TOWARDS THE SYNTHESIS OF A STABLE α-GALACTOSIDASE INHIBITOR

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i. Preface
This master thesis is the result of the laboratory work performed by the author in the bioorganic group at the Department of Chemistry, Aarhus University under supervision of associate professor Henrik Helligsø Jensen. The work was performed from September 2009 until August 2010, and this report is the final part to obtain the Master of Science degree.

First and foremost I would like to thank associate professor Henrik Helligsø Jensen for giving me the great opportunity to work in the bioorganic group on this interesting and challenging project. Although he is cheering for a rival soccer team “FC Midtjylland”, I would like to thank him for his professional assistance regarding theoretical and practical problems and for giving great advices and ideas.

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**ii. Abbreviations and acronyms**

α-GAL = α-galactosidase A  
AFG = 1-azafagomine  
AUS = australine  
AX = alexine  
bs = broad singlet  
cat = catalytic  
C = celsius  
cHex = cyclohexyl  
COSY = Correlation spectroscopy  
CSA = camphor-10-sulfonic acid  
d = doublet  
DCC = dicyclohexylcarbodiimide  
dd = double doublet  
dddd = double double double doublet  
DEPT = distorienless enhancement by polarization transfer  
DGJ = 1-deoxy-galactonojirimycin  
DMAP = 4-(N,N-dimethylamino) pyridine  
DMF = N,N-dimethylformamide  
DMP = Dess-Martin periodinane  
DMSO = dimethyl sulfoxide  
E. = Escherichia  
Eq = equivalent(s)  
ER = endoplasmatic reticulum  
ESI = electro spray ionization  
FG = fagomine  
Gal = galactose  
Gb3 = globotriasylceramide  
gem = geminal  
Glc = glucose  
hr = hour  
HMOC = heteronuclear multiple quantum coherence  
HRMS = high resolution mass spectroscopy  
HWE = Horner-Wadsworth-Emmons  
Hz = Hertz  
i = ipso  
IBX = 2-iodoxybenzoic acid  
K_i = inhibition constant  
IFG = isofagomine  
LA = lewis Acid  
LRMS = low resolution mass spectroscopy  
m = meta  
min = minutes  
Ms, mesyl = methanesulfonyl  
MS = molecular sieves  
NJ = nojirimycin  
NM = noeuroycin  
NMR = nuclear magnetic resonance  
NS = noeurostegine  
o = ortho  
PG = protecting group  
Piv, pivaloyl = trimethylacetyl  
p = para  
ppm = parts per million  
R_f = retention factor  
rt = room temperature  
s = singlet  
SET = single-electron-transfer  
t = triplet  
TBAB = tetrabutylammonium bromide  
TBAF = tetrabutylammonium fluoride  
TBDMs = t-butyldimethyl silyl  
TBDOs = t-butyldiphenyl silyl  
TES = triethyl silyl  
Tf, triflyl = trifluormethane sulphonyl  
TFA = trifluoroacetic acid  
THF = tetrahydrofuran  
THT = tetrahydrothiophene  
TIPS = triisopropyl silyl  
TMS = trimethyl silyl  
TOF = time of flight  
Tr or trityl = triphenylmethyl  
TLC = thin layer chromatography  
q = quartet  
Å = Angstrom
iii. Abstract

Glycoside hydrolases exhibit an important role in the biological world, and compounds which are able to bind to these enzymes can have a beneficial effect. The goal of this project is to synthesize a stable α-galactosidase inhibitor, in the quest for finding the optimal therapeutic treatment for the rare lysosomal storage disorder, Fabry-Andersons disease. At first thought it seems peculiar that an inhibitor in the active site of an enzyme will enhance the activity, thus it is believed that the inhibitor will act as a chemical chaperone; hence regain the enzymes activity. Chemical chaperones exist for α-galactosidase with great inhibition strength but due to the low stability of the compound, the synthesis of a novel inhibitor is of great interest.

Adding a ethylene bridge to the known inhibitor, galactonoeuromycin, will potentially retain some binding affinity for galactosidases, thus still be stable at neutral pH. Synthesizing an analog with galactose configuration of the naturally occurring pyrrolizidine, Australine, and test the properties of this compound is also of great interest.

This thesis presents a synthesis approach towards the potential α-galactosidase inhibitors, galacto-noeurostegine 1 and 2-epi-australine 2. The common synthetic pathway toward 1 and 2 includes an interesting regioselective ring opening of a benzylidine acetal, and multiple successful and unsuccessful attempts to substitute with inversion on a secondary carbon. The synthesis of 1 ended prematurely at the nitrile 76, thus the alcohol prepared 72 is potentially an intermediate towards the synthesis of 2. An inhibitor was not synthesized in the given time, but the work on this project will resume as soon as possible.
iv. Resumé

Glycoside hydrolaser udøver en vigtig rolle i den biologiske verden og stoffer som har evnen til at binde til disse enzymer kan have en gavnlig effekt. Målet med dette projekt er at syntesere en stabil α-galactosidasehæmmer i jagten efter at finde den optimale terapeutiske behandling af den sjældne lysosomale aflejringssygdom, Fabry-Anderssons sygdom. Umiddelbart virker det underligt at en hæmmer a det ”active site” af et enzym vil øge aktiviteten, men fordi at det er tænkt at hæmmeren vil opføre sig som en kemisk ”chaperone” og vil derfor gendanne enzymet aktivitet. Kemiske ”chaperones” eksisterer of α-galactosidaser med stærk bindingsstyrke men på grund af at stofferne er ustabile er interessen stor for at finde syntesen af nye hæmmer.

Ved at tilføje en ethylenebro til den kendte hæmmer, galactonoeuromycin, vil bindingsaffiniteten for galactosidaserne potentielt blive bevaret, og stadig være stabilt ved neutrat pH. Syntesen af en analog med galactosekonfiguration af det naturligt forekommende stof, australine, samt teste stoffets egenskaber er også af stor interesse.

Den syntetiske fremgangsmåde med potentielle α-galactosidasehæmmerne, galacto-noeurostegine 1 og 2-epi-australine 2, er fremlagt i denne afhandling. Syntesen mod 1 og 2 følger en fælles syntetisk vej, som involverer en interessant regioselektiv ringåbning af en benzylidenacetal og flere vellykkede og ikke mislykkede forsøg på at substituere med inversion på et sekundært carbon. Syntesen mod 1 stoppede tidligt ved nitrilen 76, men alkoholen 72 er muligvis et intermediat frem mod syntesen af 2. En hæmmer blev ikke fremstillet i den tildelte tid, men projectet vil blive genoptaget så hurtigst som muligt.
1. Introduction

1.1. Background

1.1.1. Total synthesis – aiming for the ideal synthesis

Figure 1

Total synthesis is the art of creating any compound desired, typically naturally occurring compound. Total synthesis test the new methodologies and strategies developed, and today the synthetic chemist is capable of creating almost any molecule with diverse degree of structural complexity. The new stage of organic synthesis is to aim for the “ideal synthesis”. In 1975 Hendrickson defined the “ideal synthesis” as:

“Ideally, the synthesis would start from available small molecules so functionalized as to allow constructions linking them together directly, in a sequence only of successive construction reactions involving no intermediary refunctionalizations, and leading directly to the structure of the target, not only its skeleton but also its correctly placed functionality. If available, such a synthesis would be the most economical, and it would contain only construction reactions.”[1]

Gaith and Baran recently defined percent ideality as being the number of construction- and strategic redox-reactions times 100 divided by the total number of reactions (Equation 1).[2]

\[
%\text{ideality} = \frac{(\text{number of construction reactions}) + (\text{number of strategic redox reactions})}{(\text{total number of steps})} \times 100\%
\]

Equation 1

Construction reactions are those which form skeletal bonds, and the strategic redox reactions transform a functional group into the functionality found in the final product. The rest fall into the category of concession steps (includes: non-strategic redox-reactions, protecting group manipulations and functional group interconversions), since they require extra effort but are often unavoidable. The percent ideality is a term that can evaluate the work done together with overall yield and number of steps.
1.1.2. Carbohydrates
Carbohydrates are the most abundant group of natural products, and are commonly presented in the formula \( \text{C}_n(\text{H}_2\text{O})_m \). Carbohydrates in nature are produced in the chloroplast of plant cells from carbon dioxide, water and sun-light and are the main fuel source in most cells. Carbohydrates mainly exist as the 6-membered ring; the pyranose and the 5-membered ring; the furanose ring, and the cyclic structure is formed either as an aldose or ketose. Carbohydrates exist as monosaccharides, oligo- and polysaccharides and carboconjugates. Glucose is the primary only used fuel source for the human brain, and low-glucose concentration will impair some psychological function. Sucrose (Scheme 1) is a disaccharide derived from fructose and glucose and is more commonly known as table sugar. An example of polysaccharides built of glucose units are cellulose (Scheme 1), which assist in determine the structure of plants and cannot be digested by mammals due to lack of enzymes capable of hydrolyzing the 1,4-\( \beta \)-glucosidic bonds. Glucogen and starch (Scheme 1) are other examples of polysaccharides consisting of glucose. Ribose, a furanose, is an important component of the backbone of ribonucleic acid (RNA) and in coenzymes such as adenosine triphosphate (ATP) (Scheme 1), flavin adenine dinucleotide (FAD) and Nicotinamide adenine dinucleotide (NAD).

The diversity of the high number of various glycosidic combinations introduces high informational content in carbohydrates. This is especially seen with the carbohydrates being involved in cell-cell recognition, fertilization etc.\(^3\)

Scheme 1

1.1.3. Lysosomal Storage Diseases
Lysosomes are spherical organelles in the cell responsible for digesting engulfed viruses or bacteria, old organelles and food particles. The lumen is acidic with a pH around 4.5 compared to the cytosol with a pH
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around 7.0-7.4,\textsuperscript{[4]} which allows the large number of digestive enzymes (eg. Lipases, Amylases) to function properly. These enzymes are synthesized in the cytosol and modified by attaching mannose-6-phosphate in the endoplasmatic reticulum, allowing it to be transported into the lysosomes.

Some of these enzymes are involved in the stepwise degradation of glycosphingolipids. Reduced or none activity of one of these enzyme will lead to accumulation of the substrates resulting in a specific lysosomal storage disease like Gaucher’s disease, Tay-Sachs disease and angiookeratoma corporis diffusum (Fabry-Andersons disease or in short: Fabry’s disease). A lysosomal storage disease has an estimated prevalence of 1 out of 7,700 individuals and Fabry’s disease an estimated prevalence of 1 out of 117,000.\textsuperscript{[5]} In the case of Fabry’s disease, Brady et al.\textsuperscript{[6]} discovered in 1967 that reduced activity of the lysosomal enzyme $\alpha$-Galactosidase A retarts the conversion of Globotriasylceramid to Lactosylceramide (Scheme 2, Page 5, A). The diminished activity is often caused by a genetic defect in the gene encoding for the enzyme, making it unable to fold properly. The modification by the endoplasmatic reticulum of the misfolded enzymes will be retarted. The enzymes are unstable at around pH 7.0 so it will be degraded, even though they might have enzymatic activity in the presence of a substrate.

Gb3 accumulation in the lysosomes eventually leads to rupture of the cytoplasma and as a consequence: cell death.\textsuperscript{[7]} Secondary effects of Gb3 accumulation is inflammation and induces oxidative stress.\textsuperscript{[8]}

Fabry’s disease is an X-linked recessive disorder, hence symptoms are more commonly found in male patients. The symptoms are often first experience in early childhood, and Fabry’s disease is often misdiagnosed due to the rarity of the disease The symptoms include:\textsuperscript{[8]}

1) Renal failure which is a common cause of premature death due to the disease. The first sign is often proteinuria, in which the urine possesses excess serum proteins in the urine, causing the urine to be foamy.

2) Cardiac complication due to accumulation of glycolipids in different hearts cells. Hypertension and cardiomyopathy are often observed symptoms. Cardiac involvement is also a common cause of premature death in the disease.

3) Neuropsychiatric symptoms as patients with Fabry’s disease were significantly more depressed and exhibits lower cognitive performance.

4) Angiokeratomas, which is tiny little papules that can appear anywhere on the body

5) Anhidrosis which is lack of sweating.

6) Etc.
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1.1.4. The chemical chaperone
Current treatments of these diseases are: enzyme replacement therapy, bone marrow transplantation and substrate reduction therapy, thus a novel therapy is the use of a active-site-specific chaperone. This was first demonstrated by Fan et al. in 1999[9], and some of their result are described next.

1-Deoxy-galactonojirimycin (Scheme 4, Page 9, gal-DNJ) is a competitive inhibitor of α-galactosidase A. Gal-DNJ has enhanced α-GAL activity by 8-fold in lymphoblast derived from Fabry patients, and even a slight enhanced activity in normal lymphoblast. Another experiment show that the enzyme activity was elevated with increasing gal-DNJ concentration between 0.2-20 µM, but the enzyme activity decreased at higher concentration of gal-DNJ than 20 µM. In vitro studies showed that the enzyme activity of the mutant α-galactosidase A was retained at pH 5.0 and pH 6.0 but lost activity at pH 7.0. In the presence of gal-DNJ at pH 7.0 the activity was retained. Enhanced enzyme activity was also reported in transgenic mice, with gal-DNJ added in their drinking water for a week.

A mechanism is suggested for the chemical chaperone therapy but first a description of the pathway of a wild-type α-GAL enzyme. The enzyme will be synthesized in the cytoplasm and secreted into the endoplasmatic reticulum. The enzyme will be assembled and folded correctly, including the attachment of mannose-6-phosphate. This attachment is a part of the quality-control system in the ER, which allows it to be transported to the Golgi apparatus for further maturation. From the Golgi apparatus it will be transported into the lysosomes were the enzymes function will be utilized. Genetic mutation can lead to proteins which are unable to follow the wild-type enzyme pathway. The mutant proteins will be transported out of the ER and degradated by proteasomes.

Addition of a chemical chaperone can lead the enzyme back on track by one of the following mechanism:

1) Interaction with the protein while it is folding, lowering the proteins folding time, which reduced the chance that the quality control will recognize the protein as unfolded.
2) Bind to the protein once it is fully folded, stabilizing is making it less prone to unfold or to be recognized as unfolded.
3) Stabilize the protein and promote the normal posttranslational modifications.
4) Stabilize the enzyme in the environments which otherwise would promote unfolding, like low pH.
5) Induce or stabilize interactions with a binding partner which is required for following the pathway.
6) A combination of the above.

When using an active site chemical chaperone, the chaperone will be outperformed by the natural substrate, and the chaperone can return to another cycle.
**1.1.5. The target enzyme - α-Galactosidase A**

α-GAL is in a large family of enzymes called glycosidases, or glycoside hydrolases. Glycosidases hydrolyses the glycosidic bond between carbohydrates or carboconjugates, a process that uncatalyzed is very slow as the glycosidic bond is very stable. Glycosidases are virtually found in every domain of life, as they play an important role in many biochemical functions. The glycosidases in saliva and the intestinal tract degrade complex carbohydrates which subsequently will be absorbed and used as fuel. β-galactosidases is essential in the gene expression in *E. Coli*.

α-GAL catalyzes the removal of a terminal α-galactose residue from polysaccharides, glycolipids and glycopeptides. As mentioned *vide supra*; the enzyme catalyzes the conversion of globotriaosylceramide into lactosylceramide and galactose in the lysosomes (Scheme 2, A). α-GAL also has the ability to convert human blood type B to blood type 0 by catalyzing the second reaction shown in (Scheme 2, B).[10]

![Scheme 2](image)

Guce *et al.* has investigated the mechanism of human α-GAL, by X-ray crystallography of 4 different crystal structures.[11] Human α-GAL is a homodimer. Each monomer consist of an N-terminal (β/α)₈ barrel domain which contains the active site and a C-terminal antiparallel β domain. The two active sites in the homodimer are separated by 43 Å. There is no structural evidence for cooperativity in the dimer.
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Guce et al. have determined four crystal structures at different stages of the catalytic cycle (Figure 2).

![Diagram of the catalytic cycle](image)

**Figure 2**

In the “empty” active site the ligand is replaced by water molecules. In the substrate bound crystal structure, the galactoside of melibiose (D-Gal-α(1→6)-D-Glc) is in a realxing $^4C_1$ chair conformation (Figure 2, **E-S**), which is also the most stable conformation for galactose outside the enzyme. The enzyme shows specificity for the terminal α-galactoside and only little specificity beyond the glycosidic bond. The protonated aspartic acid residue D231 is catalyzing the nucleophilic substitution of the glycosidic bond with aspartic acid residue D170. The formation of the covalent bonded intermediate, involves a $^4H_3$ half-chair conformation of the galactoside transition state (Figure 2, **TS1**). The covalent bonded intermediate exist in a $^1S_3$ skew boat conformation (Figure 2, **E-INT**), and is stabilized by van der Waals interactions between tryptophan residue W47 and C4, C5 and C6 of the galactoside (not shown). The aspartic acid residue D231 catalyzes the hydrolysis, and the transition state is again in a $^4H_3$ half-chair conformation (Figure 2, **TS2**). The product α-galactose have retained the α-configuration at the anomeric center, hence α-GAL is
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classified as a retaining glycoside hydrolases. In the animal enzyme the enzyme only bounds α-galactose when treated with an α/β-mixture of galactose (Figure 2, E-P).

