 8:1) which gave a mixture of **2** and **3** as a colorless solid. Yield: 20369 g (51%) of α, $R_f = 0.40$; 3110 mg (8%) of β; $R_f = 0.32$ (Toluene/EtOAc 8:1); **2:** $^1$H-NMR (400 MHz, $CCl_3D$): δ = 7.79 (dd, J = 8.6, 18.4 Hz, 3H, Ar-H), 7.58-7.50 (m, 3H, Ar-H), 7.29 (dd, J = 2.8, 9.2 Hz , 1H, Ar-H), 5.87 (d, J = 3.6 Hz, 1H, H-1), 5.80 (t, J = 9.8 Hz, 1H, H-3), 5.10-5.16 (m, 1H, H-4), 5.07 (dd, J = 3.6, 10.4 Hz, 1H, H-2), 3.92 (dd, J = 6, 11.2 Hz, 1H, H-5), 3.79 (t, J = 11 Hz, 1H, H-5), 2.12 (s, 3H, $CH_3$), 2.10 (s, 3H, $CH_3$), 2.07 (s, 3H, $CH_3$); **3:** Selected $^1$H-NMR (400 MHz, $CCl_3D$): δ = 5.34 (d, J = 5.6 Hz, 1H, H-1)

**2-naphthyl α-D-xylopyranoside (x3)**

**2** (4.12 g, 10.24 mmol) was dissolved into MeOH (50 mL) and NaOMe (5 mL, 1M in MeOH) was added. When all solid material was dissolved, Amberlite IR-120 $H^+$ was added after 20 minutes until the solution turned neutral. The mixture was filtered and co-concentrated with toluene which gave **4** as a colorless solid. Yield: 2.70 g (95%); $R_f$ = 0.19 (Toluene/EtOAc 8:1); $^1$H-NMR (400 MHz, MeOD): δ = 7.76 (dd, J = 8, 16.4 Hz, Ar-H), 7.50 (d, J = 2.4 Hz, Ar-H), 7.37-7.33 (m, Ar-H), 7.31 (dd, J = 2.4, 9.2 Hz, 1H, Ar-H), 5.62 (d, J = 3.2 Hz, 1H, H-1), 3.88-3.83 (m, 1H, H-5), 3.67-3.59 (m, 4H, H-2. H-3, H-4, H-5).

**2,2,3,3-tetramethoxy butane (7)**

Trimethyl orthoformate (60 mL, 538 mmol) was dissolved in MeOH (30 mL). 2,3-butadione (**6**) (20 mL, 228 mmol) and 4 drops of $H_2SO_4$ was added. The solution was heated to 66 °C and stirred with reflux for 2 h, after which it was quenched with $NaHCO_3$, filtered and concentrated. The crude was then diluted with 200mL ether and washed with 3 × 100 mL $NaHCO_3$ (sat aq.). The organic phase was co-concentrated with toluene to give orange liquid which was distilled to give **7** as a colorless liquid. Yield: 30mL (80%); $R_f$ = 0.59 (Toluene/EtOAc 10:1); $^1$H-NMR (400 MHz, $CCl_3D$): δ = 1.32 (s, 6H, $CH_3$), 3.31 (s, 12H, $OCH_3$); $^{13}$C-NMR (400 MHz, $CCl_3D$): δ = 103.0, 49.3, 19.0.

