NATURVETENSKAPLIGA FAKULTETEN

LUND UNIVERSITY

Department of Chemistry

Centre for analysis and synthesis

MASTER THESIS

SYNTHESIS OF β-D-RIBOPYRANOSIDES DERIVATIVES FROM β-D-XYLOPYRANOSIDE

CHRISTOPHER MUSONDA

Supervisor: Sophie Manner, Ph. D.

Examiner: Prof. Ulf Ellervik

Table of Contents

1. LIST OF ABBREVIATIONS

PG - Proteoglycan

GAG - Glycosaminoglycan

β4GalT7 - β-1,4-Galactosyltransferase 7

CS/DS - Chondroitin sulfate/dermatan sulfate

HS - Heparan sulfate

XylNap - 2-Naphthyl β-D-xylopyranoside

Nap - Naphthyl

KOAc - Potassium Acetate

Ac_20 - Acetic anhydride

$BF_3 \cdot OEt_2$ - Boron trifluoride diethyl etherate

Et_3N - Triethylamine

N_2 - Nitrogen gas

R.t. - Room temperature

NaOMe - Sodium methoxide

MeOH - Methanol

CSA - Camphorsulfonic acid

DMF - Dimethylformamide

Anhyd. - Anhydrous

DMSO - Dimethylsulfoxide

THF - Tetrahydrofuran

CS_2 - Carbon disulfide

MeI - Iodomethane

AIBN - 2,2'-Azobis(2-methylpropionitrile) or a,a'-Azoisobutyronitrile

n-Bu_3SnH - Tributyltin hydride

AcOH - Acetic acid

Tf_2O - Trifluoromethanesulfonic anhydride (triflic anhydride)

DAST - Diethylaminosulfur trifluoride

NMR - Nuclear magnetic resonance

Ppm - Parts per millon

TLC - Thin layer chromatography

UV - Ultra Violet

EtOH - Ethanol

Sat. aq. - Saturated aqueous

EtOAc - Ethyl acetate

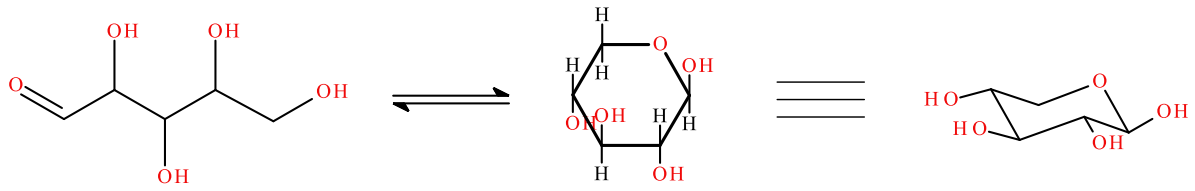
Dist. - Distilled

2. ABSTRACT

The aim of this thesis was to synthesize various β-D-ribopyranoside derivatives with different C-4 functional groups, using D-xylose as the starting material. The desired modifications were deoxygenation, fluorination, methylation and epimerization of the C-4 hydroxyl group. Methylation provided the best results with the final product being successfully synthesized. Deoxygenation was also achieved but the final de-protection step was not carried out. Epimerization was attempted using two different methods but without success, firstly probably due to the high energetic favorability of having the hydroxyl group in the equatorial position, and secondly due to harsh reaction conditions causing the cleavage of the hydroxyl group. Fluorination was not attempted.

3. INTRODUCTION

After cardiovascular diseases, cancer represents the second leading cause of death in highly developed countries.[1] It constitutes a major burden on modern societies and estimates show that if rapid improvements in medical treatments aren't made it could soon become the world's main cause of human mortality. A current development in cancer research is the interest in D-xylose (fig. 1) as a precursor for anti-cancer agents due to its biological importance in mammals and its availability in nature.[2]



1 D-xylose (Open chain form) **2** β-D-xylopyranose (Cyclic form) **3** β-D-xylopyranose (Chair conformation)

Fig. 1. *D-xylose in its open chain and pyranose form (only the pyranose form of sugars will be considered in the rest of the thesis).*

[1] Rozpedek, W.; Pytel, D.; Mucha, B.; Leszczynska, H.; Diehl. J. A.; Majsterek, I. The Role of the PERK/eIF2α/ATF4/CHOP Signaling Pathway in Tumor Progression During Endoplasmic Reticulum Stress, *Curr. Mol. Med.*, **2016**, *16*, 533-544

[2] Thorsheim, K.; Siegbahn, A.; Johnsson, R. E.; Stålbrand, H.; Manner, S.; Widmalm, G.; Ellervik, U. Chemistry of Xylopyranosides, *Carbohydr Res.*, **2015**, *418*, 65-88

3.1. Xylose, proteoglycan and cancer

Xylose is a sugar which is largely isolated from wood and is hence regularly referred to as *wood sugar*. It is one of the most common carbohydrates found on Earth and is also present in proteoglycans (PGs). PGs are produced by mammalian cells and are important for a variety of biological processes.[3] The PG is made up of a core protein attached to various GAG (Glycosaminoglycan) chains via a xylose-bearing polysaccharide linker (fig. 2).

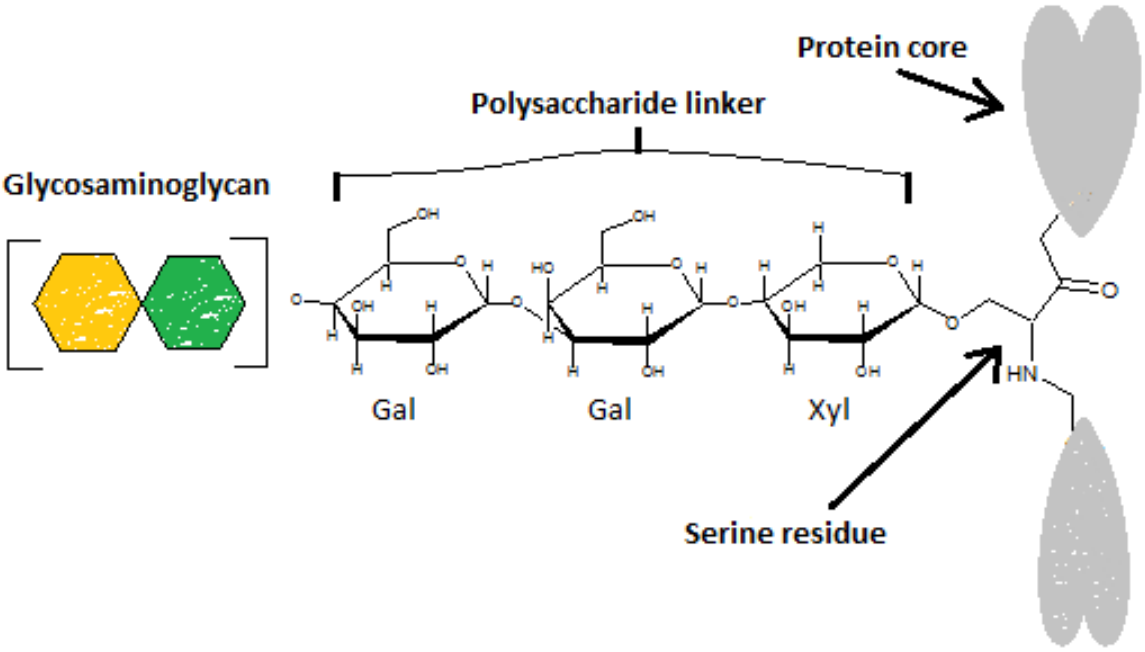


Fig. 2. *The structure of proteoglycan (Gal = Galactopyranose, Xyl = Xylopyranose).*