Another interesting report by the x-ray crystallographic studies by Guce et al. [11] was the discovery of a second ligand binding site in α-GAL. The secondary ligand binding site is specific for β-galactose when treated with an α/β-mixture of galactose. This discovery can lead to another approach for finding a chemical chaperone, which can stabilize an enzyme without being a competitive inhibitor.

Petsko et al. investigated the pH dependence in the wild-type α-GAL enzyme in detail, and found the same activity-relation as Fan et al. [9], but x-ray crystallography showed no change in the 3D structure. [12] This is probably because of the disulfide bridge at the mouth of the active site and elsewhere makes it impossible to make such conformational changes. They performed differential scanning calorimetric studies to determine that the wild-type enzyme is more stable at pH 5.2 compared to pH 7.4, towards denaturation. An inhibitor will in both cases stabilize the enzyme. From the different chemical chaperone mechanisms in the case of Fabry’s disease, Petsko et al. suggest that the chaperone stabilizes the enzyme at the neutral pH-environment of the ER, promoting the proteins export from the ER. The result suggests that the chemical chaperone needed for treatment, does not have to make conformational changes to α-GAL.

As briefly mentioned vide supra, α-GAL catalyzes the hydrolysis with retention of stereochemistry at the anomeric position. Another retaining enzyme is E. Coli β-galactosidase which retain the β-configuration of the substrate after hydrolysis. [13] A mechanistically different class of glycosidases catalyzes the hydrolysis with inversion of stereochemistry at the anomeric position; hence called inverting glycosidases. To the best of my knowledge no inverting galactosidase is discovered thus the mechanism of the inverting glycosidases will be based on inverting glucosidases. [14] The active-site is similar for the two classes as they both possess two carboxyl functionalities which are involved in the mechanism of the hydrolysis. Inverting glycosidases operate via a direct displacement of the leaving group; hence do not form a covalent intermediate and only involve one transition state (Figure 3).

The primary distinction between the two classes of glycosidases is the distance between the two carboxyl groups. In the retaining enzymes they are close together (4.5-5.5 Å) and in the inverting enzymes the distance is longer (9-9.5 Å) allowing insertion of a water molecule for direct displacement.
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**Figure 3**

**1.1.6. Sugar mimics**

Alkaloids which are sugar mimics are believed to be widespread in plants and microorganism. The fact that they are sugar-mimics makes them able to inhibit glycosidases, hence potential pharmaceutical drugs for treatment of various diseases. This field of chemistry has shown major interest in recent carbohydrate chemistry.

Currently some commercially available drugs exist. Acarbose (Scheme 3) as a \( \alpha \)-glucosidase inhibitor is a drug for the treatment of diabetes mellitus type 2 and was on the marketed in 1990 (GlucoBay\textsuperscript{TM}).\textsuperscript{[15]} Miglitol (Scheme 3), developed by Bayer Company, is another drug for this treatment and has been licensed as Glyset since 1996.

**Scheme 3**

**Piperidines**

The first naturally occurring glucose mimic discovered was nojirimycin in 1966, and was described as an antibiotic (Scheme 4, NJ).\textsuperscript{[16]} \( \text{NJ} \) is found to be a good inhibitor toward human lysosomal \( \alpha \)-glucosidase (IC\textsubscript{50} = 9 \( \mu \)M) and \( \beta \)-glucosidase (IC\textsubscript{50} = 19 \( \mu \)M).
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Scheme 4 – *naturally occurring sugar mimics*

Other naturally occurring sugar mimics are nojirimycin B (Scheme 4, man-NJ) and galactostatin (Scheme 4, gal-NJ).[16–17] Gal-NJ is a very potent inhibitor of various α-D-galactosidases (E. coli, Kᵢ ~0.7 µM) and β-D-galactosidases (E. coli, Kᵢ = 45 nM).[18] The more stable 1-deoxy derivaties (Scheme 4, DNJ etc.) can be prepared by catalytic hydrogenation with a palladium catalyst or by sodium borohydride treatment.[17] Gal-DNJ is a powerful inhibitor of α-D-Galactosidase[18b] (E. coli, Kᵢ = 240 nM; coffee beans, Kᵢ = 1.6 nM) and is selective as gal-DNJ is only a modest inhibitor of β-galactosidase (E. coli, Kᵢ = 12.5 µM).[18] More importantly gal-DNJ is also a powerful inhibitor of α-Gal (IC₅₀ = 4.7 nM).[9]

A problem with iminosugars is that they are hydrophilic, which limits the drug potential as they are not easily absorbed. Attaching lipophilic groups to the nitrogen is a way to decrease the hydrophilicity.[19] Both gal-NJ and N-butyl-gal-NJ has an IC₅₀ value of 25 µM for human β-galactosidase.[20]

Fagomine (Scheme 4, FG) is isolated from Japanese buckwheat, and is the 1,2-didexoy analog of NJ. In Isofagomine (Scheme 4, IFG) the nitrogen is moved to the pseudoanomeric position, and where synthesized in 1994 by Jespersen et al.[21] IS was proposed to be a β-glucosidase inhibitor since it was assumed that the the charge developed at the anomeric carbon would be stabilized by β-glycosidases.[22] IFG inhibits the pig liver glucogen phosphorylase (IC₅₀ = 0.7 µM) and is the best binding inhibitor compared to other synthesized Isofagomine analogs by studied performed by Jespersen et al.[23] Inhibition of the human enzyme can potentially be used in the treatment of diabetes mellitus. The galactose analog of IFG (Scheme 4, gal-IFG) is a strong inhibitor of β-galactosidase (Aspargillus Oryzae, Kᵢ = 4 nM) and only a modest inhibitor of α-galactosidase (green coffee beans, Kᵢ = 50 µM).

Noeuromycin (Scheme 4, NM) is a hemiaminal analog of IFG and was discovered by Liu et al. in 2000 on day of the Danish Euro Referendum.[24] NM is an extremely potent inhibitor of α-glucosidase (yeast, Kᵢ = 22 nM) and β-glucosidase (almonds, Kᵢ = 69 nM). The galactose analog of NM (Scheme 4, gal-NM) is

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likewise a potent inhibitor of β-galactosidase (*Aspargillus Oryzae*, $K_i = 35$ nM) and less potent inhibitor of α-galactosidase (green coffee beans, $K_i = 742$ nM).

In general the position of the nitrogen is important regarding selectivity of α- versus β-glycosidases, as seen for the the deoxynojirimycins versus the isofagomines and the noeuromycins. This is desciber further in section 1.1.7.

A problem with NJ, NM and its analogs are the fact that they can undergo Amadori rearrangement. A classic example of Amadori rearrangement is reported by Kuhn and Weygang in 1937, where p-toluidin-D-glucoside 3 was converted to the corresponding Amadori product 4 (Scheme 5, A).[^25]

![Scheme 5](image)

**Scheme 5**

NM undergoes the Amadori rearrangement at neutral pH after 7 hours and the mechanism is depicted in Scheme 5 (B). The hemiaminal initially converts to the Schiff’s base which is in a keto-enol equilibrium. The enol form is then hydrated to give the Amadori product 5. The stability is dependent upon pH as the NM hydrochloride is stable for months. The binding affinity for the NM Amadori product is highly decreased for α-glucosidase (yeast, $K_i = 8.5$ µM) and β-glucosidase (almonds, $K_i = 7.5$ µM).[^26] The 3-C-methyl analog 6 of NM has similar affinity for β-glucosidase (almonds, $K_i = 3.0$ µM) but further reduced affinity for α-glucosidase (yeast, $K_i = 110.0$ µM). These observations suggest that there is no space for an axial substituent on C-3.

Although 1-azafagomine (Scheme 4, AFG) is not a piperidine but a hydrazine and it is included here for comparison. AFG inhibits α-glucosidase (yeast, $K_i = 0.32$ µM) and β-glucosidase (almonds, $K_i = 6.9$ µM).[^27] The weaker binding affinities of AFG compared to the corresponding amines; DNJ and IFG can be explained by...
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the lower base strength. The interaction between the inhibitor and the two acid functionalities in the active site is weaker. AFG has a pKₐ value of 5.3, DNJ has a pKₐ value of 6.7 and IFG has a pKₐ value of 8.4. The galactose analog of AFG; 1-azagalactofagomine (Scheme 4, gal-AFG) has similar binding affinities for β-galactosidase (Aspargillus Oryzae, Kᵢ = 0.04 µM) and α-galactosidase (green coffee beans, Kᵢ = 0.28 µM) compared to gal-NM.

The selectivity of the fagomines is in favor of β-galactosidase, and the nojirimycins favor α-galactosidase. The lack of selectivity of the hydrazine can be explained by the higher versatility, thus it is able to be protonated at both nitrogens.

Pyrrolidines

1,4-dideoxy-1,4-imino-d-arabinitol (Scheme 6, d-AB) is a naturally occurring compound isolated first from Angylocalyx boutiqueanus and later discovered in other distinct species, indicating that it is a common secondary metabolite. The configuration of the 3 hydroxy groups of d-AB resembles that of d-glucose. d-AB is a potent inhibitor of α-glucosidase (yeast, IC₅₀ = 0.18 µM) and a poor β-glucosidase (emulsin, IC₅₀ = 0.20 mM). The synthetic enantiomer, 1,4-dideoxy-1,4-imino-l-arabinitol (Scheme 6, l-AB) is also a lesser potent inhibitor of α-glucosidase (yeast, IC₅₀ = 10 µM). Interestingly in the mouse gut digestive α-glucosidase l-AB (IC₅₀ = 2.0 µM) is more potent inhibitor than d-AB (IC₅₀ = 47 µM).

The naturally occurring 2R,5R-dihydroxymethyl-3R,4R-dihydroxypyrrolidine (Scheme 6, d-DMDP), a fructose analog of NJ, is less potent inhibitor of α-glucosidase (yeast, IC₅₀ = 3.3 µM) compared to d-AB. The synthetic enantiomer (Scheme 6, l-DMDP) is more potent inhibitor of rat intestine sucrose (IC₅₀ = 0.1 µM) than d-DMDP (IC₅₀ = 81 µM).
Towards the synthesis of a stable α-galactosidase inhibitor

1,4-dideoxy-1,4-imino-D-lyxitol (Scheme 6, D-IL) is a potent α-galactosidase inhibitor (coffee beans, \( IC_{50} = 0.5 \, \text{µM}, K_i = 0.13 \, \text{µM} \)) and the enantiomer 1,4-dideoxy-1,4-imino-L-lyxitol (Scheme 6, L-IL) is less potent (coffee beans, \( IC_{50} = 209 \, \text{µM}, K_i = 113 \, \text{µM} \)). It is not surprising that D-IL is a poor inhibitor of α-glucosidase (Rice, \( IC_{50} = 845 \, \text{µM} \)) since the configuration of the 3 hydroxy groups of D-IL resembles that of D-galactose.

Studies by Asano et al. show that the D-sugar mimics are competitive inhibitors in contrast to their L-enantiomers which are noncompetitive inhibitors, hence L-enantiomers bind to an allosteric site. \(^{[34]}\)

Indolizidines

Indolizidines are bicyclic polyhydroxyheterocycles as are the nortropanes and the pyrrolizidines. Indolizidines has one 6-membered ring fused together with a 5-membered ring.

Castanospermine (Scheme 7, CAS) was isolated from the Australian legume Castanospermum australe in 1981\(^{[35]}\) and is found to be a poor competitive inhibitor of α-glucosidase (yeast, \( K_i = 1.5 \, \text{mM} \)) and a potent competitive inhibitor of β-glucosidase (almonds, \( K_i = 1.5 \, \text{µM} \)).\(^{[36]}\) The configuration of the 3 hydroxy groups and the hydroxymethyl group of CAS resembles that of D-glucose, and the hydroxymethyl group is locked in position by the 5-membered ring (Scheme 7).

6-epi-Castanospermine (Scheme 7, 6-epi-CAS) was isolated from the same Australian legume as Castanospermine (Castanospermum australe) in 1986.\(^{[37]}\) 6-epi-CAS was reported be a better inhibitor of α-glucosidase than CAS, however did not inhibit β-glucosidase. 8-epi-Castanospermine (Scheme 7, 8-epi-CAS) has been synthesized but no binding studies has to the best of my knowledge not been performed, thus it could be interesting to evaluate 8-epi-CAS as a potential galactosidase inhibitor.\(^{[38]}\)
Towards the synthesis of a stable α-galactosidase inhibitor

**Pyrrolizidines**

Pyrrolizidines has two 5-membered ring fused together. Alexine (Scheme 8, AX) was the first pyrrolizidine isolated and identified, as it was isolated from the legume *Alexa leiopetala* in 1988.[19] Same year the epimer of alexine; australine (Scheme 8, AUS) was isolated from the same legume as CAS (*Castanospermum australe*).[40] The structure of AUS resembles CAS with the large ring contracted one carbon. AX was reported to be a moderate inhibitor of the β-glucosidase; trehalase (Porcine kidney, IC₅₀ = 4.6 µM), and AUS reported to be a potent inhibitor of α-glucosidase (rat intestinal sucrose, IC₅₀ = 4.6 µM).[41]

![AX](image1.png) ![AUS](image2.png) ![1-epi-AUS](image3.png) ![1-epi-AUS-β-glu](image4.png) ![CAU](image5.png) ![CAU-α-glu](image6.png) ![Validoxylamine A](image7.png)

Scheme 8 - *naturally occurring sugar mimics*

The naturally occurring 1-epi-australine (Scheme 8, 1-epi-AUS) exhibit moderate inhibition of α-glucosidases (rice, IC₅₀ = 280 µM; rat intestinal sucrase, IC₅₀ = 470 µM), which is poorer than AUSs inhibition strength of α-glucosidases.[41] In 1-epi-australine-2-O-β-D-glucopyranoside (Scheme 8, AUS-β-glu) with a carbohydrate attached to 2-O, the inhibition strength is increased (rice, IC₅₀ = 1.8 µM, Kᵢ = 0.22 µM) in a competitive manner. The opposite is observed for casuarine (Scheme 8, CAU) compared to its glucosidic derivative, casuarine-6-O-α-D-glucopyranoside (Scheme 8, CAU-α-glu). CAU-α-glu is reported to be a poor inhibitor of α-glucosidase (rice, IC₅₀ = 440 µM), thus it is interesting that it is a good inhibitor of β-glucosidase (almond, IC₅₀ = 7.0 µM) and a very potent inhibitor of trehalase (porcine kidney, IC₅₀ = 0.34 µM, Kᵢ = 0.018 µM). CAU is in the other hand reported to be a good inhibitor of α-glucosidase (rice, IC₅₀ = 1.2 µM) and also a potent inhibitor of trehalase (porcine kidney, IC₅₀ = 12 µM) and inhibits the enzyme in a competitive manner. It is proven that the porcine kidney trehalase has two subsites, and is the explanation of the high affinity for the disaccharide mimic of the natural substrate, trehalose.[42] Thus
Towards the synthesis of a stable α-galactosidase inhibitor

better inhibitor of porcine kidney trehalase already exist in form of Validoxylamine A (Scheme 8)(IC$_{50}$ = 2.4 nM), which is also a disaccharide mimic.$^{[43]}$

There exist additional types of pyrrolizidines including the Hyacinthacines, however they are not described.$^{[44]}$ The synthesis of pyrrolizidines still show interest as recent total synthesis of AUS and CAS etc. are performed.$^{[45]}$ To the best of my knowledge the galactose analogs of AX (Scheme 9, 2-epi-AX), AUS (Scheme 9, 2-epi-AUS) and (Scheme 9, 2-epi-CAU) have not been reported, neither as naturally occurring nor synthetic compounds. They could be potential galactosidase inhibitors.

Scheme 9 - *not yet reported

**Nortropanes**

Calystegines are in the structural class called nortropanes. The common features of the calystegines are: the nortropane ring system, a hemiaminal functionality positioned at the bridgehead and between 2 to 4 hydroxyl groups in various positions. The calystegines are categorizes into the calystegines A and B according to their relative mobility on paper electrophoresis.$^{[46]}$ Calystegines A have 2, calystegines B have 3 and calystegines C have 4 hydroxyl groups besides from the hemiaminal hydroxyl group. Calystegines N have an aminal functionality instead of the hemiacetal.