**(1S, 3R, 4R, 6R, 7S, 10R)-10-hydroxy-3,4-dimethoxy-3,4-dimethyl-7-(2-naphthoxy)-2,5,8-trioxabicyclo[4.4.0]decane (8)**

**4** (1030 mg, 3.728 mmol) and TMB (1.3 mL, 7.456 mmol) was dissolved in dry MeCN (50 mL) to which $BF_3{\cdot}Et_2O$ (0.092 mL, 0.746 mmol) was slowly added which turned the solution dark brown. The solution was stirred for 30 h after which $Et_3N$ (10 mL) was added to quench. The solution was co-concentrated with toluene and the crude was then purified with column chromatography ($SiO_2$, Heptane/EtOAc 2:1) which gave **8** as a yellow-white solid. Yield: 430 mg (30%) of **8**(4-OH)**,** $R_f$ = 0.21, 747 mg (51%) of **9** (2-OH), $R_f$ = 0.28 (Heptane/EtOAc 2:1); **8:** $^1$H-NMR (400 MHz, $CCl_3D$): δ = 7.79-7.73 (m, 3H, Ar-H), 7.48-7.33 (m, 4H, Ar-H), 5.66 (d, J = 3.6 Hz, 1H, H-1), 4.25 (t, J = 9.6 Hz, 1H, H-3), 3.93-4.01 (m, 1H, H-4), 3.90 (dd, J = 3.6, 10.4 Hz, 1H, H-2), 3.79 (dd, J = 5.6, 11.2 Hz, 1H, H-5), 3.71 (t, J = 11 Hz, 1H, H-5), 3.38 (s, 3H, $OCH_3$), 3.30 (s, 3H, $OCH_3$), 1.37 (s, 6H, $CH_3$); $^{13}$C-NMR (100 MHz, $CCl_3D$): δ = 154.48, 134.59, 130.15, 129.61, 127.95, 126.64, 124.60, 119.84, 111.96, 110.48, 99.97, 95.93, 70.18, 68.35, 68.32, 63.03, 48.41, 48.34, 18.23, 18.03; **9:** Selected $^1$H-NMR (400 MHz, $CCl_3D$): δ = 7.0-7.8 (m, 7H, Ar-H), 5.73 (d, J = 4 Hz, 1H, H-1), 4.13 (t, J = 9.6 Hz, H-3), 3.90-3.97 (m, 1H, H-2), 3.81-3.89 (m, 2H, H-4, H-5), 5.66 (m, 1H, H-5), 3.40 (s, 3H, $OCH_3$), 3.31 (s, 3H, $OCH_3$), 1.40 (s, 3H, $CH_3$), 1.33 (s, 3H, $CH_3$).

**Ethyl 2,3,4-triacetyl-1-thio-α-D-xylopyranoside (10)**

**1** (200 mg, 0.628 mmol) was dissolved in $CH_2Cl_2$ (1.5 mL) and stirred for 15 min at 0 °C, after which EtSH (0.068 mL, 0.917 mmol) was added to the solution and it was stirred for an additional 15 min at 0 °C. $BF_3{\cdot}Et_2O$ (0.103 mL, 0.836 mmol) was added to the solution and the reaction was kept at 0 °C for 5h, after which it was diluted with 10 ml $CH_2Cl_2$, washed with 20 mL NaOH (2M, aq) and 2 × 20 mL Brine. The organic phase was dried with $MgSO_4$, filtered and co-concentrated with toluene and the crude was then purified with column chromatography ($SiO_2$, Toluene/EtOAc, 5:1) which gave **10** as a yellow oil. Yield: 126.3 mg (63 %); (α:β 0.08:1, determined by NMR spectroscopy); $R_f$ = 0.45 (Toluene/EtOAc 5:1); $^1$H-NMR (400 MHz, $CCl_3D$): δ = 5.18 (t, J = 8.6 Hz, 1H, H-4), 4.97 (dt, J = 3.6, 8.6 Hz, 1H, H-5), 4.53 (d, J = 8.4 Hz, 1H, H-1), 4.23 (dd, J = 5.2, 11.6 Hz, 1H, H-2), 3.38 (dd, J = 9.2, 11.6 Hz, 2H, H-3, H-5), 2.63-2.74 (m, 2H, C$\underline{H_2}$$CH_3$), 2.12 (s, 3H, $OCH_3$), 2.05 (s, 6H, $OCH_3$), 1.26 (t, J = 7.4 Hz, 3H, $CH_2C\underline{H_3}$).

**Ethyl 1-thio-α-D-xylopyranoside (11)**

**10** (126 mg, 0.394 mmol) was added into MeOH (5 mL) followed by NaOMe (0.5 mL, 1M in MeOH). After 20 minutes, Amberlite IR-120 $H^{\oplus}$ was added until the solution turned neutral according to pH paper. The mixture was filtered and co-concentrated with toluene which gave **11** as a yellow oil. Yield: 71.5 mg (94%); $R_f$ = 0.11 (Toluene/EtOAc 5:1); $^1$H-NMR (400 MHz, MeOD): δ = 5.63 (d, J = 3.3 Hz, 1H, H-1), 3.89-3.81 (m, 1H, H-5), 3.70-3.61 (m, 4H, H-2. H-3, H-4, H-5), 2.83-2.64 (m, 2H, C$\underline{H_2}$$CH_3$), 1.41 (t, J = 7.3 Hz, 3H, $CH_2C\underline{H_3}$).