PG/GAG expression is a crucial part of the pathobiology of every stage in cancer progression including tumor invasion, proliferation and metastasis. One enzyme that plays a crucial role in the biosynthesis of PGs and GAGs is β4GalT7 (β-1,4-Galactosyltransferase 7). It has been shown that controlling the expression of this enzyme subsequently controls PG/GAG expression, thus making the enzyme a promising target in the development of anticancer agents.[4]

3.2. β4GalT7 in biosynthesis and inhibition

β4GalT7 is an enzyme which is essential in the biosynthesis of CS/DS (chondroitin sulfate/dermatan sulfate) and HS (heparan sulfate) GAG chains. Xylosyltranferase initiates the biosynthesis by xylosylating the serine residue on the core protein (fig. 2) before β4GalT7 galactosylates the xylosylated protein. Further enzymatic reactions are carried out until a

[3] Siegbahn, A.; Manner, S.; Persson, A.; Tykesson, E.; Holmqvist, K.; Ochocinska, A.; Rönnols, J.; Sundin, A.; Mani, K.; Thorsson, G. W.; Widmalm, G.; Ellervik, U. Exploration of the active site of β4GalT7: Modifications of the aglycon of aromatic xylosides, *Org. Biomol. Chem.*, **2015**, *13*, 3351-3362

[4] Siegbahn, A.; Manner, S.; Persson, A.; Tykesson, E.; Holmqvist, K.; Ochocinska, A.; Rönnols, J.; Sundin, A.; Mani, K.; Thorsson, G. W.; Widmalm, G.; Ellervik, U. Rules for priming and inhibition of glycosaminoglycan biosynthesis; probing the β4GalT7 active site, *Chem. Sci*, **2014**, *5*, 3501-3508

polysaccharide linker (fig. 2) is formed, which serves as the branching point for CS/DS and HS biosynthesis.

Alternatively, the biosynthesis can be initiated by an exogenous addition of β-D-xylopyranosides carrying hydrophobic aglycons. One such compound is 2-Naphthyl β-D-xylopyranoside (XylNap) (fig. 3) which can act as a β4GalT7 acceptor substrate in the initial galactosylation step, resulting in GalXylNap (i.e. XylNap with a galactose substrate added to it).

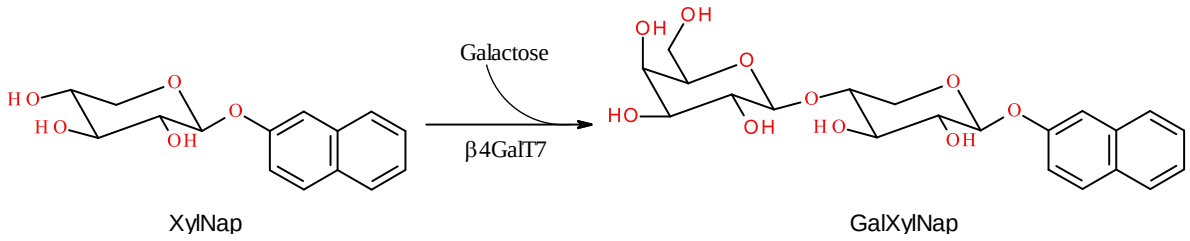


Fig. 3. *GalXylNap made from the galactosylation of XylNap by β4GalT7.*

However, the β4GalT7 binding pocket is very narrow with a specific number of important hydrogen bonds. This means that inhibitors of galactosylation can be rendered by modifying the xylose moiety of the xylopyranoside, which can be a valuable tool for exploring PG and GAG biosynthesis and for developing anti-tumor agents.

3.3. Xylose synthesis

The focus of this thesis will be the modification of the xylose moiety in the xyloside. Compared to various carbohydrates, such as galactose and glucose, xylose doesn't have a primary hydroxyl group and is therefore synthetically challenging.[5] Additionally, all three remaining non-orthogonal hydroxyl groups are secondary and of nearly equivalent reactivity (fig. 4). Small differences do exist though with the C4-OH usually being considered the most reactive and the 2-OH being the second most reactive.

[5] Manner, S.; Ellervik, U. Release of Ring Strain as Driving Force for Inversion of Stereochemistry – Application to the Synthesis of Ribopyranosides from Xylopyranosides, *Synlett*, **2014**, *25*, 1271-1274

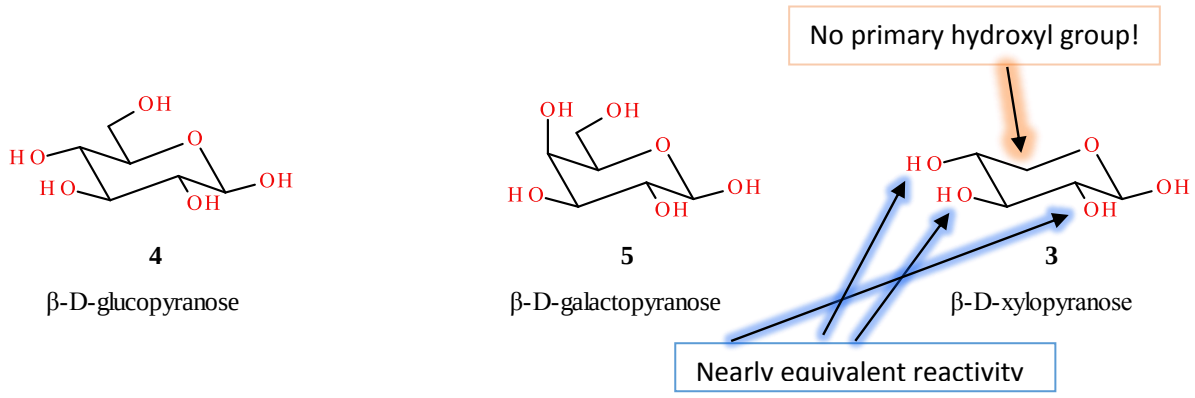


Fig. 4. *Hydroxyl groups contributing to the synthetic challenges of xylose compared to glucose and galactose.*

Nevertheless, it is possible to achieve selective epimerization of the C3 hydroxyl group, turning the xylopyranoside into a ribopyranoside. This is a desired transformation in this thesis and is done via a Swern oxidation-epimerization-reduction sequence (fig. 5). The driving force for the epimerization is the release of ring strain when going to a 1,2-cis-acetal from a 1,2-trans-acetal.