Calystegines in general do not inhibit α-glucosidases. Nortropane inhibitors of almond β-glucosidase are Calystegines A3, B1, B2 and C1 and calystegine B₂ is also a potent inhibitor of green coffee bean α-galactosidase (Scheme 10, Table 1).$^{[47]}$ Despite of that calystegine B₂ reported to have greatly lowered the inhibition activities toward the liver α-galactosidase enzymes.$^{[48]}$

Scheme 10 – *naturally occuring sugar mimics

~ 14 ~
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<table>
<thead>
<tr>
<th>Enzyme</th>
<th>A₃ IC₅₀ (µM)</th>
<th>Kᵢ (µM)</th>
<th>B₁ IC₅₀ (µM)</th>
<th>Kᵢ (µM)</th>
<th>B₂ IC₅₀ (µM)</th>
<th>Kᵢ (µM)</th>
<th>C₁ IC₅₀ (µM)</th>
<th>Kᵢ (µM)</th>
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<tr>
<td>β-glucosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>almonds</td>
<td>26</td>
<td>20</td>
<td>4</td>
<td>1.8</td>
<td>2.6</td>
<td>1.2</td>
<td>0.82</td>
<td>0.45</td>
</tr>
<tr>
<td>human liver</td>
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<td>.ᵇ</td>
<td>50</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>α-galactosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>coffee beans</td>
<td>180</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>1.9</td>
<td>0.86</td>
<td>360</td>
<td>90</td>
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<tr>
<td>human liver</td>
<td>410</td>
<td>ndᶜ</td>
<td>-</td>
<td>-</td>
<td>140</td>
<td>185</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β-galactosidase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bovine liver</td>
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<td>30</td>
<td>9.8</td>
<td>1.6</td>
<td>240</td>
<td>46</td>
<td>16</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Table 1 - ⁺less than 50% inhibition at 1000 µM, -a no inhibition, -b not determined.

A general structural feature of the mentioned calystegines is that the hydroxyl groups (2-O, 3-O) are equatorial in the piperidine ring. In the case of Calystegine B₂, the inhibitor can potentially be placed in the binding site with two different orientations (Scheme 10, B₂). A crystal structure determines DNJ to bind a β-glucosidase with the nitrogen placed at the pseudoxygen position (Figure 4, A), and Calystegine B₂ could potentially bind in the same manner (Figure 4, B).⁴⁹ This is not observed as a crystal structure of Calystegine B₂ binding to a β-glucosidase determine the inhibitor to bind in the conformation where the nitrogen is position at the pseudoanomeric position (Figure 4, C).⁵⁰ IFG and CAS bind in the same manner.⁴⁹⁻⁵⁰

![DNJ](image)

![Calystegine B₂](image)

![Calystegine B₂](image)

Figure 4

According to Bredt’s rule, double bonds cannot be placed at the bridgehead unless the rings are large enough.⁵¹ As described vide supra hemiaminals are unstable, but as a consequence to Bredt’s rule, the calystegines remain stable as they are unable to undergo the Amadori rearrangement (Figure 5, A).
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![Diagram A](image1.png)

Figure 5

Recently a novel β-glucosidase inhibitor, noelurostegine (Figure 5, B, NS) was synthesized by Rasmussen and Jensen, which is a hybrid between the strongest binding inhibitor, NM and the stable calystegine B₂.[52] The hemiaminal functionality was stable, as NS dissolved in D₂O at room temperature for 10 days did not show any sign of degradation product. NS inhibits β-galactosidase (Aspargillus Oryzae, $K_i = 23 \mu M$) and α-galactosidase (green coffee beans, $K_i = 2.5 \mu M$). More important, NS is found to be a powerful inhibitor of β-glucosidase (almonds, $K_i = 50 \text{ nM}$) when pre-incubated for 30 minutes, and NS is selective as did not inhibit yeast α-glucosidase at 1mM. The selectivity is presumed to originate from the congested bicyclic structure of NS. When no pre-incubation of the inhibitor with almond β-glucosidase is performed, the $K_i$ value drops to 1.5 µM. The slow binding process is speculated to be a consequence of the slow dynamics of almond β-glucosidase in the binding of the rigid inhibitor. Competitive inhibition was found.

1.1.7. Inhibition of α- versus β-retaining glycosidases

As describes vide supra in section the positioning the nitrogen is important regarding selectivity towards α-versus β glycosidases. When the nitrogen is on the pseudoanomeric position, life IFG the selectivity increases in favor of β-glycosidases. An explanation is proposed by Heightman and Vasella.[53] The active of configuration-retaining glycosidases show similar location for the hydrophobic and hydrophilic amino acid residues interacting with the pyranoside ring. The main differences are the carboxyl functionalities involved in the glycosidic bond hydrolyzes of the substrate. The catalytic nucleophilic acid residue is positioned at the α-face in retaining β-glycosidases, and vice versa for α-glycosidases. When a α-glycosidase binds DNJ, ionic interaction between the protonated amine and the carboxylate exist (Figure 6, A). The corresponding ionic interactions when β-glycosidase binds DNJ is not possible (Figure 6, B). Ionic interactions are present when β-glycosidase binds IFG (Figure 6, C).
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Bols propose a different explanation as the inhibitors are not charged at the time of binding to the enzymes.\(^{[54]}\) In the hydrolysis of an α-glucoside the oxocarbenium ion is readily formed (Figure 7, A); hence the charge in the protonated DNJ is placed at the same position as the charge in the glucosyl cation. Due to stereoelectronic effect the oxocarbenium ion cannot be directly formed from β-glycosides but first form a unstabilized carbocation (Figure 7, B). The charge in IFG mimics the charge of this carbocation; hence IFG is a better inhibitor of β-glycosidases compared to DNJ.

Figure 6 - A DNJ binding in the active site of a α-glycosidase, \(^8\)DNJ binding in the active site of a β-glycosidase, IFG binding in the active site of a β-glycosidase

1.1.8. Transition state mimics
Wolfenden et al. emphasized that glycosidases are exciting target for inhibition since their rate enhancements of hydrolyses are estimated to be over \(10^{17}\) fold compared to the uncatalyzed reaction.\(^{[55]}\) If a compound is designed to mimic the transition state, they could potentially have high affinity for the binding site. The dissociation constant of the glycosidases transition state ES\(^+\) complex is not greater than \(10^{-22}\) M.

Withers encourages some ‘rigorous’ riteria classify whether glycosidase inhibitors are transition state analogue or the so-called fortuitous binders.\(^{[56]}\) To be an analogue the criteria include that the inhibitor should have the best binding affinity at the optimal pH of the enzyme, and the inhibitor should bind to the active form of the enzyme. A satisfactory free-energy relationship must exist between inhibition and catalysis when the inhibitor and the substrate are analogously modified. According to these ‘rigorous’
Towards the synthesis of a stable α-galactosidase inhibitor

criteria the iminisugars cannot be classified as transition state analogues, though it has only been investigated for a few cases.

Still the glycosidase inhibitors can roughly be placed in two different categories: those that mimic the charge of the transition state and those that mimic the conformation. Binding of an inhibitor should also be associated with a large negative enthalpy.

The transition state for the substrate in α-Gal A is in \( ^4 \text{H}_3 \) half-chair conformation and has developed a positive charge (Figure 2, Page 6); hence the ideal inhibitor would have \( ^4 \text{H}_3 \) or the \( ^4 \text{E} \) envelope conformation and a positive charge in the right position. Since no galactosyl transition state mimics have been prepared yet the description is given for the β-glucosidase from Thermotoga maritima (TmGH1). Sinnot suggested that the \( ^4 \text{H}_3, ^3 \text{H}_4 \) (Or their equivalent \( ^4 \text{E} \) and \( ^3 \text{E} \) \( ^2,5 \text{B} \), or \( B_{2,5} \) half- or boat-chair conformations are necessary for transition state (Scheme 11).\(^{[57]}\)

![Scheme 11](image)

The X-ray crystallographic studied for TmGH1 determines the conformations of inhibitor in the active site.\(^{[58]}\) NM, IFG, AFG and Calystegine B\(_2\) exist in the relaxed \( ^4 \text{C}_1 \) chair conformation, hence the conformation are substrate or product mimics.\(^{[50]}\) DNJ exists in a \(^1 \text{S}_3 \) skew-boat conformation. CAS exists in a \(^1 \text{A}_B \) boat conformation. The mentioned substrates are likely to be protonated, in which they are mimicking the charge of the transition state. The mimics with a heteroatom in the position of the anomeric center exhibit hydrogen bonding with the aspartic acid residues. The mimics that posses a nitrogen in the endocyclic oxygen is either distorted or is hydrogen bonding with a water molecule. Gluco-hydroximolactam NR exist as a \( ^6 \text{H}_3 \) half-chair conformation, hence the conformation is mimicking the
conformation of the transition state. NR with a pKa of 4.8 is not basic enough to be protonated, hence is not mimicking the charge.

1.2. Project Idea
Based on the recent results by Rasmussen and Jensen in the discovery NS as a stable and powerful inhibitor of β-glucosidase, the search continues to discover NS-analogs to inhibit other glycosidases. The galactose analog of NS, galacto-noeurostegine 1, could potentially be an inhibitor for galactosidases, hence be used as a chemical chaperone in the treatment of Fabry’s Disease. The galacto-noeurostegine is also expected to be stable against the Amadori rearrangement.

![Scheme 12](image)

The aim for this project is primarily to synthesize the potential inhibitor: galacto-noeurostegine 1. Stability studies of the inhibitor are also an objective in this project. Finally inhibition studies will be performed, to determine how good an inhibitor it is towards relevant glycosidases.

On the path of synthesizing 1, a common intermediate could be used to synthesize a different novel potential inhibitor, 2-epi-australine 2. Inhibition studies will also be performed.
Towards the synthesis of a stable α-galactosidase inhibitor

2. Organic manipulation
This part of the thesis introduces the background regarding a series of the organic manipulation. They are discussed as they are of great relevance regarding the produced results of the master thesis.

2.1. Protecting groups
Protecting groups is an important tool in organic chemistry to induce chemoselectivity, hence prevent unwanted side reactions. A good protecting group is conveniently prepared selectively in a high yield, stable towards the desired conditions and conveniently removed selectively in a high yield. Protecting group is often also employed to make the compounds more lipophilic, hence dissolvable in organic solvents. This section describes the relevant protecting group considerations regarding my project, starting with the acetals.

2.1.1. Isopropylidene ketals
Acetals and ketals are a convenient method to protect diols. In general 5-membered ketals are preferred when prepared it is from the corresponding ketone, and 6-membered acetals are preferred when it is prepared from the corresponding aldehyde. The 5-membered ketal is preferred because the 1,3-diaxial interaction in a 6-membered ring is avoided.

The isopropylidene ketal is also known as an acetonide. The reaction of a carbohydrate like D-glucose with acetone under acidic conditions usually results in a diisopropylidene.\(^{[59]}\) The formation of a 5-membered isopropylidene ketal in a cyclic system requires that the hydroxyl groups of the vicinal diol are cis-configurated to each other. This is exemplified by the hexoses, D-glucose 7 and D-galactose 9, which after reaction adopts the products 8 and 9 respectively in furanose (Scheme 13, A) and in pyranose (Scheme 13, B) configuration. Since the anomeric center is able to mutarotate the 1,2-isopropylidene ketal can potentially always be prepared from carbohydrates bearing a 2-hydroxy group.

Scheme 13

The starting material for the synthesis of 1 has been D-Ribose 11. Even though the compound has been known for a very long time, the crystal structure of crystalline 11 has been unknown until recently.\(^{[60]}\) In an
Towards the synthesis of a stable α-galactosidase inhibitor

aqueous solution, 11 exist as a mixture of α- and β-furanoses and α- and β-pyranoses with β-pyranose configuration as the dominating form.

11 also contain two pairs of cis-hydroxyl groups and thus the diisopropylidene product 12 is expected. Levene and Stiller found that the reaction will however result in the monoisopropylidene ketal product 13 or the condensation product 14 instead (Scheme 14).[61]

Scheme 14
Steric interference from the gem-dimethyl groups hinders the formation of 12, explaining the formation of the 13.

The mechanism is shown in Figure 8. It should be noted that all steps are reversible. Acetone is usually the reagent of choice but another is 2,2-dimethoxypropane. The mechanism is almost identical to the one as described later in the formation of a benzylidene acetal (Figure 14, Page 26). One advantage of using 2,2-dimethoxypropane is that it eliminates methanol instead of water which subsequently can be removed by evaporation. It is however much cheaper to use acetone in excess forcing the equilibrium towards the product.
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Cleavage of the isopropylidine ketal

The method commonly employed to cleave an acetal or a ketal, are with Brøndsted acids like acetic acid. The pivaloyl ester is stable towards these acids but which silyl ethers are not as stable. Aprotic conditions and using lewis acids has also been employed in acetal removal. Ribes et al. report a very mild and efficient method to selectively cleave acetals in the presence of some of the bulky silyl ethers.\cite{62} Initially they used 4 equivalents of anhydrous zinc bromide in dry dichloromethane is used. The reaction stirred at room temperature for a couple of hours, yielding the selective acetal removal in the high yields as shown on Figure 9. Later Ribes et al. discovered that adding a few drops of water did not cause noticeable effect in neither reaction time nor product yield.

\[
\begin{array}{cccc}
\text{O} & \text{CH}_2\text{CH}_2\text{OPG} & \text{ZnBr}_2 & \text{CH}_2\text{Cl}_2, \text{rt} & \text{O} & \text{CH}_2\text{CH}_2\text{OH} & \text{PG} \\
\end{array}
\]

\[
\begin{array}{c}
\text{PG} = \text{TBDMS} & \text{Yield} & 65 \% \\
\text{PG} = \text{TIPS} & 95 \% \\
\text{PG} = \text{TBDPS} & 94 \% \\
\text{PG} = \text{Bn} & 86 \% \\
\end{array}
\]

Figure 9

This procedure works at room temperature, but one should be aware of that heating it to 45-50 °C in wet conditions have been reported to cleave off the TIPS ether after 24 hours by Crouch et al.\cite{63} The mechanism is not clarified for the mild acetal cleavage but proposed by Ribes et al. and shown in Figure 10.\cite{62}

\[
\begin{array}{cccc}
\text{a} & \text{fast} & \text{b} & \text{slow} \\
\end{array}
\]

Figure 10

The Brøndsted acid catalyzed cleavage follow pathway a (Figure 10) and is straightforward. The leaving group in the elimination step is poor, so the cleavage is proposed to be specific-acid-catalyzed. The ZnBr\textsubscript{2} promoted cleavage under anhydrous conditions is suggested to follow pathway b (Figure 10). In a control experiment with cyclohexanone acetals, Ribes et al. was not able to detect the formation of 1,1-dibromocyclohexane,\cite{62} but formation of A (Figure 10) seem likely.
2.1.2. Protecting of a primary alcohol

After the isopropylidene ketal formation, two unprotected functional groups are still present – the hemiacetal at the anomeric center and the primary alcohol. The hemiacetal still needs to be unprotected in the next step of the synthesis; the alcohol however needs to be protected. The protecting group should be capable of selective deprotection in a later stage of the synthesis. As both groups are nucleophilic, the task is to find a protecting group and reaction conditions that will selectively protect the primary alcohol. The protecting group should also be stable towards mild reducing agents and mild acidic conditions. The ability of the protecting group to migrate should also be taken into consideration. A few candidates (Figure 11) are chosen primarily because of the selectivity and a brief description of the preparation and removal is given.

**Figure 11**

**Triphenylmethyl ether (Tr)**

Trityl ether can selectively protect a primary alcohol in the presence of secondary alcohols. Using trityl chloride, triethyl amine and a catalytic amount of DMAP (in DMF, 25 °C, 12 hr) affords the product in 88% yield.\(^{[64]}\) Trityl ether can also selectively protect a primary alcohol in the presence of hemiacetals, using 2,3-isopropylidene ribofuranose as substrate the product is obtained in a yield of 95%.\(^{[65]}\) The trityl group has been known to migrate under acidic conditions.\(^{[66]}\) The trityl group can be cleaved under acidic conditions using reagents like acetic acid\(^{[67]}\) (56 °C, 7.5 hr, 96%), \(p\)-toluene sulphonylic acid\(^{[68]}\) (in MeOH, 25 °C, 5 hr), boron trifluoride etherate\(^{[68]}\) (in CH\(_2\)Cl\(_2\) and MeOH, rt, 2 hr, 80%) or excess zinc bromide\(^{[69]}\) (in CH\(_2\)Cl\(_2\) and MeOH (6:1), rt, 83%). TIPS and TBDPS ethers are stable towards zinc bromide at room temperature.

**Silyl ethers\(^{[70]}\)**

The standard method to prepare a trialkylsilyl ether is using the corresponding trialkylsilyl chloride and a base like triethyl amine, pyridine or imidazole in DMF. The least hindered alcohols are in general silylated, thus the primary alcohol will probably be favored in selectively protection compared to the hemiacetal. The stability of common silyl ethers towards acid-catalyzed hydrolysis are: TMS < TES < TBDMS < TIPS < TBDPS, and the stability towards base catalyzed hydrolysis: TMS < TES < TBDMS ≈ TBDPS < TIPS. Another useful method to deprotect silyl ethers is to use fluoride based reagents like tetrabutylammonium fluoride,
as non-silyl ethers are usually stable towards these conditions. The Si-F bond strength is greater than the Si-O bond strength explaining why the alcohol is deprotected.