**Ethyl 2,3,4-tribenzyl-1-thio-α-D-xylopyranoside (12)**

**11** (100 mg, 0.515 mmol) was dissolved in freshly distilled DMF (1.3 ml) at 0 °C to which NaH (106 mg, 2.45 mmol) was added and the solution was stirred for 30 min at 0°C, after which BnBr (0.257 mL, 2.16 mmol) was added and the solution was stirred overnight at r.t. The reaction was quenched with MeOH (5 ml), diluted with 50 mL $CH_2Cl_2$ and washed with 2 × 50 mL $H_2O$ and 50 mL Brine (sat. aq.). The organic phase was dried with $MgSO_4$, filtered and co-concentrated with toluene. The crude was purified with column chromatography ($SiO_2$, Toluene/EtOAc, 5:1) which gave **12** as a yellow oil. Yield: 220 mg, (92%); $R_f$ = 0.69 (Toluene/EtOAc 5:1); $^1$H-NMR (400 MHz, $CCl_3D$): δ = 7.8-7.0 (m, 15H, Ph-H), 4.92 (d, J = 10.8 Hz, 1H, C$H_2$-Ph), 4.72-4.61 (m, 5H, C$H_2$-Ph), 4.42 (d, J = 9.6 Hz, 1H, H-1), 4.02 (dd, J = 7.2, 11.6 Hz, 1H, H-5), 3.62 (m, 2H, H-3, H-4), 3.36 (t, J = 9.6 Hz, 1H, H-2), 3.21 (dd, J = 10, 11.2 Hz, 1H, H-5), 2.80-2.65 (m, 2H, C$\underline{H_2}$$CH_3$), 1.33 (t, J = 7.4 Hz, 3H, $CH_2C\underline{H_3}$).

**(1S, 3R, 4R, 6R, 7S, 10R)-10-(2,3,4-tri-O-benzyl-α-D-xylopyranoside)-3,4-dimethoxy-3,4-dimethyl-7-(2-naphthoxy)-2,5,8-trioxabicyclo[4.4.0]decane (14)**

**12** (1527 mg, 3.29 mmol) was dissolved in dry $CH_2Cl_2$ (10 mL) and $Br_2$ (0.147 mL, 0.21 mmol) in 1 mL $CH_2Cl_2$ was added. The solution was stirred for 15 minutes under $N_{2(g)}$ and quenched with cyclohexene (5 mL), after which it was concentrated and diluted with $CH_2Cl_2$ (12 mL). The solution was added to a mixture of **8** (628 mg, 1.61 mmol), 4Å MS (6029 mg), *n*-$Bu_4NBr$ (937 mg, 2.91 mmol), and $CH_2Cl_2$/DMF (5:3, 12.9 mL). The mixture was stirred for about 60 h under $N_{2(g)}$ after which pyridine (4.9 mL) was added and stirred for an additional 3 h. The mixture was filtered through Celite, diluted with 15 mL $CH_2Cl_2$ and washed with 3 × 40 mL $H_2O$ and 2 ×40 mL Brine (sat. aq.). The organic phase was dried with $MgSO_4$, filtered and co-concentrated with toluene. The crude was purified with column chromatography ($SiO_2$, Heptane/EtOAc 2:1) gave **14** as a yellow-white solid. Yield: 373 mg (31 %); $R_f$ = 0.22 (Heptane/EtOAc 2:1); $^1$H-NMR (400 MHz, $CCl_3D$): δ = 7.80-7.26 (m, 22H, Ar-H, Ph-H). 5,64 (d, J = 3.6 Hz, 1H, H1), 5.24 (d, J = 3.6 Hz, 1H, H1'), 4.95-4.86 (m, 3H, C*H*$_2$-Ph), 4.72 (m, 2H, C*H*$_2$-Ph), 4,57 (d, J = 11.6 Hz, 1H, C*H*$_2$-Ph), 4,43 (t, J = 9.6 Hz, 1H, H-4), 3.95-3.88 (m, 3H, H-3, H-5, H-3', H-5'), 3,81 (d, J = 8.4 Hz, 2H, H-, H-2, H-5'), 3.61-3.30 (m, 3H, H-5, H-2'. H-4'), 3.30 (s, 3H, $OCH_3$), 3.19 (s, 3H, $OCH_3$), 1.33 (s, 3H, $CH_3$), 1.24 (s, 3H, $CH_3$); $^{13}$C-NMR (400 MHz, $CCl_3D$): δ = 154.41, 139.13, 138.73, 138.48, 134.54, 130.07, 129.55, 129.36, 128.75, 128.70, 128.60, 128.58, 128.51, 128.40, 128.26, 128.12, 128.06, 127.90, 127.83, 127.73, 127.41, 126.55, 125.59, 124.50, 119.80, 111.76, 100.37, 99.76, 98.67, 95.57, 81.30, 79.35, 78.23, 75.87, 74.02, 73.79, 72.66, 69.06, 68.60, 48.31, 48.21, 21.75, 18.12, 17.93; **15**: Selected $^1$H-NMR (400 MHz, $CCl_3D$): δ = 5.62 (d, J = 3.6, 1H, H1), 4,56 (d, J = 7.6, 1H, H1').