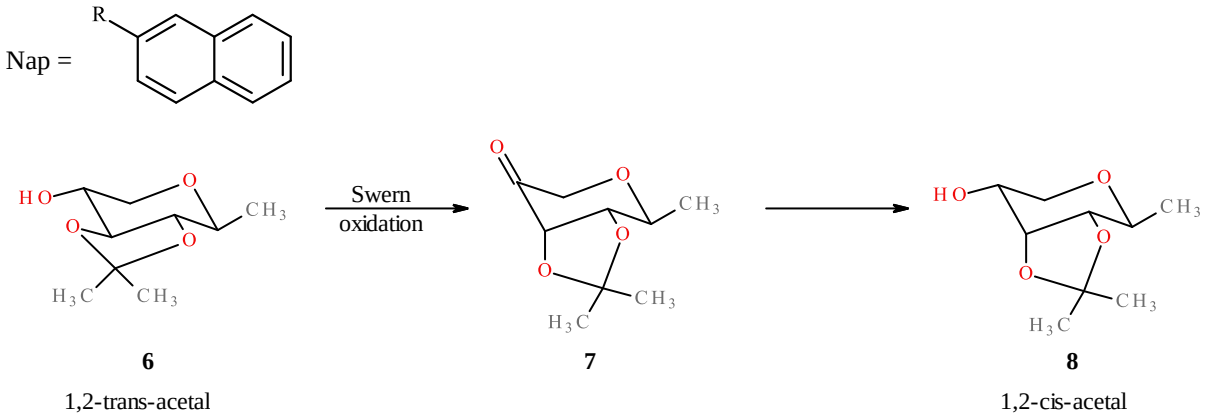


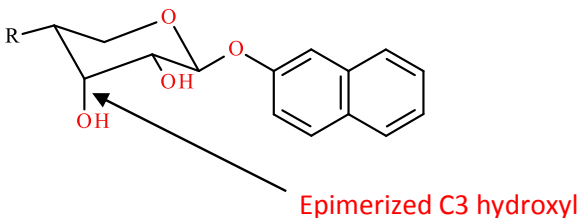
Fig. 5. *Swern oxidation leading to the release of ring strain when going from 1,2-trans-acetal to 1,2-cis-acetal.*

3.4. Synthetic protocol

A synthetic protocol based on selective benzylation and acetal protection was developed by Siegbahn et al. (2011) for the modification of the hydroxyl groups in xylose. Twelve XylNap analogs were synthesized where each hydroxyl group was replaced by hydrogen (deoxygenated), methoxy or fluoro or had been epimerized.

A similar protocol was explored in this thesis. Firstly, the hydroxyl group on position C3 on the xylose moiety was to be epimerized. The hydroxyl group on C4 was then to be replaced by hydrogen, fluorine, a methoxy group and be epimerized, resulting in 4 XylNap analogs (fig. 6). The ability of the analogs to inhibit β4GalT7 and subsequent PG/GAG expression can then be explored, potentially leading to the development of an anticancer agent.

9a: R = OMe
9b: R = epi-OH
9c: R = F
9d: R = H

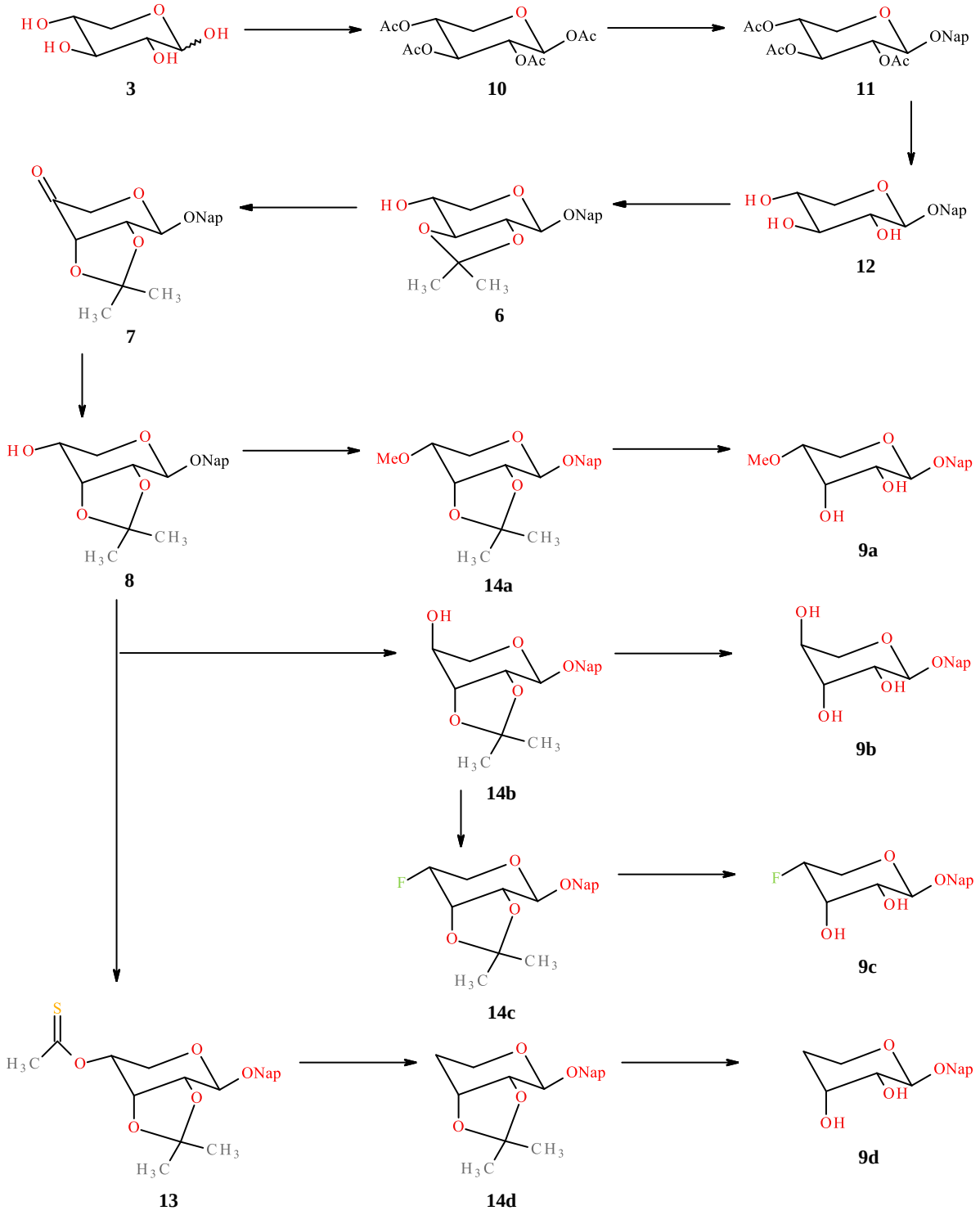


Epimerized C3 hydroxyl

Fig. 6. *Synthetic protocol of compounds intended to be synthesized in this thesis.*