Silyl ethers are able to migrate and the migrations are usually acid- or base-catalyzed.

**TMS ether**

The trimethyl silyl ether is the smallest of the silyl ethers given, and the primary ethers are acid and base labile. Selectivity in protection of primary hydroxyls in presence of secondary hydroxyls is probably hard to achieve. Conditions besides using TBAF for deprotection are potassium carbonate\(^{(71)}\) (in MeOH, 0 °C, 45 min, 100%) or citric acid\(^{(72)}\) (MeOH, 10 min, 20 °C, quant.), which are relatively mild conditions.

**TIPS ether**

Selective protection of primary hydroxyls with the triisopropyl silyl group is possible in presence of secondary hydroxyls. Reaction of deoxygenylidine with 1.1 molecular equivalents of TIPSCI and imidazole in DMF yield 82% of the primary hydroxyl-group protected product, 2% of the secondary hydroxyl-group protected product and 4% of the biprotected product.\(^{(73)}\) Direct silylation of D-ribose with TIPSCI yield the primary protected furanose in 80% yield,\(^{(74)}\) thus it should be possible using 2,3-isopropylidene ribofuranose as a substrate. The reaction times however is approximately 50,000 times longer using TIPSCI silylation agent as compared to TMSCl as a result of the increased bulkiness.\(^{(75)}\)

The TIPS ether group can be cleaved with a TFA-THF-H\(_2\)O (1:3:3) mixture\(^{(76)}\) (25 °C) or KOH \(^{(77)}\) (40% in methanol, water, 18 hr, reflux). The last example is pretty harsh due to the strong basic conditions. The reaction time needed for total deprotection with TBAF (1.1 equivalents in THF, rt) is 15 min.\(^{(78)}\)

**TBDMS ether**

The t-butyldimethylsilyl ether can be prepared by the standard method with the same selectivity. Using the 2,3-O-isopropylidene ribofuranose as substrate with the hemiacetal present, the primary alcohol is protected in a yield of 90%.\(^{(65)}\) The TBDMS ether is approximately 10’000 times more stable than the TMS ether towards base hydrolysis, but it is still sensitive towards acid hydrolysis. The TBDMS ether on a primary hydroxyl group can be cleaved by a acetic acid-H\(_2\)O-THF (13:7:3) mixture\(^{(79)}\) (30 °C, 15 hr, 79%), K\(_2\)CO\(_3\)\(^{(80)}\) (in MeOH, 2 days) or DIBALH\(^{(81)}\) (in DCM, toluene, 23 °C, 1-2 hr.). The reaction time needed for total deprotection with TBAF (1.1 equivalents in THF, rt) is 20 min.\(^{(78)}\)

**TBDPS ether**

The t-butyldiphenylsilyl ether is approximately 100 times more stable towards acid hydrolysis, but less stable towards base hydrolysis, compared to the TBDMS ether. This is due to the fact that the methyl
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groups are more electron donating than the phenyl groups. The same selectivity is observed as before with 2,3-isopropylidene ribofuranose, as substrate, resulting in a impressive yield of 99%.[⁶⁵] The primary TBDPS ether, in the presence of a secondary TBDDS ether, can cleaved by NaOH (10% in MeOH, reflux, 3 hr) in high yield.[⁸²] The TBDPS ether is stable towards most acidic conditions[⁸³] such as 50 % aqueous TFA (25 °C, 15 min) or aqueous formic acid (25 °C, 6 hr). The reaction time needed for total deprotection with TBAF (1.1 equivalents in THF, rt) is 50 min.[⁷⁸] Thus the TBDPS ether is relatively more stable compared to TMS-, TBDMS- and TBDPS ethers.

Piv ester

The pivaloyl ester is a bulky ester group compared to the acetyl group, and it can selectively acylate on primary alcohols over a secondary alcohol,[⁸⁴] with the standard procedure using PivCl in pyridine. Selectivity is also found with a 2,3-O-isopropylidene furanose.[⁸⁵] It is more stable than other acyl groups such as acetyl and benzoyl towards acid and base. Prolonged heating in sodium methoxide will cleave the pivaloyl group.[⁸⁴] HCl (3M in dioxane, reflux, 18 hr) will also cleave the pivaloyl ester.[⁸⁶] The ester can be reduced by DIBAlH[⁸⁴] (2.5 equivalents in DCM, - 78 °C) or Grignard reagent if no other, more labile acyl is present.

2.1.3. Protection with benzyl ether

I am familiar with the benzyl ether protecting group in my previous project in the fall of 2008. The benzyl ether is very robust and is often first removed in some of the latest steps in a synthesis. The standard method of alkylating an alcohol to the benzyl ether is to use benzyl bromide and sodium hydride in DMF. The mechanism is the Williamson ether synthesis (Figure 12, A). Mobashery et al. recently investigated the multiple properties of sodium hydride as a base and reducing agent, as it can result in a possible side reaction (Figure 12, B).[⁸⁷]

![Diagram](image)

Figure 12

To the best of my knowledge it is not reported whether the alkoxide could substitute on the N,N-dibenzyl-N,N-dimethylammonium substituents. Otherwise this would explain if more reagents is needed.

~ 25 ~
The 4-methoxybenzyl ether protecting group

The 4-methoxybenzyl and benzyl ethers are not orthogonal protecting groups, since conditions that will cleave the benzyl ether will also cleave the PMB ether. The formation of the PMB ether can be performed with essentially the same method as with the benzyl ether. The PMB is labile towards Brønsted acids like 2% TFA in CH₂Cl₂. Benzyl ethers are stable towards these conditions, and the PMB ether is labile since the cation intermediate is stabilized by resonance (Figure 13).

![Figure 13](image1)

In addition the PMB ether can selectively be removed by 2,3-dichloro-5,6-dicyanobenzoquinone oxidation. The products are 2,3-dichloro-5,6-dicyanohydroquinone, 4-methoxybenzaldehyde and the alcohol. This method should be avoided if groups sensitive toward single-electron-transfer oxidations are present. SnCl₄ and thiophenol at very low temperature have also been reported to remove the PMB ether, and can be attempted if the other methods do not work.

2.1.4. Preparation of a monobenzylated alcohol from a diol

Regioselective reductive ring opening of a benzylidene acetal is a useful method of selectively protect one of the alcohols in a 1,2-diol or 1,3-diol with a benzyl ether protecting group, leaving the other unprotected. A convenient preparation of the benzylidene is using benzaldehyde dimethyl acetal and an acid such as camphor sulphonylic acid in toluene. The mechanism is essentially the same as in the formation of a ketal described in section 2.1.1 and is shown in Figure 14.

![Figure 14](image2)
A mixture of two diastereomers is possible, and when a substrate is acyclic it is hard to predict the ratio. It can possibly have an effect for the outcome of the the next step of the synthesis and it will be discussed later in this section. The reaction is reversible.

Initially, in the general mechanism for benzylidene acetal reduction, coordination of the acid to one of the two oxygens occurs (Figure 15), which weakens the O-CHPh bond. By the assistance of the other oxygen atom and cleavage of the weakened O-HPh bond, an oxocarbenium ion is formed. The oxocarbenium ion is reduced irreversibly to the benzyl ether by the added reducing agent.

![Figure 15](image1)

A classic example in carbohydrate chemistry is seen with a 4,6-O-benzylidene acetal as substrate. Battach and Gorin used lithium aluminum hydride and aluminum chloride to obtain the free 6-hydroxy group in 1969.[91] The free 4-hydroxy group can be obtained by using sodium cyanoborohydride and HCl, as reported by Garegg et al. in 1982,[92] who also proposed an explanation. The 4-O is secondary and more sterically hindered than the primary 6-O, thus using a Lewis acid like aluminum chloride will primarily coordinate to the 6-O, and after elimination, and reducing it gives the free 6-hydroxy group (Figure 16). A proton is significantly smaller, so it can also protonate at the 4-O. The product formed is then governed by the acid/base properties of the two possibilities, and since the 4-O is more basic than the 6-O, protonation occurs here.

![Figure 16](image2)

With the same conditions Garegg et al. also reduced the 5-membered acetal with the benzylidene acetal at 2-O and 3-O. In this case the product formed is dependent of the stereochemistry of the benzylidene acetal. The endo-benzylidene acetal 14 result in the benzyl ether on 2-O (Scheme 15, A) forming 15, where as the exo-benzylidene acetal 16 result in the benzyl ether on 3-O (Scheme 15, B) forming 17. The same result was also produced with lithium aluminum hydride and aluminum chloride by Lipták.[93]
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Scheme 15

The regioselectivity of the benzylidene reduction is not only controlled by the size of the acid and the basicity of the oxygens. Shie et al. used cupper(II) triflate as Lewis acid but used different reducing agents.\cite{94} When employing the borane tetrahydrofuran complex as reducing agent on a 4,6-O benzylidene acetal, the alcohol liberated was on the 6-O position, and when using triethyl silane the other regioselectivity was found. These result show that a more bulky reducing agent favors the reduction to occur on the least hindered oxocarbenium ion.

Reactions of 18 using a deuterated reducing agent result in a diastereomeric mixture of the products 19 and 20 or 21 and 22, which is an experimental evidence showing that the mechanism follows the $S_{N}1$ pathway when using borane or triethyl silane (Scheme 16). If the reaction hypothetical followed the $S_{N}2$ pathway; hence by Walden inversion, only diastereomer 22 or 19 would be observed. This observed is not seen as deuterium is not a good enough nucleophile.

Scheme 16

So far only bicyclic substrates have been shown. Regioselectivity can also be achieved when having the benzylidene acetal as the only ring, but can be harder to predict. Morelli et al. illustrated this using the simple diasteriomic substrates in Scheme 17.\cite{95} They isolate very high yields of the benzyl ethers and in a high regioselectivity.
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Scheme 17

The main product is the same from the syn- and anti-substrate, indicating that both reaction pathways follow exactly the same equilibrium and reduction step progresses from the same intermediate. The regioselectivity arises from the stronger electronic-withdrawing effect of the trifluoromethyl group compared to the methyl group. As a consequence the positive charge from the oxocarbenium ion, developed in the intermediate, is relatively more stabilized the more away from the electron-withdrawing group it is. Morelli et al. also use other aromatic groups with similar results.

DIBALH\textsuperscript{[96]} has also shown to make the regioselective reductive ring opening. An advantage of using DIBALH is that it acts both as the Lewis acid and the reducing agent.

Another possibility to make a monobenzylated product from a diol is to first stanny late the diol with di(n-butyl)tin oxide, forming a stannylene acetal, then treat it with benzyl bromide and an activator such as tetrabutylammonium iodide in DMF. It generally enhances the nucleophilicity of one of the hydroxyl groups. An example of alkylation with one equivalent of benzyl bromide to give the monobenzylated product, was reported by Nashed and Anderson with a yield of 72\% (Figure 17).\textsuperscript{[97]}

Figure 17

The substrate is still cyclic and the regioselectivity is the consequence of the different electronic properties of the two oxygens involved.

2.2. Redox

In organic chemistry the redox-terminology always focused on the organic compound. To reduce an atom other atoms have to be equally oxidized. The carbon atom has all the nine naturally numbered oxidation states from -4 to +4. Reduction and oxidations are methods commonly employed to convert a functional group into a new functional group, with new properties.
Towards the synthesis of a stable α-galactosidase inhibitor

2.2.1. Reduction of a hemiacetal
A reduction is the part of the redox-reaction where an atom has oxidation state reduced. A hemiacetal is in a fast equilibrium with the alcohol and aldehyde in acidic or alkaline conditions. The aldehyde can then be reduced from oxidation state +1 to the primary alcohol with oxidation state -1 (Figure 18).

![Figure 18](image)

To the best of my knowledge the pivaloyl and TIPS protected substrates have not been reduced to the corresponding ribositol. Some related examples are those 2,3-O-isopropylidene-5-O-t-butyldimethylsilyl ribofuranose which has been reduced using sodium borohydride and 2,3-O-isopropylidene-5-O-trityl ribofuranose which has been reduced using NaBH₄ (in EtOH, 0 °C, 72 %).[98]

The precise mechanism of reduction with sodium borohydride is uncertain. Deuterium experiment have been performed to determine from where the hydrogens originate (Figure 19, A).[99] Using LiAlH₄/LiAlD₄, experiments have shown the hydride/deuteride is added to the carbonyl first, and then the other hydrogen/deuterium is added by the solvent (Figure 19, B).[100]

![Figure 19](image)

2.2.2. Reduction of a nitrile to aldehydes
The nitrile is a carboxylic acid derivative, and the carbon atom has oxidation state + 3. An uncontrolled reduction of carboxylic acid and the derivatives will reduce the carbon atom to the oxidation state – 1 (primary alcohols, amines). A classic example is reducing carboxylic acid with borane will result in the alcohol, and oxidation is required to make the aldehyde.
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Nitriles are sp hybridized and have some of the same properties as an alkyne. The pKₐ of the corresponding acid is ~1, and are more unreactive towards nucleophiles than the imine (pKₐ of corresponding acid is about 4), as a negative charge on nitrogen is disfavored. Hence nitriles require acid catalyst, unless a really strong nucleophile is used.

Nitriles can be reduced to the primary amines by reduction with lithium aluminum hydride. Nystrom and Brown reported in 1948[101] that 0.5 molecular equivalents LiAlH₄, will convert one equivalent of nitrile into an intermediate, which will be hydrolyzed upon work-up (Scheme 18). The intermediate is stable in nitrogen atmosphere but pyrophoric in air. Amundsen and Nelson suggested in 1951[102] that at 35 °C only two of the hydrides from LiAlH₄ are available. They also discovered complications when α-hydrogen was present next to the nitrile.

\[
2 \times R-C\equiv N + \text{LiAlH}_4 \xrightarrow{\text{ether, reflux}} \text{(RCH}_2\text{N)}_2\text{LiAl} + \text{H}_2\text{O} \xrightarrow{\text{work-up}} 2 \times R-\text{NH}_2
\]

Scheme 18

Friedman suggested that using 0.25 equivalents of LiAlH₄ at 0 °C could reduce a nitrile into an aldehyde. This was performed by Smith and Rogier, who reduced cyclopropyl cyanide to cyclopropyl aldehyde in 48 % yield.[103]

The common method employed to reduce a nitrile into an aldehyde in modern organic chemistry is by using DIBALH. An early report was by LeBel et al. in 1964, who reduced trans-1-cyano-4-hexene into trans-5-heptenal in 64% yield.[104] DIBALH is synthesized by heating triisobutyl aluminum by β-elimination, and is suggested to exist as a dimer.[105] DIBALH is an electrophilic reducing agent. The reason that DIBALH does not reduce the nitrile into the amine is that the aluminum forms a relatively stable complex with the imine. Acid work-up with this complex will result in the aldehyde (Figure 20).

![Figure 20](image_url)

Nitriles can be converted to alcohols by a two-step reduction, initially with DIBALH followed by NaBH₄.[106]

In general, DIBALH usually reduces esters, acyl chlorides, ketones, aldehydes and carboxylic acids into the corresponding alcohol. An example where an ester is not reduced by DIBALH is reported by Zoretic et al.[107] as they have reduced a ketone to the secondary alcohol and an a nitrile to the aldehyde without reducing...
the ester (Scheme 19, A). With a similar compound where the ketone has been removed the ester is easily reduced (Scheme 19, B). Their explanation to this phenomenon is that the formed diisobutylaluminum alkoxide is can chelate with the carbonyl of the ester, preventing complexation with another DIBALH molecule.

Scheme 19

Other methods of reducing nitriles to aldehydes are known. One old method is by the Stephen’s aldehyde synthesis named after Henry Stephen’s research in 1925.\cite{108} The Stephen’s aldehyde synthesis reduces a nitrile with stannous chloride and hydrogen chloride. Though Stephen claimed that, in general, this synthesis would result in a quantitative yield, Williams discovered that this was not as general.\cite{109} High yields are obtained when the substrate is an aromatic nitrile. Another method is using Raney Nickel and like formic acid in mediocre yields.\cite{110} This appears to work only on aromatic nitriles.

2.2.3. Oxidation of alcohols

Oxidations in organic chemistry can be performed in many ways. A short description of relevant types is given in this section.

Oxidation with “activated DMSO”

The first published oxidation of an alcohol dissolved in dimethyl sulfoxide originated from Pfitzner and Moffat in 1963 by serendipity.\cite{111} In a coupling attempt they treated a primary alcohol of a nucleoside with dicyclohexylcarbodiimide in the presence of mild acid in DMSO. Instead this resulted in an oxidation of the alcohol to the aldehyde with no traces of the over-oxidized acid, by the reaction now called Pfitzner-Moffat oxidation. At this time, no other efficient method for this reaction had been discovered. The reason that the species is “activated”, is because a good leaving group is formed, which is ready for a substitution by a nucleophilic attack of the alcohol. Other of “activated DMSO” species have been discovered (Figure 21).