**(1S, 3R, 4R, 6R, 7S, 10R)-10-α-D-xylopyranoside-3,4-dimethoxy-3,4-dimethyl-7-(2-naphthoxy)-2,5,8-trioxabicyclo[4.4.0]decane (16)**

Degaussa-type Pd/C (10 wt%, 150.2 mg) was suspended in freshly distilled DMF (1 mL) and was put under $H_2$ atmosphere and stirred for 10 minutes. To the mixture HCl (37%) (0.34 mL, 10.94 mmol) in 5 mL DMF was added and the mixture was stirred for 10 minutes under $H_2$ atmosphere. **14** (363 mg, 0.504 mmol) in 5 mL DMF was added to the mixture and stirred for 2 h under $H_2$ atmosphere, after which $Et_3N$ (0.48 mL) was then added to quench the reaction. The mixture was filtered through Celite with EtOAc and washed with 2 × 75 mL $H_2O$ and 75 mL Brine (sat. aq.). The aqueous phases were extracted with EtOAc three times. The pooled organic phases were co-concentrated with toluene. The crude mixture was purified with column chromatography ($SiO_2$, Toluene/EtOAc 20:1) which gave **16** as a colorless solid. Yield: 142 mg (68%); $R_f$ = 0.05 (Toluene/EtOAc 20:1); $^1$H-NMR (400 MHz, $CCl_3D$): δ = 7.73-7.79 (m, 3H, Ar-H), 7.48-7.42 (m, 2H, Ar-H), 7.394-7.34 (m, 2H, Ar-H), 5.64 (d, J = 3.6 Hz, 1H, H-1), 5.11 (d, J = 3.6 Hz, 1H, H-1'), 4.37 (t, J = 9.3 Hz, 1H, H-4), 3.95-3.88 (m, 2H, H-2, H-3), 3.83 (dd, J = 5.6, 11.2 Hz, 1H, H-5), 3.72

(m, 1H, H-5), 3.64-3.61 (m, 3H, H-3', H-4', H-5'), 3.51-3.44 (m, 2H, H-2', H-5'), 3.36 (s, 3H, $OCH_3$), 3.29 (s, 3H, $OCH_3$), 1.35 (s, 6H, $CH_3$, $CH_3$); $^{13}$C-NMR (400 MHz, $CCl_3D$): δ = 129.5, 129.2, 128.4, 127.7, 126.5, 125.4, 124.47, 119.6, 119.5, 11.7, 100.6, 100.3, 99.9, 95.5, 75.6, 75.4, 72.7, 69.7, 69.0, 68.2, 62.3, 61.9, 48.3, 61.9, 48.3, 48.2, 18.0, 17.8.

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