4. RESULTS & DISCUSSION

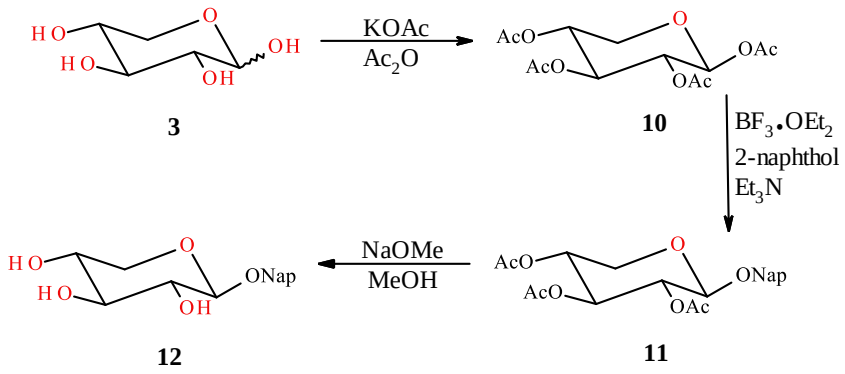
The following scheme summarizes the synthetic procedures that were intended to be carried out in the thesis:



Scheme 1. *Schematic overview of synthetic work.*

4.1.XylNap (2-Naphthyl β-D-xylopyranoside) synthesis

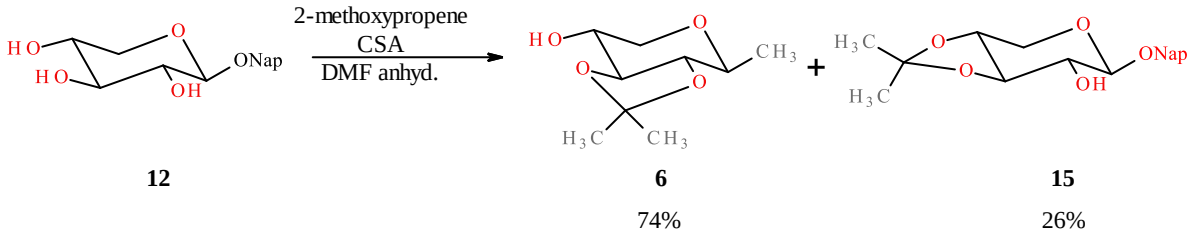
Synthesis of XylNap (sch. 2), **12**, was performed successfully without any significant problems. Peracetylation gave yields slightly lower than reported values (37% compared to 50%) while glycosylation of 2-naphthol gave **11** in good yield as expected. The final de-protection step also gave XylNap in an expected good yield of 76%.



Scheme 2. *Synthesis of 2-Naphthyl β-D-xylopyranoside (XylNap) from D-Xylose.*

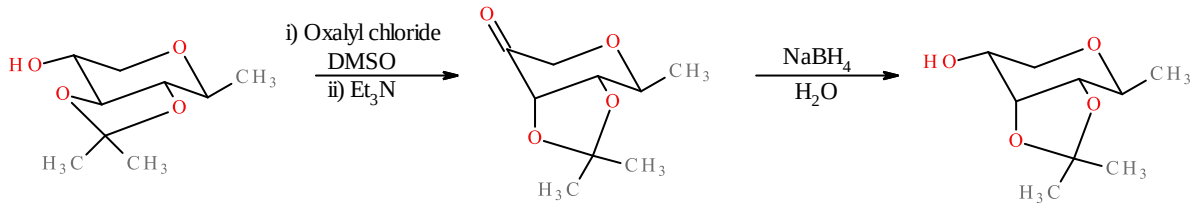
4.2.From XylNap to 2,3-acetal protected RiboNap

The acetal isopropylidene is used to protect the 2-OH and 3-OH of XylNap in order to leave the C4-OH free to be modified at a later step. The reaction gave **6** and **15** in a ratio of 74:26 which is consistent with the ratio of 77:23 published by S. Manner and U. Ellervik (2014) (sch. 3). A fair yield of 42% for **6** was slightly lower than the published value of 52%, probably due to product dilution during flash chromatography resulting in some of the product remaining in the column.



Scheme 3. *Addition of isopropylidene protecting group giving both the 2,3- and 3,4-acetal.*

As explained by S.Manner and U. Ellervik (2014), the strategy for epimerizing the 3-oxygen of the xylopyranoside, and turning it into a ribopyranoside, involves the oxidation of the C4-OH under Swern conditions and the subsequent release of ring strain through the epimerization of the 3-oxygen (sch. 4). **6** was successfully oxidized and epimerized to **7**, but this compound was not isolated. It was instead immediately reduced to give **8** in a very good yield of 83%.



6 **7** **8**

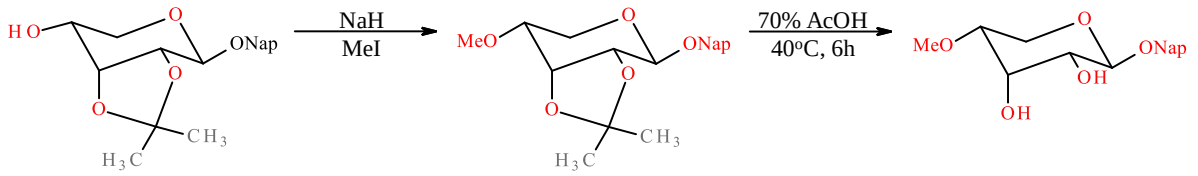
Scheme 4. *Swern oxidation-epimerization-reduction sequence to give ribopyranoside.*

4.3.Modifications of C4-OH

4.3.1. Methylation

Sodium hydride is used to deprotonate the C4 hydroxyl group of **8** before methylation by methyl iodide to give **14a** (sch. 5). A fair yield of 57% was obtained which is similar to published values of 66% for the same reaction with **8** having two benzyl protecting groups instead.

14a was then deprotected by AcOH to give **9a** and the product was identified via ^{1}H NMR and HRMS.



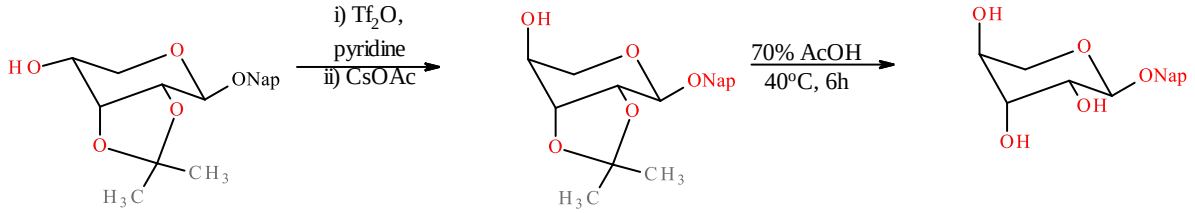
8 **14a** **9a**

Scheme 5. *C4-OH methylation followed by acetal de-protection.*

4.3.2. Epimerization

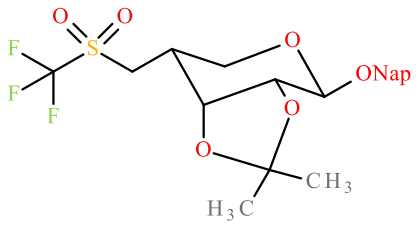
4.3.2.1. Standard method

9b is synthesized in two steps (sch. 6). Firstly, **8** is turned into a triflate (fig. 7), **17**, by trifluoromethane sulfonic anhydride. The triflate was identified by ^{1}H NMR and then immediately substituted in the presence of cesium acetate with the intention to give **14b**. However, ^{1}H NMR indicates the synthesis of a different compound. The two protons at 2.22 and 1.99 ppm indicate that the C4-OH has been deoxygenated, resulting in **14d** (sch. 8). The C4 oxygen was probably cleaved due to a prolonged reaction time of 3 days with cesium acetate and this is further suggested by multiple compounds on the TLC plate obtained after the reaction. Only one isolated compound was detectable and this product was obtained in trace amounts. **9b** was not synthesized.