Figure 21
Towards the synthesis of a stable α-galactosidase inhibitor

**Parikh-Doering oxidation**

Doering and Parikh used a pyridinium sulphur trioxide complex and triethyl amine at room temperature to activate DMSO in 1967.\(^1\) Optimized conditions suggest that the reaction should be cooled to about 0 °C and combined with a co-solvent like CH\(_2\)Cl\(_2\), as the melting point of DMSO is 18 °C. The most convenient way is to add the SO\(_3\)·Py complex as a solid, rather than dissolved in DMSO. The problem is that nucleophiles, such as alcohols, will react with the SO\(_3\)·Py complex forming the sulfonic acid group.\(^2\) Premixing the SO\(_3\)·Py complex with DMSO to form the “activated DMSO”, subsequently mixing this mixture with the alcohol might improve the yield.

The suggested mechanism for the Parikh-Doering oxidation has an “activated DMSO” intermediate (Figure 22).\(^3\)

![Figure 22](image)

Addition of sulfurtrioxide pyridine complex creates an activated DMSO, where a substitution by the substrate alcohol occurs. Then the sulfur ylide is formed and the oxidation occurs, forming the carbonyl product and the other byproduct, dimethyl sulfide. Elimination of the “activated DMSO” can also occur and addition of alcohol to the highly reactive sulfonium species results in the methylthiomethyl ether as an unwanted byproduct. Low polar solvents disfavor this side reaction.

Performing this oxidation under dry conditions has also been reported to generate increased yields.\(^4\) The byproducts from the SO\(_3\)·Py complex are easily removed during work-up, and the Parikh-Doering rarely result in a considerable amount of the methylthiomethyl ether byproduct.

The Parikh-Doering oxidation usually cannot be employed in the presence of nucleophiles. Exceptions are tertiary alcohols (Swern oxidation cannot be employed) and tertiary amines. Acid and base sensitive protecting group are stable to the Parikh-Doering oxidation. Even oxidation sensitive protecting groups like the PMB ether, remain stable.\(^5\)

Another DMSO based oxidation is the Swern oxidation, which uses oxalyl chloride to active the DMSO, and are probably the most common method applied.\(^6\) The Swern oxidation is very mild and highly acid labile protecting groups, like trityl ethers, remain stable under these conditions.\(^7\) The Swern oxidation is prone
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to form methylthiomethyl ethers of tertiary alcohols with excess “activated DMSO”,\textsuperscript{[121]} and in some cases with primary and secondary alcohols as well.\textsuperscript{[122]}

**Oxidation using hypervalent iodine compounds**

Iodine (III) compounds have been known and used for various different applications in organic chemistry.\textsuperscript{[123]} Application of iodine (V) compounds is a fairly new but interesting field of oxidation agents for alcohols.

**Oxidation with Dess-Martin Periodinane**

Hypervalent iodine compounds in oxidation state +5 have been known for a long time as oxidation agents of alcohols. It was not often applied because of poor solubility in organic solvents and lack of stability. Not until 1983 when Dess and Martin performed efficient oxidations of alcohols, with the compound named Dess-Martin periodinane, which is stable and is highly soluble in most organic solvents.\textsuperscript{[124]} This increased the interest of using hypervalent iodine compounds as oxidizing agents.

The DMP can be prepared from 2-iodoxybenzoic acid by an acetylation with acetic anhydride in acetic acid as described by Dess and Martin (Scheme 20). IBX can be synthesized by oxidation of 2-iodobenzoic acid by oxone as developed by Santagostino et al.\textsuperscript{[125]}

\begin{center}
\begin{tikzpicture}
\node[anchor = north west] (a) at (0,0) {2-iodobenzoic acid};
\node[anchor = north west] (b) at (1,0) {2-iodobenzoic acid};
\node[anchor = north west] (c) at (2,0) {IBX};
\node[anchor = north west] (d) at (3,0) {Dess-Martin Periodinane};
\node[anchor = north west] (e) at (0.5,0) {oxone $\rightarrow$ Ac$_2$O, AcOH $\rightarrow$ Ac$_2$O, AcOH $\rightarrow$ Ac$_2$O, AcOH};
\node[anchor = north west] (f) at (1.5,0) {H$_2$O, 70 $^\circ$C, 3 h};
\node[anchor = north west] (g) at (2.5,0) {100 $^\circ$C, 40 min};
\end{tikzpicture}
\end{center}

**Scheme 20**

A standard protocol for oxidation is to dissolve the alcohol and DMP in CH$_2$Cl$_2$ at room temperature. It should be noted that DMP has shown to be explosive in presence of impurities. Experimental data show that the initial displacement step is fast and the oxidation step is slower (Figure 23, A).\textsuperscript{[126]}
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Figure 23

The iodine is reduced from oxidation state +5 to +3. Using excess DMP can actually decrease the oxidation speed. Two displacement reactions can occur, which by employing excess alcohol will result in a lower yield, as alkoxyperiodinane is formed as a by-product (Figure 23, B). Addition of t-butanol can inhibit this formation as it is unable to be oxidized and forms the t-butoxyperiodinane instead. The periodinane formed can easily be hydrolyzed by sodium hydroxide into the water soluble 2-iodosobenzoic acid, or the periodinane can be reduced by sodium thiosulfate and the resulting 2-iodobenzoic acid can be recycled.

DMP is a mild reagent. Sulfides can be oxidized by DMP, but it is possible to selectively oxidize alcohols in its presence. The same can be achieved for alcohols in the presence of a lactol, even though lactols can be oxidized to lactones. Tertiary and secondary amines remain stable by oxidation of an alcohol. Acetic acid is produced as the oxidation proceeds and addition of a base, like sodium bicarbonate, will prevent acid catalyzed side reactions. So TMS and trityl ethers that otherwise would be claved by the acid can potentially remain stable. The PMB ether is also resistant of being oxidized by DMP. Alkenes can be epoxidated by acetylated IBX, which is the product from hydrolyzed DMP with water, but the oxidation of alcohols is faster.

Oxidation with 2-Iodoxybenzoic acid

As it was supposed that IBX was insoluble in organic solvent it was mainly used as a precursor of DMP until 1994 when Santagostino et al. dissolved IBX in DMSO and oxidized alcohols extremely efficient and very selectively. Selective oxidation of alcohols were achieved in the presence of amines, thiols, thioacetals by the group and later Corey and Palani reported that 1,4-diol can be oxidized to the γ-lactols without over-oxidizing to the γ-lactone.

The most conventional method of oxidizing an alcohol using IBX is actually not to dissolve it. Nicolaou et al. reported that IBX can react with the solvent, which will attach as a ligand, and it result in different
Towards the synthesis of a stable α-galactosidase inhibitor

oxidation properties of the hypervalent iodine. Finney and More[136] found the most efficient method to be a heated suspension in solvents like ethyl acetate or 1,2-dichloroethane, though other solvents like acetone resulted in higher yield at lower temperature. Ethyl acetate and 1,2-dichloroethane was chosen because they are inert under these conditions and the only form of purification needed is a simple filtration.

IBX has also shown to be explosive, but a pure sample requires above 200 °C to be able to explode. IBX exist as two tautomers. The mechanism resembles the oxidation with DMP (Figure 24).

IBX exist as two tautomers. The mechanism resembles the oxidation with DMP (Figure 24). Although dry conditions are not required, the ligand exchange equilibrium are shifted towards the left when water is added, which slow down the reaction. Goddard and Su[137] suggest that the rate-determining step is the hypervalent twist, required for the oxidation to proceed, and predict that an ortho-methyl group will enhance the reactivity of the oxidizing agent.

2.3. Organometal additions
An organometal addition is an excellent way of forming a carbon-carbon bond. Organometal reagent makes the carbon nucleophilic and makes the carbonyl more electrophilic. Organometal substitution is not obtained. The section will first give an overview of additions to ketones and aldehydes, then of addition to nitriles.

2.3.1. Addition to ketones and aldehydes
Grignard
The additions of Grignard reagents to aldehydes and ketones was discovered by Victor Grignard in 1900.[138] A Grignard reagent is a magnesium halide bound to a carbon compound, and has to be prepared just prior to the addition step. Grignard reagents are not water-stable.

The Grignard reactions are often accompanied by a lot of different side reactions like enolization, reduction[139], conjugated addition etc. due to their reactivity. A lot of different optimizations have been
performed, but many are efficient in specific cases and are not general. Swayne and Boyle used magnesium bromides as an additive, to change a preference for reduction of the ketone into addition of the ketone (Figure 25, A). They also use these results to redefine the mechanisms. The Grignard reagent initially coordinates to the ketone, making the carbonyl carbon more electrophilic. The reduction of the ketone occurs intramolecularly, together with an elimination of the Grignard reagent (Figure 25, B). The addition reaction occurs with another Grignard reagent (Figure 25, C). Magnesium bromide is a slightly stronger Lewis acid than the Grignard reagent, and coordination of magnesium bromide to the ketone will hinder the intramolecular reduction. Chastrett and Amouroux reported an increase of addition product to 70% when using LiClO₄ or TBAB as additive. The greatest enhancement occurs when they premix the salt with the Grignard reagent, compared premixing the salt with the ketone. Their explanation is that the salt complexes with the Grignard reagent, thus enhancing the ionic character. Addition is then favored since the transition state of the addition is more polar than the transition state of the reduction.

![Reaction mechanism](image)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>1,2-Addition</th>
<th>1,4-Addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>74 %</td>
<td>2 %</td>
</tr>
<tr>
<td>THT</td>
<td>82 %</td>
<td>9 %</td>
</tr>
<tr>
<td>Et₂O</td>
<td>54 %</td>
<td>17 %</td>
</tr>
<tr>
<td>THF</td>
<td>53 %</td>
<td>39 %</td>
</tr>
</tbody>
</table>

Table 2 – reaction of cyclohexen-2-one with EtMgBr

Hydrocarbon solvents like benzene also increase the preference for addition compared to ether solvents. The solvent has an effect in the ratio of 1,2-addition versus 1,4-addition for the reaction on α,β-unsaturated ketones. The reaction of cyclohexen-2-one with ethyl magnesium bromide in different
solvent are shown on Table 2, and the 1,2-addition is again favored in benzene compared to the more basic ethereal solvent\textsuperscript{[143]}

The mechanism has also been proposed to be a single-electron-transfer mechanism from ketone 23 by studies performed by Ashby et al., and it involves a ketyl anion 24 as an intermediate.\textsuperscript{[144]} 24 is stabilized when it is in conjugation with phenyl groups or alkenes. Evidence for this theory is the products 27 is formed by hydrogen abstraction with the solvent, and by the fact that the pinacol byproduct 26 is formed (Figure 26). Addition of \textit{p}-dinitrobenzene as a radical scavenger show inhibition in the formation of the pinacol byproduct without affecting the formation of the 1,2-addition product. The fact that the scavenger does not affect the 1,2-addition product confirm that the radicals are in a solvent cage, as the radicals need to be free to be captured by the scavenger.

Imamoti et al. thoroughly investigated the use of the lanthanide salt cerium chloride, as an additive and generally found higher selectivity towards the 1,2-addition product.\textsuperscript{[145]} Their explanation is that cerium chloride has a strong oxophilicity, and that the basicity of the Grignard reagent is weakened by cerium chloride. Knochel et al. improved these yields by using other lanthanide salts as an additive.\textsuperscript{[146]} A simple example is shown on Scheme 21, but more complicated ketone and Grignard reagents have been used efficiently.
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Scheme 21 – i) Optimized yield by Dimitrov et al.[147]

Furthermore the procedure by Knochel et al. also reduced the rate of the unwanted side reactions when using a Grignard reagent with cyclohexen-2-one (Scheme 22).[146]

Scheme 22

Barbier

The Barbier reaction is named after Victor Grignard’s teacher Philippe Barbier. The major difference between the Barbier reaction and the Grignard reaction is that the metal halide in the Barbier reaction is generated in situ. In addition Barbier reactions can be performed in water. The metals used in the Barbier reactions are zinc,[148] tin,[149] indium[150] etc.

Stereoselectivity

Different models have been created to predict the stereochemistry of the product. In the Felkin-Anh model, the largest substituent on the α-carbon will be placed perpendicular to the carbonyl, allowing two different conformations (Figure 27, B).[151] The carbonyl π- and π*-orbitals are now parallel, allowing delocalization of electron density by hyperconjugation (Figure 27, A). The nucleophilic attack occurs in the Bürgi-Dunitz angle,[152] and is preferred at the conformation with the least steric hindrance. Karabatsos model uses the same argument regarding steric hindrance and the preferred conformation is when the medium sized group is eclipsed to the carbonyl (Figure 27, C).[153] When a heteroatom is present as a substituent on the α-carbon, chelation effect is directing the stereochemistry of the addition. Using the Felkin-Anh model the heteroatom is placed perpendicular to the carbonyl (Figure 27, D), using the same orbital considerations as before (Figure 27, A).
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Figure 27

Chelation effect also occurs when a heteroatom is present as a substituent on the β-carbon. This effect and can make it more complicated to predict the stereochemical outcome. This is exemplified by the addition of nucleophiles on 2,3-isopropylidene glyceraldehyde, where chelation with the α-oxygen will result in a different product compared to chelation with the β-oxygen (Scheme 23). The ratio here is dependent on the nucleophile.

Scheme 23

2.3.2. Addition to nitriles
Grignard reagents can be used to form imines, amines or ketones. Without additives the nitrile will react slowly with the Grignard reagent. In 1987 Weiberth and Hall found copper bromide as an additive. The reaction was high yielding on aromatic nitriles, and depending on the work-up method they got either the imine, the ketone or the primary amine (Scheme 24).
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Scheme 24

Using two bulky aliphatic reactants result in a lower yield (Scheme 25, A). They propose a pathway where the cuprate react with the nitrile by an oxidative addition, followed by an unsymmetrical reductive elimination. (Scheme 25, B).

Scheme 25

Imamoto et al. was successful with cerium chloride as an additive in the Grignard additions on ketones and aldehydes, and a small enhancing effect was also seen on nitriles.\[145\] Grignard addition by n-butyl magnesium bromide on phenylacetonitrile with equimolar quantities cerium chloride resulted in a yield of 28% after 30 min. Lee and Lin performed a Barbier reaction with allyl bromide, phenylacetonitrile, 4.0 equivalents of zinc and 0.4 equivalents of aluminum chloride in a yield of 85% after 30 minutes.\[156\] They also estimated the mechanism to proceed via the S$_{2}$2’ pathway.

2.4. Substitution and elimination

Substitution is a valuable tool in organic chemistry to attach a group to a carbon skeleton. The substitution often occurs by a heteroatom nucleophile, but also some carbon nucleophiles can be employed. Elimination is a competing reaction when a leaving group is attached to the carbon skeleton, and is a useful procedure of preparing olefins.
2.4.1. Olefination of carbonyl compounds

The Wittig reaction

The classic Wittig reaction was developed by Wittig and Geissler in 1953 (Figure 28).[157]

\[
\text{Ph} \quad \text{Ph} \quad \text{Ph}_3\text{P} \quad \text{CH}_2 \quad \text{Ph} \quad \text{Ph} \quad 84 \%
\]

Figure 28

A phosphonium ylide is usually prepared by the reaction of a phosphonium salt with a base. An aldehyde or ketone then reacts with the phosphonium ylide, and results in the corresponding olefin and phosphonium oxide. The currently accepted mechanism and the stereoselectivity is well understood (Figure 29).

Figure 29

The Wittig reaction is suggested to go through the oxaphosphetane intermediate and the stereochemistry is directed from here as it undergoes stereospecific syn-elimination. The Z-alkene is preferred under kinetic control and the E-alkene is favored under thermodynamic control. The most important factor influencing whether it is thermodynamic or kinetic control is which kind of ylid is used. Stabilized ylids have an electron withdrawing group attached, and are usually so unreactive that they only react with aldehydes under thermodynamic control, resulting in the E-alkene. Non-stabilized ylids have an electron donating group attached and reacts with both aldehydes and ketones under kinetic control, resulting in the Z-alkene. Other alternative conditions to alter this selectivity are known.

Levine discovered in 1958 that the Wittig reaction can be used in the synthesis of a homologues aldehydes.[158] First a preparation of the enol ether by reaction of an aldehyde or ketone with the methoxymethyl phosphonium ylid, and then treatment with acid will result in the homologated aldehyde (Figure 30).

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Towards the synthesis of a stable α-galactosidase inhibitor

Figure 30

Peterson Olefination

The Peterson olefination was developed by Peterson in 1968 and is the formation of an alkene from a ketone or aldehyde in a two step procedure shown in Figure 31.

Figure 31

The addition step can be performed with a Grignard reaction or by using a lithium reagent as described in section 2.3. The elimination step can be performed by either treating the β-silyl alcohol with acid or a hydride base. Peterson and Hydlik reported that the elimination is stereospecific and the stereoselectivity is dependent on the conditions (Figure 32).