8 **14b** **9b**

Scheme 6. *Standard epimerization of C4-OH followed by acetal de-protection.*

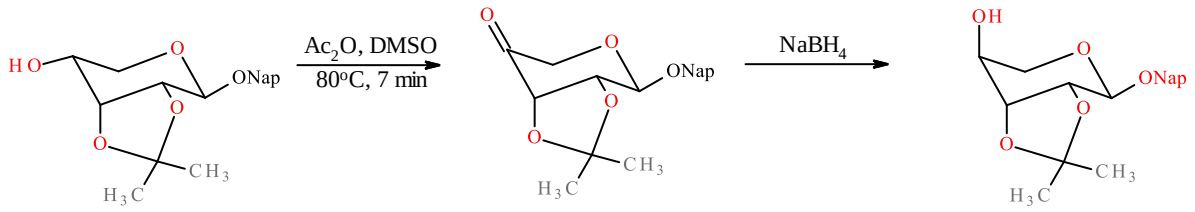


17

Fig. 7. *Molecular structure of the triflate intermediate **17**.*

4.3.2.2. Microwave method

8 was first heated under microwave radiation and oxidized by DMSO to give **7**, which was then reduced by $NaBH_4$ with the intention to give **14b** (sch. 7). ^1H NMR showed that **8,** instead of **14b,** had formed after the reduction of **7**. This is probably because having the OH group in an equatorial position makes it much more electron withdrawing than when in an axial position.[6] This effect is further enhanced by the axially positioned C3 oxygen. If the C4 oxygen is in an axial position as well it will make the molecule very unstable. This will cause the OH group to return to its preferred equatorial position and hence result in the regeneration of **8**. **9b** (sch. 6) was not synthesized.



8 **7** **14b**

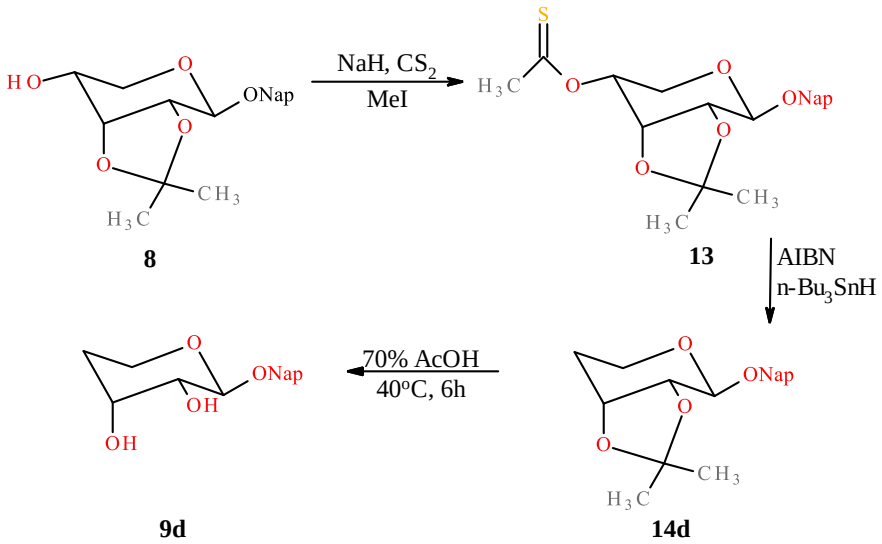
Scheme 7. *Epimerization of C4-OH via the microwave method.*

[6] Rönnols, J.; Manner, S.; Ellervik, U.; Widmalm, G. Conformational effects due to stereochemistry and C3-substituents in xylopyranoside derivatives as studied by NMR spectroscopy, *Org. Biomol. Chem.*, **2014**, *12*, 8031-8035

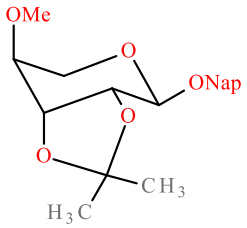
4.3.3. Deoxygenation

The C4-OH of **8** is deoxygenated in two steps. The first step results in the formation of a xanthate, **13**, using methyl iodide and carbon disulfide (sch. 8). The product yield was poor at 34% and the compound was identified using ^{1}H NMR but the peak-values were only used to quickly identify the product before the entire amount was used in the next step.

In the second step AIBN and n-Bu_3SnH is used to deoxygenate the xanthate intermediate to give **14d** (sch. 8). However, ^{1}H NMR indicates that a different product was synthesized. The peak at 2.61 ppm suggests the presence of a methoxy group on C4. Comparing with the ^{1}H NMR of **14a** (sch. 5), the signal is further upfield while the multiplet signal for C4-H at 6.30-6.26 ppm is further downfield for the intended product. This indicates that the methoxy group in the intended product is in an axial position, ultimately giving **16** (fig. 7). The isolation of this substrate could have occurred as a consequence of harsh reflux conditions giving multiple products, as shown on the TLC plate, followed by the solitary detection of the most abundant compound. The product yield was also very poor only giving trace amounts, further indicating that decomposition took place during reflux. This was however not confirmed.



Scheme 8. *Xanthate formation followed by deoxygenation and final acetal de-protection.*



16

Fig. 7. *Molecular structure of the unintended product **16** (2-Naphthyl-2,3-O-isopropylidene-4-epimethoxy β-D-ribopyranoside).*

5. CONCLUSION AND FUTURE WORK

XylNap was successfully synthesized without any significant problems. Subsequent 2,3-acetal protection and C3 epimerization to RiboNap also proceeded well with yields consistent with published values. The modification of C4-OH was more problematic with three of the four reactions giving unintended products. The attempted deoxygenation gave an axial methoxy group instead due to unfavorable reflux conditions, while attempted epimerization either gave the starting material back when oxidizing under microwave radiation or gave a deoxygenated product due to a prolonged reaction time of 3 days after the addition of cesium acetate. Methylation of C4-OH was successful however with a fair yield of 57%. Subsequent de-protection of the methylated compound was also successful, giving one of the intended β-D-ribopyranoside derivatives.