Figure 32

The mechanism of the acid catalyzed olefination is different from the mechanism of the base catalyzed olefination (Figure 33).
Towards the synthesis of a stable α-galactosidase inhibitor

The acid catalyzed reaction forms a carbocation by dehydration (Figure 33, A). Dehydration from intermediate b is favored since the leaving group is anti-positioned against silicon and the staggered conformation makes in less sterical hindered. This carbocation is stabilized by the β-silicon effect, where electrons in the σ(Cα-Si) bond is donated into the empty p(Cβ)-orbital (Figure 33, B). It is also this effect that forms the desired elimination. The elimination must be faster than the rotation from b to a since the E-alkene is the major product.

In the hydride promoted elimination mechanism, the alcohol is deprotonated and the alkoxide subsequently form a 4-membered ring by nucleophilic attack on silicon (Figure 33, C). The ring has to be formed when the alkoxide and silicon are syn to each other. Elimination can then from the E-alkene. Using t-butoxide as a base result in the homo-Brook rearrangement,\textsuperscript{[161]} and will finally result in the de-silylated alcohol.

If the substrate is acid or base sensitive, acetyl chloride or thionyl chloride can be employed.\textsuperscript{[162]} Treatment with the acetyl chloride or thionyl chloride converts the alcohol into an β-silyl ester, which then quickly eliminates.
2.4.2. Nucleophilic bimolecular substitution

Nucleophilic bimolecular substitution (S\(_{\text{n2}}\)) is one of multiple competing with reactions including S\(_{\text{n1}}\), and eliminations E1, E2 and E1cB etc. (Figure 34). A lot of factors influence on the preference between these reaction: the substrate carbon skeleton, the leaving group, the nucleophile and solvent effects.

\[
\text{Figure 34}
\]

An S\(_{\text{n2}}\) always occurs by Walden inversion.\[^{[163]}\]

The substrate carbon skeleton

One of major contributor to the chemoselectivity is the carbon skeleton of the substrate. To get a better view at the intermediates and transition states in the different pathways, they are is depicted in Figure 35.

\[
\text{Figure 35}
\]

Both of the unimolecular pathways shown, have the same cationic intermediate. R-substituents which are able to stabilize a carbocation will increase the preference toward the unimolecular pathways. Easily formed carbocations are the tertiary, allylic, benzylic, \(\alpha\)-hetero substituted and anchimeric assisted carbocations. Secondary carbocations are less stable, and primary even less.
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Alkyl groups stabilize the carbocation by hyperconjugation of electrons from the σ-orbital into the empty p-orbital, which is the same effect as for the β-silicon effect (Figure 36, A). Allylic and benzylic groups stabilize the carbocation by resonance from the π-system. As a consequence, allylic substrates can follow the $S_N1'$ (or $S_N2'$) pathway (Figure 36, B, C). A π-system further away can also stabilize carbocation by homoconjugation. An example is the 7-norbonadienyl carbocation as a non-classical carbonium ion investigated by Hoffmann (Figure 36, D). The homoconjugation also exist in the homoallyl cation, and exists as a bicyclobutonium ion as proposed by Roberts et al. (Figure 36, E).

When the substrates are less able to stabilize the carbocation, the bimolecular pathways are favored. Steric effects from the substituent contributes to the reaction rate of the $S_N2$, as more bulky substrates will substitute at a lower rate.

The leaving group

From a neutral substrate the leaving group will develop a negative charge in the rate determining step. Better leaving groups are more able to stabilize this charge. This ability is correlated by the $pK_a$ of the corresponding acid, but only within a class of leaving groups. Halides is a class of leaving groups and the iodides ($pK_a(HI) \approx -10$) are better than bromides ($pK_a(HI) \approx -9$) which is better than chlorides ($pK_a(HI) \approx -7$). Alcohol in itself is a poor leaving group, but the protonated oxonium ion is better. If a substitution of an alcohol functionality is wanted it is more efficient to convert it into a better leaving group. Commonly mesylates, tosylates or triflates are prepared. The mesylate is synthesized from mesyl chloride and triethyl amine through an E1cB mechanism (Figure 37), and the triflate from trifluoromethanesulfonic anhydride.
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![Chemical reaction diagram](image)

Figure 37

The chloride has shown to substitute the mesylate, but this process is slower than the mesylation. Dichloromethane has often been used as solvent, but recently it is reported that pyridine reacts with this solvent at a slow rate.\[167\]

**The nucleophile**

In general, a good nucleophile is a bad leaving group. Negatively charged nucleophiles are better compared to neutral nucleophiles. Less stable nucleophiles are more nucleophilic. The nucleophilicity is usually in correlation with the basicity of the nucleophile, as both involve a donation of electrons into an electrophile.

Solvation effects have a major role in this, as the nucleophilicities of the halides in polar protic solvents are in the order: iodide > bromide > chloride > fluoride, and this order is reversed in polar aprotic solvents in various reactions. This is caused by the ability of a protic solvent to form hydrogen-bonds to the nucleophile, lowering the reactivity. Solvation is stronger for small ions than bigger ions, and the lesser solvated ions are more nucleophilic. Hence nucleophilicity is highest in aprotic solvents with low polarity. But since the nucleophile often is negatively charged, low polarity solvents will not dissolve it. The most commonly used solvents are DMF and DMSO.

The counter ion can influence on the nucleophile, as the more dissociated these ions are, the higher nucleophilicity. The dissociation can be enhanced by addition of a crown ether or other macrolides.\[168\]

Another effect in solvation is the α-effect, where the nucleophilicity is increased though the basicity is not. The α-effect appears when the nucleophile is adjacent to a heteroatom. An example is from the discoveries by Edwards and Pearsons in 1962.\[169\] The nucleophilic replacement rates on p-nitrophenyl acetate of hydroperoxid (pKₐ (H₂O₂) = 11.5) was found to be about 200 times higher compared with hydroxide (pKₐ (H₂O) = 15.7) (Figure 38). An indication that the α-effect is a solvent effect is that it is not apparent in gas phase reactions.\[170\] The magnitude of the α-effect is dependent on the solvent composition, and the optimal composition of DMSO and water is about 1:1 in a reaction with p-nitrophenyl acetate and a nucleophile.\[171\]
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Figure 38
The shape of the nucleophile also influence on the nucleophilicity, as bulky nucleophiles suffer from severe sterical hindrance. Hence linear nucleophiles like cyanide and azide are good nucleophiles

**Solvation effect**
As previously discussed, the polarity of the solvent has an influence of the reaction rate, and exactly what influence is dependent on the charges. When the reactants are neutral, charge develops in the transition state, and increasing the polarity will speed up the reaction. When any of the reactants have a charge, increasing the polarity will stabilize the reactants more than the transition state will be stabilized, thus the reaction rate will be retarded.

**Alkylation of amines**
Direct alkylation of a primary amine by treating it with an alkyl halide will often overalkylate in an uncontrolled fashion. Traditional methods to prepare a secondary amine, is to use large excess of amine compared to alkyl halides. This method is inconvenient as the excess amine have to be recovered, and it can still give a mixture of products depending on a lot of conditions. Extending the carbon chain by two carbons of a primary amine resulted in the monoalkylated product instead of the bialkylated product, as reported by Srivastava et al. (Scheme 26).

![Scheme 26](image)

An optimized method for mono-\(N\)-alkylation with an alkyl halide on the primary amine was developed by Salvatore et al. The method was optimized to include the use of cesium hydroxide as base in DMF (Scheme 27, A). Cesium hydroxide is usually stored as the monohydrate, and the water will prevent full conversion. Using activated 4 Å molecular sieves and prestirring it with the cesium hydroxide was equally as efficient as drying the cesium hydroxide monohydrate for 24 hours. It is apparent from a control experiment, that cesium hydroxide inhibits the alkylation of secondary amine (Scheme 27, B).
Towards the synthesis of a stable α-galactosidase inhibitor

Scheme 27[174]

The chemoselectivity is reversed compared to normal alkylation reactions, and the involved mechanism is depicted on Figure 39. The primary amine forms a complex with cesium making the amine proton acidic. Deprotonation with hydroxide occurs and removal of water by the dessicant will push the equilibrium towards the cesium amide. This is more nucleophilic and the alkylation occurs. Cesium again coordinates to the amine, but the steric hindrance retard the deprotonation of the ammonium salt.

Other common ways of making secondary amines from primary amines is by reductive amination or addition to imines etc., but these procedures are not further described.
3. Results and discussion

3.1. Synthesis strategy
The initial strategy involved an iodide catalyzed acylation of D-ribose followed by an attempt to prepare the corresponding orthoester. This approach was unsuccessful; hence quickly scrapped and is not further described in this thesis.

A retrosynthetic strategy is proposed for galacto-noeurostegine 1 (Scheme 28). The early part of the retrosynthetic strategy involves a lot of protecting group manipulations from D-ribose 11, which are necessary to synthesise the intermediate alcohol 28. Protecting groups that are able to selectively protect the primary alcohol is needed. The trityl ether and the TMS ether are surely too labile to be used as a protecting group, but the pivaloyl ester and the TIPS-, TBDMS- and TBDPS ethers can probably be used in this case. After the preparation of compound 28 the alcohol will be transformed into a leaving group, a cyanide substitution will be performed. Reduction and organometal addition etc. will prepare the compound 29, which will undergo an olefination protocol. Ring closing metathesis, followed by hydroboration-oxidation and benzoylation will potentially give a mixture of product, hopefully with high selectivity for the heptanes 30. Removal of the PMB-ether followed by azide introduction, debenzylation and oxidation prepares 31, which upon reduction will synthesise the congested compound 1, in an estimated 23 step synthesis from D-ribose.

![Scheme 28](image-url)
Towards the synthesis of a stable α-galactosidase inhibitor

3.2. Synthesis

3.2.1. Synthesis of 2,3-isopropylidene ribofuranose
The first few steps are proposed and are depicted in Scheme 29. The exact same pivaloylation step has been performed by Mahmood et al. in 1991.\textsuperscript{[85]} The hemiacetal 32 can then be reduced and the two hydroxy groups can be protecting as benzyl ethers, as they do not need to be removed before the last part of the synthesis.

Scheme 29

Ribose was used as the starting material. The yields given in this section are reported as crude yield and/or isolated yields after two steps. This isopropylidene ketal formation (Scheme 30) was not performed as smoothly as expected. Various different methods have been used. Concentrated sulphuric acid have in most cases been employed as the Brøndsted acid. A large excess of acetone has been used as both reagent and solvent. As D-Ribose is not fully soluble in acetone, it will initially remain a solid before the addition of the acid. The product is soluble in acetone, and as the reaction proceeds, more starting material will be dissolved. Addition of the acid is, therefore, shifting the equilibrium towards the product. After short time, TLC analysis will show the same pattern, with different intensities, at any point of the reaction. Multiple spots appear as expected, with one major, as discussed in section 2.1.1. The equilibrium was fully established, when the solution was homogenized. Copper sulphate has once been added as a drying agent, to push the equilibrium but no apparent effect was visible and it also made it more difficult to see when the starting material was fully dissolved.

Scheme 30

The troublesome part of this reaction is the work-up. Different work-up methods have been used to obtain the product. It is very important that the solution is not acidic when removing the acetone, as the
Towards the synthesis of a stable α-galactosidase inhibitor

equilibrium will be pushed towards either the dehydrated product or the starting material (Scheme 14, Page 21). The product did not turn into the colorless oil as reported in literature.\textsuperscript{[85]} Instead a red oil was formed. Different bases have been applied to quench the reaction. Triethyl amine was used once to quench a reaction with only two drops of sulphuric acid, in a crude yield of about 40% and a two-step yield of 17% (PG = Piv). The triethyl amine was necessary to be removed by a wash with 1 M aqueous hydrogen chloride, and then saturated aqueous sodium bicarbonate, with ethyl acetate as the organic phase. The yield was not satisfying.

Another method was to use a known, larger amount of the sulphuric acid and to quench with calcium hydroxide until pH around 7. The solution was filtered through celite and yielded 91% of the crude product and the two step yield of 51% (PG = Piv). This yield was very satisfying but the result was not reproducible.

Extracting with a 2:1 chloroform/ethanol mixture combined with sodium bicarbonate wash, resulted in a 78% crude yield and a yield over two step of 46% (PG = TIPS). This method was more reliable than the calcium hydroxide method, but not as convenient.

A solution to the problem with acid sensitive acetone evaporation and the inconvenience of extracting in large quantities of organic solvent, it was attempted to use a solid phase acid catalyst. Amberlite IR-120 in hydrogen form is a sulphonic acid moiety bound to a resin. With a few beads, the reaction was stirred at very low speed. As the suspension is slow to homogenize, it is clear that the solid phase catalyst is slower than the solution phase. The reaction was finished over night, in a crude two step yield of about 7.5 g from 5.0 g D-Ribose. The resin was simply just filtered off and the acetone removed under reduced pressure without worrying about any acid remaining. An isolated yield is not given because this batch was used, combined with other batches, to perform the next step from a crude substrate.

3.2.2. **Regioselective synthesis of the pivaloyl ester**

At first it was difficult to predict which protecting group, of the candidates in section 2.1.2 would be the best choice. The pivaloyl was initially chosen as protecting group for the primary alcohol. The ribofuranose can mutarotate in basic solution, and are in equilibrium with the ribopyranose form, so the other nucleophilic oxygens are O1 and O4. The result in this and the following steps will tell if it was a choice well made. The mechanism is shown in Figure 40.
Towards the synthesis of a stable α-galactosidase inhibitor

Figure 40

Pyridine was used as the solvent but also catalyzes the reaction. Pyridine substitutes the chloride first by an addition-elimination mechanism. As the pyridinium ion is a better leaving group than chloride, so it will speed up the reaction. The alcohol then substituted the pyridine by the same mechanism. The ester is then formed together with the pyridinium chloride salt, which precipitates in the reaction vessel. The easiest way to remove the excess pyridine is to wash it with aqueous hydrogen chloride as it will make the salt, which is water soluble.

Starting from a premade isopropylidene 13, the reaction resulted in the mediocre yield of 59% of the monopivaloylated product 32 as a α/β-mixture (Scheme 31). In addition 16% combined yield of the bipivaloylated byproducts 35 as the α-anomer and the β-anomer was obtained. The reaction completed within a couple of hours. The mediocre yield is acceptable as it is just the second step of the synthesis.

Scheme 31

3.2.3. Regioselective synthesis of the TIPS ether

The TIPS ether protection group was later chosen in favor of the Pivaloyl group for reasons which will be discussed in section 3.2.11. In this and the following sections both pathways are described as they were performed consecutive, as to making the comparison easier.

The conditions used are almost the same as for the Piv protection step, except that a catalytic amount of DMAP was added as a nucleophilic catalyst, and the temperature is raised to 50 °C. Imidazole has not been used as it in the standard protocols is dissolved in DMF, and pyridine and DMAP is easier to remove completely by washing. The general mechanism for a silylation of the isopropylidene is shown in Figure 41.
Towards the synthesis of a stable α-galactosidase inhibitor

Figure 41

DMAP substitutes chloride by an $S_n2$ at Si mechanism, silicon will go through a pentacoordinated intermediate. The pyridinium ion from DMAP is stabilized by resonance, which will lower the intermediate energy level and the corresponding transition state energy level resulting in a faster reaction than compared to only using pyridine.$^{[176]}$ When the alcohol has substituted irreversible the products are the silyl ether and 4,4-$N$-dimethylpyridinium hydrochloride, which can be deprotonated by pyridine and DMAP can be reused for another cycle.

Even though DMAP is used as catalyst and in spite of the increased temperature, the reaction time is longer for the TIPS silylation than the pivaloylation. The reaction was stirred for about 24 hours and yielded only 44% of 36 from the premade isopropylidene 11 (Scheme 32). The $\alpha/\beta$ relation is approximately 1:5. The biTIPS silylated product 37 was not isolated from other byproducts.

Scheme 32

It was investigated if the anomeric TIPS group of 37 could be removed selectively by treatment with TBAF (Figure 42). It should be noted that the sample was not purified further than the column chromatography, where the mono TIPS ether was isolated, and contain multiple products.
Towards the synthesis of a stable α-galactosidase inhibitor

Adding TBAF to a 446 mg mixture in THF at –50 °C afforded only 20 mg isolated yield of the desired product 36, making this not worthwhile.

### 3.2.4. Reduction of the hemiacetal

At this point in the synthesis, the only other susceptible functionality besides the hemiacetal, towards reduction is the pivaloyl ester. The pivaloyl group should be stable towards NaBH₄ at room temperature. The reduction of the hemiacetal was performed using NaBH₄ in methanol.

The reaction was usually stirred over night; the reaction was probably complete before this. The yields were generally very good. The Piv-protected yielded 95% of 33 and the TIPS-protected compound resulted in a quantitative yield of the corresponding ribitol 38 (Figure 43).

### 3.2.5. Protecting the ribositol with benzyl ethers

**With pivaloyl ether**

The standard procedure for a benzylation is to add NaH to the alcohol, dissolved in DMF and then add the benzyl bromide after 10 minutes. To my experience this is a good procedure, thus the same method was initially used. The applied reaction mechanism for the product is shown in Figure 44.
Towards the synthesis of a stable α-galactosidase inhibitor

Unfortunately this did not work as planned. This method resulted in an isolated yield of 62% of the desired product 34, and multiple by-products with similar polarity based on TLC analysis. Based on crude H$^1$-NMR and LRMS some of the by-products are compounds where the pivaloyl group has migrated. Even though they have not been completely isolated and characterized, some of the proposed by-products are shown on Figure 45.

These migrations occur intermolecular, but whether they also occur intramolecular is unknown. Intermolecular acyl migration have been observed before with the acetyl group in alkaline solution, but the pivaloyl group should be more stable. Intramolecular migration studies performed by Roslund et al. on benzyl β-galactopyranoside at pH = 8.0, has shown that the first-order rate constant from 2-O-acyl to 3-O-acyl to be about 4.9 h$^{-1}$ for the acetyl group and 0.06 h$^{-1}$ for the pivaloyl group. With the reverse migration neglected, this corresponds to a half-life of about 12 hours for the pivaloyl group. Although it is not directly comparable, it should give an indication that the intermolecular migration is slow and should be able to survive the 10 minutes without the benzyl bromide.

A solution to overcome this problem is to add the benzyl bromide before the base. By this order of addition, the yield of the desired product 34 increased to 77%, which is acceptable (Scheme 33). The migration was not seen to the same extent.
Towards the synthesis of a stable α-galactosidase inhibitor

With TIPS ether
From the experience with the pivaloyl group migration, the addition order of the reagents was retained.
With these conditions the isolated yield of the benzylation product was 68% (Scheme 33). None of the migration product was detected.

3.2.6. Cleavage of the isopropylidene ketal
The next few steps are proposed in Scheme 34. The first two steps should be straightforward, but the reduction step will be crucial.

With pivaloyl ester
The initial attempt to cleave the isopropylidene ketal was with TFA and CH₂Cl₂ (1:1 v/v) over night and was unsuccessful as the pivaloyl group once again migrated in a yield of 40% and none of the non-migrated 2,3-diol was detected. The migrated product has not been characterized, but indications from LRMS and H¹-NMR suggest that the pivaloyl group has migrated to one of the secondary alcohols. Whether it is intermolecular or intramolecular is unknown. Another experiment resulted in the formation of the 2,3-diol 40 with TFA and CH₂Cl₂ (1:1 v/v) at 0 °C after 30 minutes, but it did not fully convert the substrate to completion. TFA is too strong an acid so a reaction of 34 with acetic acid and water (3:1 v/v) at 60 °C over night was investigated. This resulted in a quantitative yield of the product 40, while none of the migrated product was detected (Scheme 35).
Towards the synthesis of a stable α-galactosidase inhibitor

Scheme 35

With TIPS ether

Treatment of the isopropylidene ketal with TFA and acetic acid did not result in any product formation, but was attempted because it would be a more convenient way to handle, if it was succesful. The method used was developed by Ribes et al. and described in section 2.1.1. The substrate is, however more complex which might have consequences in the form of the reaction not reacting as smoothly. As describe vide supra, large amount of ZnBr₂ is needed, and that requires a large amount of ethylenediaminetetraacetate to extract the zinc. It gave 41 in 87% yield on a 6 gram scale (Scheme 36).

Scheme 36

3.2.7. Synthesis of the benzylidene acetal

The next step was introduction of a benzylidene acetal on the diol 40 or 41. The reaction was performed following the same protocol for the pivaloyl ester protected substrate, and the TIPS ether protected substrate resulted in good yields of 83% (3:7 diasteromeric mixture) and 75% (3:10 diasteriomeric mixture) respectively (Scheme 37). The equilibrium between benzaldehyde dimethyl acetal and the the desired product acetal, 42 and 43 respectively, under acidic conditions exist, and is shifted toward the product acetal 42 or 43. A method to further force the equilibrium towards the product acetals, is to use excess benzylidene dimethyl acetal reagent or to remove the methanol produced by evaporation under reduced pressure. The second method was applied in every benzylidene acetal formation to push the reaction forward, but whether it had a significant effect is unknown. The diasteriomeric mixtures were not separated for the next step, even though it could be interesting to see whether the two epimers will have different regioselectivity.
Towards the synthesis of a stable α-galactosidase inhibitor

Scheme 37

3.2.8. Regioselective reduction of the benzylidene acetal

With pivaloyl ester

As described *vide supra* a number of conditions and reagents are used to make a regioselective reductive ring openings. The mechanism for this reaction can give two possible products (Figure 46) and is essentially the same as described for the general mechanism (Figure 15, Page 27).

Figure 46

Benzylic ethers are not prone to hydrogenolysis under these comparably mild conditions. The benzyl ether deprotection is often performed by hydrogenation with Pd/C, and not by borane or aluminum based reducing agents. The pivaloyl protecting group, on the other hand, can be reduced if the reducing agent is too reactive like the reducing reactivity of DIBALH.

It is difficult to predict what the outcome will be, since both products are secondary alcohols and the adjacent groups both are sterical hindered due to the benzyl ether group. The desired product is the alcohol 28, and one thing that would favor the formation of product 28 over 45, is the pivaloyl ester group at 5-O, due to the bulkiness of the group. How significant an effect on the regioselectivity the group has, experiments will have to show, but a moderate yield will be acceptable.

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Towards the synthesis of a stable $\alpha$-galactosidase inhibitor

If, however, the regioselectivity are in favor of product 45, and selectivity towards the desired product 28 cannot be achieved, all hope is not lost. Reducing a $p$-methoxybenzylidene acetal will potentially give the same selectivity as reducing a benzylidene acetal, and the hydroxyl group can subsequently be benzylated to prepare the desired product.

A various set of conditions have been used to prepare the desired product 28. The results of the reaction, depicted in Scheme 38, are listed in Table 3.

![Scheme 38](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reducing agent</th>
<th>Acid</th>
<th>solvent</th>
<th>Time</th>
<th>Temp.</th>
<th>Yield 28</th>
<th>Yield 45</th>
<th>selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 eq. Et$_3$SiH</td>
<td>4 eq. TfOH</td>
<td>CH$_2$Cl$_2$</td>
<td>45 min.</td>
<td>- 78 °C</td>
<td>0 %</td>
<td>18 %</td>
<td>full</td>
</tr>
<tr>
<td>2</td>
<td>5 eq. Me$_3$N-BH$_3$</td>
<td>5 eq. AlCl$_3$</td>
<td>THF</td>
<td>3 hr</td>
<td>0 °C</td>
<td>12 %</td>
<td>53 %</td>
<td>1:4.4</td>
</tr>
<tr>
<td>3</td>
<td>1 eq. NaCNBH$_3$</td>
<td>1 eq. TiCl$_4$</td>
<td>MeCN</td>
<td>3 hr</td>
<td>0 °C</td>
<td>28 %$^a$</td>
<td>28 %$^a$</td>
<td>1:1</td>
</tr>
<tr>
<td>4</td>
<td>4 eq. Et$_3$SiH</td>
<td>1.5 eq. TiCl$_4$</td>
<td>CH$_2$Cl$_2$</td>
<td>45 min.</td>
<td>- 78 °C</td>
<td>63 %</td>
<td>13 %</td>
<td>4.8:1</td>
</tr>
<tr>
<td>5</td>
<td>4 eq. Et$_3$SiH</td>
<td>1 eq. Cu(OTf)$_2$</td>
<td>CH$_2$Cl$_2$</td>
<td>78 °C to 20 °C</td>
<td>0 %</td>
<td>0 %</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>4 eq. Et$_3$SiH</td>
<td>0.5 eq. TiCl$_4$</td>
<td>CH$_2$Cl$_2$</td>
<td>1 hr</td>
<td>- 78 °C</td>
<td>64 %</td>
<td>24 %</td>
<td>2.7:1</td>
</tr>
</tbody>
</table>

Table 3 –$^a$ A/B-ratio estimated by NMR

The two products were separable by column chromatography, thus the isolated yield is given, except in entry 3 (Table 3). The order of the entries is in the consecutive order in which the reactions were performed. The first reaction was performed with triethyl silane and triflourmethane sulphonic acid and resulted in a low yield of 18%, and with full selectivity to product 45. Switching to a borane reducing agent with a more bulky Lewis acid, aluminum chloride, gave a moderate overall yield of 65%. The selectivity though was still in favor of product 45. Using sodium cyanoboro hydride with titanium chloride instead of aluminum chloride showed an even distribution of product 28 and 45, so titanium chloride was used when switching back to triethyl silane. Interestingly the selectivity turned to favor product 28 instead and in a higher overall yield. Using fewer equivalents of titanium did not lower the yield of product 28. Cupper(II) triflate was also used as Lewis acid but it was unable to convert any starting material according to TLC analysis. Satisfied with the moderate yield, no further optimization was performed, and using the stannane monobenzylating approach was not attempted.
Towards the synthesis of a stable α-galactosidase inhibitor

<table>
<thead>
<tr>
<th></th>
<th>Small reducing agent</th>
<th>Bulky reducing agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small acid</td>
<td>-</td>
<td>0% 28</td>
</tr>
<tr>
<td>Bulky acid</td>
<td>28% 28</td>
<td>64% 28</td>
</tr>
<tr>
<td>Bulky acid</td>
<td>28% 45</td>
<td>24% 45</td>
</tr>
</tbody>
</table>

Table 4 gives an overview of the differences of the steric effects between the reagents, using arguments from the introduction section. Which of the two oxygens that is the most basic cannot be determined because an experiment using a small acid and a small reducing agent have not been performed. When a small acid is used with a bulky reducing agent, it can be guessed which of the two possible oxocarbenium ions is the most accessible, here the one leading to product 45, but the basic properties can also influence on this. The best selectivity was given with a bulky reducing agent and a bulky acid. This gives an indication that O-2 is more accessible compared to O-3.

**With TIPS ether**

Compared to having an ester group in the O-5 position the molecule, the silyl ether is more stable towards stronger reducing agents. As seen before, the TIPS ether group is more acid labile than the pivaloyl ester group, but stable enough to the normal conditions used in reductions. This gives the opportunity of using DIBALH to make the reductive ring opening. It is an bulky lewis acid and a bulky reducing agent, so the proposed steric effect shown in Table 4 will probably be observed here as well. Since the TIPS group is more bulky than the pivaloyl group, the effect can be expected to be even larger.

The reaction of the benzylidene acetal with DIBALH was a delightful experience. Full regioselectivity towards product 44 was found and the yield was as high as 94% - even higher than the combined yield in the best of the previous results with the pivaloyl group (Scheme 39). None of the other alcohol 46 was detected. Purification by column chromatography was not necessary, as the product was pure enough to be used en the following step.

![Scheme 39](image)

**3.2.9. Substitution - part 1**

This and the following section only discuss the pivaloyl ester protected compounds. An overview of the substitution reaction is shown on Figure 47.
Towards the synthesis of a stable α-galactosidase inhibitor

The plan is to convert the alcohol 28 into a functionality which is a better leaving group than hydroxide. Leaving groups can be a mesylate, tosylate, triflate moiety or creating the leaving group in situ by a coupling reagent under Mitsunobu conditions.

Scheme 40

The first leaving group formed was the mesylate, and was employed because it is relatively stable, making it more convenient to work with. The synthesis converts the alcohol 47 to the mesylate 48 in a high yield of 94%. Several different conditions were attempted to substitute with cyanide. Potassium cyanide and sodium cyanide was insoluble in DMF, DMSO, and THF even at elevated temperature. Prolonged exposure with this suspension was not able to convert any of the mesylate.

Tetrabutylammonium cyanide was soluble in THF because of the apolar butyl substituents. The cyanide ion will probably be more dissociated due to the large counterion. Though the mesylate 48 was converted it was unfortunately unable to produce the substitution product 47 even at microwave induced heating. A mess was observed and none of the products were isolated.

The mesylate is apparently not a good enough leaving group, and the triflate was prepared instead. The triflate 49 was prepared in a crude yield of 92%. The triflate being a much better leaving group initially did not substitute with potassium cyanide dispersion in THF, but resulted in a fairly good yield of 52% of the substitution product, when using TBACN in THF at room temperature. The elimination 50 product was also formed in 23% yield. The other potential elimination products were not observed. The isonitrile was not observed either although it has been known to be another byproduct of the substitution.\[179\] Formation of the isonitrile can be performed with reagents like silver cyanide, as the silver is bonded to the carbon, making nitrogen the only nucleophile. To the best of my knowledge, the substitution has been with complete inversion since only one nitrile compound has been formed according to NMR.

Knowing now that an S<sub>N</sub>2 substitution on this particular secondary carbon atom is difficult to perform; we were satisfied with the result and.
3.2.10. Reactions with the nitrile – part 1
As previously mentioned, in the retrosynthetic analysis, the plan is to synthesize 29 by a reduction step, an organometallic nucleophilic addition followed by a protection of the alcohol. Two pathways are proposed as depicted in Scheme 41.
Towards the synthesis of a stable α-galactosidase inhibitor

Pathway a (Scheme 41) is the most obvious way, as reduction of nitriles to aldehydes is well-known. The Grignard addition can result in two diastereomeric products. Applying Karabatsos' model to the substrate actually predicts the unwanted product to be favored, but the two substituents are not significantly different in size (Figure 48). Chelation can occur with the oxygens, so the stereochemical outcome is hard to predict.

If this was shown to be the unwanted diastereomeric product, pathway b can be employed. Reduction of the obtained ketone can be stereoselectively controlled. A possible problem with pathway b is that the olefin potentially can migrate into conjugation with the ketone.

With the discussion above in mind, the real problem arises. The pivaloyl ester is labile towards strong reducing agents and Grignard reagents, and whether the chemoselectivity is in favor of not removing the protecting group is doubtful. At this point it was realized that the pivaloyl ester was not a good choice as the protecting group.

An attempt to reduce the nitrile with one equivalent of DIBALH at –78 °C, was not successful. A much more polar compound appeared by TLC analysis and the reaction was far from full conversion. Obviously the ester was more reactive than the nitrile, and so the goal of the reaction was now to fully convert the ester moiety. So an additional equivalent of DIBALH was added to remove the pivaloyl group and gave 51 in 60% yield (Scheme 42).

3.2.11. Switching protecting group
It was then realized that a different protecting group was needed. The TIPS ether protecting group was chosen and the retrosynthetic strategy would be the same, as for the pivaloyl groups (Scheme 28, Page 50). A lot of the reactions have already been described. The amount of compound 51 available at this point was
Towards the synthesis of a stable α-galactosidase inhibitor

low, but since this was after 10 steps, there was in point of wasting it. The alcohol 51 was TIPS ether protected by TIPSOTf using 2,4,6-coliidine as base, but this resulted in a low yield of only 31% of product 52 (Scheme 43).

\[
\text{Scheme 43}
\]

3.2.12. Substitution – part 2
With the experience from the previous substitution experiments in mind the triflate was the desired leaving group. Unfortunately the reaction of 44 with triflic anhydride and pyridine does not result in the triflate 53 as a final product. Instead cyclization occurs resulting in the tetrahydrafuran 54 in 82 % yield (Scheme 44).

\[
\text{Scheme 44}
\]

This was indeed very puzzling. Based on previous results, the mesylate was probably not good enough as a leaving group to substitute with cyanide, but a poorer leaving group was needed to potentially prevent this intramolecular cyclization. The mesylate 55 was synthesized in a crude yield of 94%, without any cyclization product observed. Unfortunately when treating the mesylate 55 with TBACN in refluxing THF, the compound cyclizes into 54 (Scheme 45) (yield not given).

\[
\text{Scheme 45}
\]

The cyclization is a 5-exo-tet, hence it is allowed according to Baldwin’s rules. Yamauchi et al. \cite{180} have experienced a similar 5-exo-tet cyclization, but under different conditions. Their cyclization occurs during prolonged treatment of compound 55 with mesyl chloride (Figure 49, A). Other differences are that they have a triethylsilyl ether group, and the carbon where the substitution occurs is next to an aromatic group. Shorter reaction times result in the diol 57.
Towards the synthesis of a stable α-galactosidase inhibitor

They proposed a mechanism which involves an initial removal of the TES ether (Figure 49, B). It is highly unlikely that the cyclization of 44 to the tetrahydrofuran 54 occurs by the same mechanism as the mesylate 55 was isolated. This indicates that the triflate also is an intermediate in the first reaction. The TIPS ether group is also more stable towards nucleophiles than TES.

Quallich and Woodall experienced a similar cyclization of the benzylic mesylate 58. Upon standing in deuterated chloroform the compound cyclized to the optical pure tetrahydrofuran 59 with full inversion of stereochemistry (Scheme 46).