Future work should focus on the epimerization of the C4 hydroxyl group as it was shown to be a problematic transformation. Methods that take into account the large difference in electronegativity between the axial and equatorial positions is advocated as well as appropriate catalysts and reactionary conditions as some of the substrates were shown to be sensitive to the environment. Having appropriate reflux conditions during C4 deoxygenating is also encouraged.

6. EXPERIMENTAL

6.1. General methods

Oven dried glassware was used for all air- and moisture-sensitive reactions and they were carried out under a dry nitrogen atmosphere. All NMR spectra were recorded using a Bruker AVANCE II spectrometer operating at 296 K. All chemical shifts are indicated by ppm values downfield from Me_4Si, with reference to the following residual internals; MeOH-d_4 (3.31), C_6H_6-d_6 (7.16) and $CHCl_3$-d (7.24). Values for coupling constants are given in Hz. COSY (2D homonuclear shift correlation) was used to assign certain ^1H NMR spectra. Monitoring of reactions was done by TLC using alumina plates that were coated with silica gel and the compounds were visualized by staining with para-anisaldehyde or by UV-light. Silica gel (35-70 µm, 60 Å) was used when performing flash chromatography. Solvents were only dried prior to use if stated. No further purification was performed on purchased reagents.

6.2. Synthesis

6.2.1. 1,2,3,4-Tetra-*O*-acetyl β-D-xylopyranose (10)

KOAc (12 g, 123 mmol) was suspended in Ac_20 (165 ml) and the mixture was heated to 140 ^{0}C. (1) (β-D-xylose) (15 mg, 104 mmol) was added in equal proportions over 30 min before being left to react for 2h. The mixture was poured onto ice-water (400 ml), the organic phase was extracted with CH_2Cl_2 (3 x 250 ml) and the combined phases were washed with $NaHCO_3$ (400 ml). The washed solution was dried with $MgSO_4$ and charcoal (4 teaspoons) was added. The mixture was stirred for 30 min before being filtered through a pile of SiO_2 which was then eluted with CH_2Cl_2. The solvent was evaporated and the precipitate was co-evaporated with toluene (twice) before being recrystallized from EtOH to give **10** (12 g, 38 mmol, 37%). TLC (Hep/EtOAc 1:1, r.f. = 0.46); ^1H NMR ($CDCl_3$): δ 5.71 (d, 1H, J = 7.2, C1-H), 5.20 (dd, 1H, J = 8.4, 8.4, C3-H), 5.04 (dd, 1H, J = 8.4, 6.8, C2-H), 4.98 (ddd, 1H, J = 8.4, 8.0, 5.2, C4-H), 4.15 (dd, 1H, J = 12.0, 4.8, C5-H), 3.52 (dd, 1H, J = 12.0, 8.4, C5-H), 2.11 (s, 3H, C1Ac-H), 2.05 (s, 9H, Ac-H).

6.2.2. 2-Naphthyl-2,3,4-*O*-acetyl β-D-xylopyranoside (11)

10 (8.0 g , 25.1 mmol) and 2-naphthol (5.4 g, 37.7 mmol) were dissolved in dry CH_2Cl_2 (80 ml) and Et_3N (1.8 ml, 12.6 mmol) was added followed by a dropwise addition of $BF_3 \cdot OEt_2$ (17ml, 62.8 mmol) and the solution was left to stir for 3h. The reaction was quenched with sat. aq. $NaHCO_3$ (125 ml) and the organic phase was extracted with EtOAc (2 x 250 ml). The combined phases were dried with Na_2SO_4, the solvent was evaporated and the resulting precipitate was recrystallized from EtOH to give **11** (6.9 g, 17.2 mmol, 68%). TLC (Hep/EtOAc 1:1, r.f. = 0.73); ^1H NMR ($CDCl_3$): δ 7.80-7.73 (m, 3H, ArH), 7.48-7.44 (m, 1H, ArH) 7.41-7.36 (m, 2H, ArH) 7.18 (dd, 1H, J = 9.2, 2.4, ArH) 5.34 (d, 1H, J = 6.0, C1-H), 5.29-5.22 (m, 2H, C2&3-H), 5.04 (ddd, 1H, J = 7.6, 7.4, 4.8, C4-H), 4.28 (dd, 1H, J = 12.4, 4.4, C5-H_1), 3.60 (dd, 1H, J = 12.0, 7.6, C5-H_2), 2.10 (s, 9H, Ac-H).

6.2.3. 2-Naphthyl β-D-xylopyranoside (12)

11 (6.5 g, 16.2 mmol) was suspended in MeOH (54 ml), 1M NaOMe (2.7 ml, 52 mmol) was added and the mixture was left to stir for 1h. AcOH (90%) was then added dropwise until pH 6 and the solvent was evaporated. The precipitate was co-evaporated with toluene (4 x 125 ml) and recrystallized from EtOH to give **12** (3.4 g, 12.4 mmol, 76%). TLC (CH_2Cl_2/MeOH 10:1, r.f. = 0.61); ^{1}H NMR (MeOH-d_4): δ 7.79-7.75 (m, 3H, ArH), 7.45-7.41 (m, 2H, ArH), 7.37-7.33 (m, 1H, ArH), 7.26 (dd, 1H, J = 9.2, 2.4, ArH), 5.03 (d, 1H, J = 7.2, C1-H), 3.98 (dd, 1H, J = 11.6, 5.2, C5-H), 3.63-3.58 (m, 2H, C-H), 3.51-3.45 (m, 2H, C-H).

6.2.4. 2-naphthyl-2,3-*O*-isopropylidene β-D-xylopyranoside (6)

12 (2.00 g, 7.24 mmol) and CSA (0.25 g, 1.09 mmol) were suspended in anhyd. DMF (12 ml). 2-methoxypropene (0.47 ml, 4.89 mmol) was added every 20 min in 4 portions and 30 min after the last addition, Et_3N (4 ml) was added. The solvent was evaporated, the precipitate was co-evaporated with toluene (10 times) before the crude was purified via flash chromatography (SiO2, petroleum ether (40-60):diethyl ether + 1% Et_3N 2:1 → 1:1) to give **6** (0.96 g, 3.03 mmol, 42%). TLC (petroleum ether:diethyl ether 1:3 + 1% Et_3N, r.f. = 0.50); ^{1}H NMR (C_6D_6): δ 7.61-7.53 (m, 3H, ArH), 7.49 (d, 1H, J = 9.0, Ar-H), 7.32 (dd, 1H, J = 9.0, 2.5, Ar-H), 7.25 (ddd, 1H, J = 8.0, 6.5, 1.0, Ar-H), 7.17 (ddd, 1H, J = 8.0, 6.5, 1.0, Ar-H), 5.28 (d, 1H, J = 7.5, C1-H), 3.81 (dd, 1H, J = 12.0, 5.0, C5-H), 3.76 (dd, 1H, J = 9.5, 7.5, C2-H), 3.66 (ddd, 1H, J = 8.5, 6.5, 5.0, C4-H), 3.57 (dd, 1H, J = 9.5, 9.0, C3-H), 3.12 (dd, 1H, J = 12.0, 6.5, C5-H), 1.40 (s, 6H, i.p.-H).

6.2.5. 2-naphthyl-2,3-*O*-isopropylidene β-D-ribopyranoside (8)

Anhyd. DMSO (1.05 ml, 14.22 mmol) was added to a solution of 2M oxalyl chloride (3.6 ml, 7.1 mmol) at -78 °C and stirred for 1h. **6** (0.75 g, 2.37 mmol) in anhyd. CH_2Cl_2 (60 ml) was then added dropwise and stirred for 2h followed by Et_3N (5 ml). After an additional 30 min the solution was allowed to reach rt. and left to stir overnight. MeOH (50 ml) was then added and the resulting solution was cooled to 0 °C followed by the addition of $NaBH_4$ (0.25 g, 6.40 mmol). After 5h stirring the reaction mixture was quenched with H_2O, allowed to reach room temperature and the organic phase was extracted with CH_2Cl_2 (4 x 110 ml). The organic phase was dried with $NaSO_4$ and the solvent was evaporated before the crude was purified via flash chromatography (Hep/EtOAc 7:3) to give **8** (0.62 g, 1.96 mmol, 83%). TLC (Hep/EtOAc 1:1, r.f. = 0.49); ^{1}H NMR (MeOH-d_4): δ 7.80-7.76 (m, 3H, ArH), 7.46-7.41 (m, 2H, ArH), 7.35 (ddd, 1H, J = 8.0, 6.8, 1.2, ArH), 7.21 (dd, 1H, J = 8.8, 2.4, ArH), 5.40 (d, 1H, J = 4.0, C1-H), 4.63 (dd, 1H, J = 6.0, 3.6, C3-H), 4.35 (dd, 1H, J = 6.0, 4.0, C2-H), 4.24 (ddd, 1H, J = 9.6, 6.4, 3.6, C4-H), 3.83-3.74 (m, 2H, C5-H), 1.55 (s, 3H, i.p.-H), 1.43 (s, 3H, i.p.-H).

6.2.6. 2-Naphthyl-2,3-*O*-isopropylidene-4-methoxy β-D-ribopyranoside (14a)

8 (150 mg, 474 µmol) was dissolved in dist. DMF (4 ml), the solution was chilled to 0 °C and NaH (40 mg, 948 µmol) was added. The solution was stirred for 20 min, MeI

(60 µL, 948 µmol) was added and the reaction mixture was allowed to reach room temperature before being left to stir overnight. The mixture was again chilled to 0 °C, NaH (19 mg, 474 µmol) was added, the mixture was stirred for another 15 min and MeI (30 µL, 474 µmol) was added. The reaction mixture was allowed to reach rt. and stirring continued for 2h 30 min before the reaction was quenched with sat. aq. NH_4Cl. The organic phase was extracted with CH_2Cl_2 (2 x 10 ml), washed with brine (12 ml), dried with $NaSO_4$ and the solvent was evaporated. The crude was then purified via flash chromatography (Hep/EtOAc 5:1) to give **14a** (90 mg, 272 µmol, 57%). TLC (Hep/EtOAc 2:1, r.f. = 0.40); ^{1}H NMR ($CDCl_3$): δ 7.79-7.74 (m, 3H, ArH), 7.45 (ddd, 1H, J = 8.4, 7.2, 1.6, ArC5/6-H), 7.41 (d, 1H, J = 2.0, ArC1-H), 7.37 (ddd, 1H, J = 8.0, 6.8, 1.2, ArC5/6-H), 7.22 (dd, 1H, J = 8.8, 2.4, ArC2-H), 5.38 (d, 1H, J = 3.6, C1-H), 4.76 (ddd, 1H, J = 4.0, 4.0, 2.4, C3-H), 4.38 (dd, 1H, J = 6.4, 3.6, C2-H), 3.96 (m, 2H, C5-H), 3.83 (m, 1H, C4-H), 3.51 (s, 3H, OMe), 1.60 (s, 3H, i.p.-H_1), 1.45 (s, 3H, i.p.-H_2); ^{13}C NMR ($CDCl_3$): δ 129.6, 127.8, 127.3, 126.6, 124.5, 118.9, 110.8, 98.4, 76.0, 73.0, 72.1, 60.8, 57.9, 27.2, 25.6; HRMS calcd for $C_{19}H_{22}O_5Na^+$ [M+Na]: 353.1365; found: 353.1364.

6.2.7. 2-Naphthyl-4-methoxy β-D-ribopyranoside (9a)

14a (84 mg, 254 µmol) was dissolved in EtOAc (40 ml), H_20 (6.5 ml) and 90 % AcOH (23 ml) was added and the solution was stirred for 4h at 40 °C. Toluene (25 ml) was added and the solvent was evaporated to give crude (74 mg). ^{1}H NMR showed **9a** with some impurities. No further purification was done. TLC (CH_2Cl_2/MeOH 20:1, r.f. = 0.61); ^{1}H NMR (MeOD): δ 7.77 (dd, 3H, J = 13.6, 9.2, ArH), 7.45-7.41 (m, 2H, ArH), 7.37-7.32 (m, 1H, ArH), 7.24 (dd, 1H, J = 8.8, 2.4, ArH), 5.55 (d, 1H, J = 4.4, C1-H), 4.22 (dd, 1H, J = 3.2, 3.2, C3-H), 3.95 (dd, 1H, J = 12.0, 5.6, C5-H), 3.84 (dd, 1H, J = 12.4, 2.8, C5-H), 3.77 (dd, 1H, J = 4.0, 3.6, C2-H), 3.57 (ddd, 1H, J = 5.6, 3.2, 2.4, C4-H), 3.48 (s, 3H, MeO); HRMS calcd for $C_{16}H_{18}O_5Na^+$ [M+Na]: 313.1052; found: 313.1052.

6.2.8. 2-Naphthyl-2,3-*O*-isopropylidene-4-epi β-D-ribopyranoside (14b) (standard method)

8 (60 mg, 285 µmol) was dissolved in dry CH_2Cl_2 (3.6 ml) and the solution was chilled to -10 °C. Tf_2O (50 µL, 285 µmol) was added followed by a slow addition of pyridine (50 µL, 570 µmol) before the reaction mixture was left to stir for 3h. The mixture was quenched with 0.5 N HCl (3 ml), firstly washed with sat. aq. $NaHCO_3$ (8 ml) and then brine (10 ml). The resulting solution was dried with Na_2SO_4 and the solvent was evaporated, leaving a precipitate which was dissolved in dry DMF (6 ml). Cesium acetate (149 mg, 760 µmol) was added and the solution was heated to 50 °C and left to stir overnight. H_2O (15 ml) was then added, the organic phase was extracted with EtOAc (3 x 25 ml), dried with Na_2SO_4 and the solvent was evaporated before the crude was purified via flash chromatography to give **14d** (trace amounts). TLC (Hep/EtOAc 1:1, r.f. = 0.83); ^{1}H NMR ($CDCl_3$): δ 7.79-7.73 (m, 3H, ArH), 7.43 (ddd, 1H, J = 8.0, 6.8, 1.2, ArH), 7.41 (d, 1H, J = 2.8, ArH), 7.36 (ddd, 1H, J = 8.4, 7.2, 1.6, ArH), 7.23 (dd, 1H, J = 8.8, 2.4), 5.47 (d, 1H, J = 4.0, C1-H), 4.57 (dt, 1H, J = 6.0, 4.4, C3-H), 4.25 (dd, 1H, J = 6.0, 4.0, C2-H), 3.89-3.86 (m, 2H, C5-H), 2.22-2.14 (m,

1H, C4-H), 1.99 (dq, 1H, J = 14.8, 4.4, C4-H), 1.56 (s, 3H, i.p.-H), 1.42 (s, 3H, i.p.-H);

6.2.9. 2-Naphthyl-2,3-*O*-isopropylidene-4-epi β-D-ribopyranoside (14b) (microwave method)

8 (200 mg, 632 µmol) was dissolved in Ac_2O (1.5 ml) and DMSO (3 ml). The solution was microwaved for 7 min at 80 °C and H_2O (5 ml) was added. The organic phase was extracted with CH_2Cl_2 (3 x 15 ml), washed with brine (40 ml) and dried with Na_2SO_4 before the solvent was evaporated. The resulting precipitate was dissolved in dry MeOH (15 ml) and the solution was chilled to 0 °C. $NaBH_4$ (74 mg, 1706 µmol) was added and the reaction mixture was stirred for 4h and then quenched with H_2O (25 ml). The organic phase was extracted with CH_2Cl_2 (4 x 50 ml), dried with Na_2SO_4 and the solvent was evaporated to give **8** (94 mg, 297 µmol, 44%). TLC (Hep/EtOAc 1:1, r.f. = 0.46); ^{1}H NMR ($CDCl_3$): δ 7.79-7.74 (m, 3H, ArH), 7.46 (ddd, 1H, J = 8.0, 6.8, 1.2, ArH), 7.42 (d, 1H, J = 2.4, ArH), 7.37 (ddd, 1H, J = 8.0, 6.8, 1.2, ArH), 7.20 (dd, 1H, J = 8.8, 2.4, ArH), 5.59 (d, 1H, J = 3.2, C1-H), 4.60 (dd, 1H, J = 6.8, 4.4, C3-H), 4.44 (dd, 1H, J = 6.8, 3.2, C2-H), 4.23-4.16 (m, 1H, C4-H), 3.92 (dd, 1H, J = 11.2, 4.4, C5-H), 3.77 (dd, 1H, J = 10.8, 7.6, C5-H), 1.60 (s, 3H, i.p.-H), 1.46 (s, 3H, i.p.-H);

6.2.10. 2-Naphthyl-2,3-*O*-isopropylidene-4-deoxy β-D-ribopyranoside (14d)

8 (60 mg, 190 µmol) was dissolved in dry THF (4.4 ml) and the solution was chilled to 0 °C. 60 % NaH (23 mg, 380 µmol) was added and the reaction mixture was stirred for 20 min before being allowed to reach rt. After a further 40 min, CS_2 (0.12 ml, 1.90 mmol) was added. Stirring continued for another hour before MeI (40 µL, 570 µmol) was added. 2h later, the solvent was evaporated and the resulting precipitate together with AIBN (29 mg, 152 µmol) was partially dissolved in dry toluene (5 ml). n-Bu_3SnH (0.77 ml, 2.85 mmol) was added, the solution was heated to reflux and refluxed for 1h. The solvent was evaporated and the crude was purified via flash chromatography to give **16** (trace amounts). TLC (Hep/EtOAc 3:1, r.f. = 0.54); ^{1}H NMR ($CDCl_3$): δ 7.77 (dd, 3H, J = 14.4, 8.4, ArH), 7.48-7.43 (m, 2H, ArH), 7.38 (ddd, 1H, J = 8.0, 6.8, 1.2, ArH), 7.24 (dd, 1H, J = 9.2, 2.8, ArH), 6.30-6.26 (m, 1H, C4-H), 5.60 (d, 1H, J = 2.4, C1-H), 4.83 (dd, 1H, J = 6.8, 4.0, C3-H), 4.47 (dd, 1H, J = 6.8, 2.4, C2-H), 4.05-3.96 (m, 2H, C5-H), 2.61 (s, 3H, OMe), 1.63 (s, 3H, i.p.-H), 1.44 (s, 3H, i.p.-H);

7. ACKNOWLEDGEMENTS

Firstly, I would like to give thanks to Ulf Ellervik for giving me the opportunity to do my thesis work in group UE. I would also like to thank Sophie Manner for her guidance and support when I needed it the most and the additional help received from the other group members, Dennis and Daniel. Lastly, I would also like to thank my late mother and brother for the love I was blessed to share with them. You remain always in my heart.

8. REFERENCES

1. Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*, New York, Oxford University Press Inc., New York, **2009**, 1041
2. Manner, S.; Ellervik, U. Release of Ring Strain as Driving Force for Inversion of Stereochemistry – Application to the Synthesis of Ribopyranosides from Xylopyranosides, *Synlett*, **2014**, *25*, 1271-1274
3. Rönnols, J.; Manner, S.; Ellervik, U.; Widmalm, G. Conformational effects due to stereochemistry and C3-substituents in xylopyranoside derivatives as studied by NMR spectroscopy, *Org. Biomol. Chem.*, **2014**, *12*, 8031-8035
4. Rozpedek, W.; Pytel, D.; Mucha, B.; Leszczynska, H.; Diehl. J. A.; Majsterek, I. The Role of the PERK/eIF2α/ATF4/CHOP Signaling Pathway in Tumor Progression During Endoplasmic Reticulum Stress, *Curr. Mol. Med.*, **2016**, *16*, 533-544
5. Siegbahn, A.; Manner, S.; Persson, A.; Tykesson, E.; Holmqvist, K.; Ochocinska, A.; Rönnols, J.; Sundin, A.; Mani, K.; Thorsson, G. W.; Widmalm, G.; Ellervik, U. Rules for priming and inhibition of glycosaminoglycan biosynthesis; probing the β4GalT7 active site, *Chem. Sci*, **2014**, *5*, 3501-3508
6. Siegbahn, A.; Thorsheim, K.; Ståhle, J.; Manner, S.; Hamark, C.; Persson, A.; Tykesson, E.; Mani, K.; Thorsson, G. W.; Widmalm, G.; Ellervik, U. Exploration of the active site of β4GalT7: Modifications of the aglycon of aromatic xylosides, *Org. Biomol. Chem.*, **2015**, *13*, 3351-3362
7. Thorsheim, K.; Siegbahn, A.; Johnsson, R. E.; Stålbrand, H.; Manner, S.; Widmalm, G.; Ellervik, U. Chemistry of Xylopyranosides, *Carbohydr Res.*, **2015**, *418*, 65-88