Scheme 46

Funder et al. experiences a similar cyclization in an attempt to attach a linker by substitution (Scheme 47). They added 60 drop wise to the sodium hydride treated linker alcohol 61 in DMF. The result was a 6-exo-tet cyclization which is also allowed according to Baldwin’s rules.
Towards the synthesis of a stable α-galactosidase inhibitor

Scheme 47

In carbohydrate chemistry the 4-pentenyl glycoside 62 can be used as a donor when activated by a halonium ion.\[183] This activation involves a cyclized intermediate 63, which subsequently leaves and the oxocarbenium ion 64 is formed (Figure 50). This indicates that the cyclization reaction involving a trisubstituted oxyctation like 64 is a common phenomenon.

Figure 50

An explanation for silyl ether induced cyclization can be that the less electronegative silicon atom makes the oxygen more nucleophilic. Just nucleophilic enough to make the cyclization, when the molecule is in the right conformation (Figure 51).

Figure 51

3.2.13. Comparison of different pathways

The easiest way to compare the two pathways is to compare the overall yield and number of steps. From the isopropylidene 13 to the alcohol 28 and 44, the overall yield is 23 % for the pivaloyl pathway and 18 % for the TIPS pathway respectively, both pathways in 6 steps (Scheme 48). From this the argument could be that the better pathway of the two is the pivaloyl pathway, but the comparison is just not that straight forward. Both pathways have different complications. The protecting of the primary alcohol is for both pathways difficult to obtain selectively, thus the TIPS pathway was the one resulting in the lowest yield. The overall yields from the protected compounds 32 and 36 to alcohols 28 and 44 are 39% for the pivaloyl pathway and 42% for the TIPS pathway respectively – the reversed order of yields compared to the previous argument (Scheme 48).
Towards the synthesis of a stable α-galactosidase inhibitor

When dealing with a long synthesis it is preferable to have the low yielding steps early in the synthesis compared to later. The atom economy of the reagents in the overall synthesis will be better, and it is more convenient to work with lesser amounts of compound. The reduction of the benzylidene acetal, was much more efficient in the TIPS pathway, and avoided the need of column chromatography of two compounds, having almost the same polarity. In my opinion, the TIPS pathway is the preferred choice of the two.

3.2.14. New synthetic strategy – a detour
The TIPS protected nitrile was synthesized but required the removal of the pivaloyl ester and convert the alcohol into the TIPS ether. These steps were low yielding, so a better strategy was proposed (Scheme 49). The overall idea is basically the same as the old strategy, and continues from the alcohol 44. Some of the major problems with the substitution step can potentially be avoided by removing the protecting group on 5-O, and hopefully the olefin will not result in the same problems. The order of addition has changed, which unfortunately requires two more steps, as the alcohol needs to be protected.

Scheme 49
A suggestion for the first few steps are depicted in Scheme 50
Towards the synthesis of a stable α-galactosidase inhibitor

### 3.2.15. Protection of the 2-ol with 4-methoxybenzyl ether and removal of O-5-protecting group

The new strategy utilizes a protection of the secondary alcohol with PMB. The Williamson ether synthesis is used with PMBCl, NaH in DMF. This reaction has only been performed with the TIPS ether protected compound but should also be applicable for the pivaloyl ester at 5-O position. The reaction was performed without any problems and resulted in 65 in an acceptable yield of 76% (Scheme 51). Normally this reaction is quenched with water or methanol, but based upon previous experiences in the group of Rasmussen and Jensen the quench is performed with n-butylamine for easier purification.\[52]\n
![Scheme 50](attachment:image.png)

Scheme 50

The silyl ether was easily removed by TBAF in THF, to give the primary alcohol 66 in an excellent yield of 97% (Scheme 51).

![Scheme 51](attachment:image.png)

Scheme 51

The pivaloyl ester can also be removed selectively with methoxide in methanol at elevated temperature. This has not been performed for 28, but for 34 at the time when the pivaloyl ester strategy was changed to TIPS ether strategy.

### 3.2.16. Oxidation of the primary alcohol

IBX was initially used as the oxidizing agent and the Parikh-Doering oxidation conditions were applied at larger scale. IBX is synthesized from 2-iodobenzoic acid by the procedure developed by Santagostino et al.\[125]
Towards the synthesis of a stable α-galactosidase inhibitor

The oxidation using IBX usually reacts without any problems in nearly quantitative yield. Filtration was the only purification method used, as the aldehyde 67 was unstable and has to be used as soon as possible after the formation. Sometimes, traces of the iodine compound, in some form, was present after filtration.

Parikh-Doering conditions were also used to oxidize the alcohol. The SO₃·Py complex was added in portions as a solid and not dissolved in DMSO first. An isolated yield is not reported for this step, but the combined yield over 2 steps is 86% (2nd step is the Grignard addition, Scheme 52, Page 71)

3.2.17. Olefination and removal of the PMB protecting group

The first attempt to synthesize the olefin was by the Wittig protocol. Unfortunately this did not result in the desired alkene. The isolated products were instead the starting material and the α,β-unsaturated aldehyde 68 (Figure 52)(yield not given).

Figure 52

These alkaline conditions have previously been reported by Ziegler and Wang to form the β-elimination of benzyl alcohol as a byproduct,¹⁸⁴ and they olefinated using Tebbe’s reagent instead. Two potential β-eliminations products – either the Z- or E-alkene - can potentially be formed, but only one product is isolated. Syn-elimination will result in the E-alkene and anti-elimination the Z-alkene. As no proof is given, the best suggestion is that the E-alkene is the product because the syn-elimination will have less sterical interference compared to the anti-elimination.

The olefination in the synthesis was then performed by Peterson olefination. A Grignard addition was performed on the aldehyde to synthesize the β-hydroxysilyl products 69 in a 2 step yield of 86% (from the alcohol 66) as a diastereomeric mixture (Scheme 52). These were not separated as they were assumed to have the same reactivity and selectivity.
Towards the synthesis of a stable α-galactosidase inhibitor

Scheme 52

The diastereomeric mixture was treated with thionyl chloride in pyridine. This resulted in several elimination products, as it was not only the TMS group which eliminates (Figure 53).

Figure 53

Product 70 was purified by column chromatography but still not isolated from the byproduct 71 as they were almost copolar. 244 mg β-hydroxysilyl compounds 69 resulted in 156 mg combined yield, with 70 being the major product. The rest of the synthesis will most likely work just as well without removing the TMS group, and it will be removed at the ring closing metathesis step, thus so the final product will not be changed. It is however more convenient for characterization and scientifically correct to work with pure compounds. An attempt to remove the silyl group with TBAF was unsuccessful.

Before optimization of the olefination was achieved, the mixture was treated with 2% TFA in CH₂Cl₂, and it was found, that the PMB protecting group was removed as expected to compound 72. An indication that the deprotection occurs is that the solution becomes pink. Knowing that the PMB ether is acid labile and the Peterson olefination can occur with acid catalysis, the idea is to perform the two steps in one reaction. Boron trifluoride was the first acid used and gave a good yield of 77% of the olefinated alcohol after 2 hours. During the second attempt of the reaction, it was monitored by TLC analysis in 15 minutes intervals. After 15 minutes the known desired product 72 and the known intermediate 70 appeared. In addition a more polar compound than the desired product 72 appeared. The β-hydroxysilyl compounds 69 were fully converted after 45 minutes, and the more polar compound was still appearing. None of 70 was present. The more polar compound gradually disappeared while the product spot became more intense. This indicated that it was a new intermediate, and even though it has not been isolated, it was assumed to be the diol 73.
Towards the synthesis of a stable α-galactosidase inhibitor

In this reaction the product was isolated in a lower yield of 63% of 71. Other elimination products were isolated and they were the other regioisomers in a Z/E-mixture in a combined yield of 33% (Scheme 53).

![Scheme 53](image)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>72</th>
<th>74</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOCl₂ then TFA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64%</td>
<td>0%</td>
</tr>
<tr>
<td>BF₃·Et₂O</td>
<td>77%</td>
<td>Not isolated</td>
</tr>
<tr>
<td>BF₃·Et₂O</td>
<td>63%</td>
<td>33%</td>
</tr>
<tr>
<td>1M HCl (aq)</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>p-TsOH</td>
<td>57%</td>
<td>&lt; 43%&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 M HCl</td>
<td>66%</td>
<td>28%</td>
</tr>
<tr>
<td>NaH then TFA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87%</td>
<td>0%</td>
</tr>
</tbody>
</table>

<sup>a</sup>two step procedure, <sup>b</sup>not fully isolated from other byproducts.

The β-silicon effect assists in generating the carbocation, but is not directing the regioselectivity of the elimination.

Other acids have been tested, but resulted in lower yields than boron trifluoride (Table 5).

At this point it was realized that a change to a hydride base - which follows a different mechanism – would prevent the formation of byproduct 74. A drawback is that two steps are needed, but they are relatively easy performed steps.

Initially sodium hydride and the diastereomeric mixture of the substrate was dissolved in THF and reacted over night at reflux. According to TLC, the major diastereomer of the substrate was fully converted, but the minor diastereomer was still present. Removing the THF and replacing it with DMF resulted in full conversion of the minor diastereomer after an hour. This demonstrates the importance of the dissociation ability of the solvent. Performing this step, followed by removal of the PMB ether with TFA, without column chromatography of intermediate 70, resulted in a two step isolated yield of 87%.

This is an improvement of the initial method, as is gives a higher overall two step yield, and is more convenient to purify as byproducts are avoided.

### 3.2.18. Substitution – part 3

The substitution was performed with the knowledge from the previously results. The triflate was again used as a leaving group and TBACN as the nucleophile. The reaction with the triflate 75 resulted in 52% yield over 2 steps of the substitution product 76 and a significant amount of an unidentified by product (Scheme 54). No elimination product was isolated.
Towards the synthesis of a stable α-galactosidase inhibitor

Scheme 54

3.2.19. Reactions with the nitrile – part 2
The options are essentially the same as previously described in Scheme 28 (Page 50). The only difference is an olefin instead of a pivaloyl ester (Scheme 28).

Treating the nitrile with DIBAL-H did unfortunately not result in the aldehyde as expected. Solvents used were CH$_2$Cl$_2$, THF and toluene, and the reaction temperature ranged from –78 °C to room temperature. Additional equivalents of DIBAL-H did not help. A desperate attempt using Raney-Nickel and NaH$_2$PO$_3$ did not work either. Barbier reactions were attempted with zinc and the additives aluminum chloride and iron triflate, and did not make the addition. The Grignard reagent did not react with copper bromide as an additive even at 110 °C. A summary of the reactions performed to convert the nitrile are shown at Scheme 56 (A).
Towards the synthesis of a stable α-galactosidase inhibitor

A theory is that treating the nitrile with a base will form the nitrile carbanion which will not react with the nucleophile (Scheme 56, B). Formation of this carbanion requires deprotonation of the nitrile carbanion. Some known $pK_a$ values for α-proton at nitriles are shown in Table 6. The $pK_a$ of the α-proton of the nitrile 76 is probably around 30-35. The $pK_a$ of propene in DMF is around 32. The nitrile can be converted to the nitril anion, which is unreactive towards nucleophiles (Figure 54).

### Table 6

<table>
<thead>
<tr>
<th>compound</th>
<th>$pK_a$ (DMSO) 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-PrCN</td>
<td>32.7[^185]</td>
</tr>
<tr>
<td>CH₃CN</td>
<td>31.3[^186]</td>
</tr>
<tr>
<td>PhOCH₂CN</td>
<td>28.1[^186]</td>
</tr>
<tr>
<td>PhCH(Me)CN</td>
<td>23.0[^186]</td>
</tr>
<tr>
<td>PhCH₂CN</td>
<td>21.9[^186]</td>
</tr>
<tr>
<td>CH₂(CN)₂</td>
<td>11.0[^186]</td>
</tr>
</tbody>
</table>

**3.2.20. Oxidation of the secondary alcohol**

The nitrile 76 seems to be a dead end and a different strategy is needed to proceed. An attempt to make the desired aldehyde was to oxidize the alcohol into a ketone, followed by a Wittig olefination with the methoxymethyl ylid, followed by acid treatment (Figure 55). The number of steps in this homologation procedure would be the same. One major problem is the loss of the stereogenic center.
Towards the synthesis of a stable $\alpha$-galactosidase inhibitor

The oxidation was performed with IBX in ethyl acetate. The ketone 77 was synthesized in a mediocre yield of 64% after purification by column chromatography. The ketone 77 was treated with methoxymethyl triphenylphosphine ylid, but unfortunately the reaction did not result in the enolether, but a mess of products.

### 3.2.21. Substitution – Part 4

Desperate times need desperate measures. Using a malonate as a nucleophile followed by decarboxylation is a known procedure to make a two carbon elongations of organic compounds. Dimethyl sodiomalonate is known to substitute triflates giving reasonable yields.$^{[188]}$ Diethyl sodiomalonate is able to substitute on secondary carbons as well.$^{[189]}$ Using the nitro ethyl enolate anion as nucleophile followed by decarboxylation will extend the carbon chain by one and introduce a nitrogen. The new strategy is depicted in Figure 56 (A).

![Figure 56](image)

The decarboxylation step will occur by heating in acid, and the mechanism is depicted in Figure 56 (B). If this idea worked the synthetic route will be shortened by a few steps compared to the original plan.

The nitro ethyl enolate anion was prepared by treating ethyl nitroacetate with either sodium ethoxide or sodium hydride. Treatment of the triflate with the formed anion did not result in the desired substitution product. The major product was the alcohols 78 and 72 as a 3:1 mixture according to $H^1$-NMR in a combined yield of 33% (Scheme 57).
=None of the elimination product was observed. The reaction was performed in dry conditions so it is puzzling that the alcohols are formed in such high yields. It is obvious that the mechanism does not entirely follow a $S_n2$ pathway with water as the nucleophile, since a diastereomeric mixture is found. If the hydration follows the $S_n1$-pathway, the carbocation could potentially be stabilized by homoconjugation from the olefin (Scheme 57).

3.3. New synthetic target: 2-epi-Australine

3.3.1. Synthesis strategy

As it became apparent that the alcohol 72 was a dead end as well, and a reasonable amount of alcohol 72 was synthesized in 13 steps, there was no point in wasting it. An epimer of Australine could potentially be synthesized by a strategy which resembles the one by Lauritsen and Madsen (Scheme 58).[190]

They used an epimer of the ketone 77 and performed a reductive amination. Following their pathway the ketone 77 could be used (Scheme 59). A problem with the reductive amination is that a mixture of the two diastereomers is generated, and this could potentially be avoided when using a different pathway.
Towards the synthesis of a stable α-galactosidase inhibitor

Scheme 59

The ideal pathway would be if homoallylamine could substitute the triflate 75, but the amine is probably too basic to be used as a proficient nucleophile. Better nucleophiles exist, which introduce a nitrogen atom by substitution. The isocyanide could probably be formed with silver cyanide,\textsuperscript{[179]} which subsequently can be converted into the amine by HCl.\textsuperscript{[191]} The azide is also an excellent nucleophile, which can subsequently be reduced by triphenyl phosphine in the Staudinger reduction.\textsuperscript{[192]} The amine can then substitute a homoallyl halide, using cesium hydroxide as an additive to prevent double alkylation.\textsuperscript{[174]} Hydroxyl amines are also excellent nucleophiles and due to the α-effect, they are not as basic as the amines. The substrate might be too bulky to double alkylate. The α-effect of the hydroxyl amine 78 could perhaps again be exploited, to alkylate the less bulky homoallyl halide, but if this does not work the nitrogen oxygen bond can be cleaved by zinc and acetic acid.

Scheme 60

3.3.2. Substitution – Part 5

Homoallyl amine is a relatively expensive compound, so homoallylammonium bromide was synthesized in a three step procedure. 3-buten-1-ol was first mesylated then substituted with a bromide, then substituted with ammonia (Scheme 61). Unfortunately the overall yield was low, and the desired compound is not pure.

\~ 77 \~
Towards the synthesis of a stable α-galactosidase inhibitor

Scheme 61

The homoallyl ammonium bromide was separated with aqueous potassium carbonate to deprotonize the ammonium and to remove the bromide ion which otherwise would be a competing nucleophile. When the substitution was attempted on the triflate 75, no substitution product was observed.

The O-benzyl-hydroxyl amine was prepared by deprotonation of the ammonium bromide salt with triethylamine. Treating the triflate 75 with O-benzyl-hydroxyl amine, resulted in the substitution product 78 in 62% two step yield, which is the highest substitution yield achieved until now on the secondary carbon (Scheme 62, B). This reaction demonstrates the power of the α-effect. The byproducts have not been isolated and have not been characterized.

Homoallyl iodide was prepared in a two step procedure. 3-buten-1-ol was first mesylated then substituted with iodide. N-alkylation was then attempted on the hydroxyl amine 78, but no alkylation product was observed by a mass spectrum of the crude mixture. Adding potassium carbonate and heating it over 3 days did not result in any alkylation product. The hydroxylamine 78, is probably too bulky to be a decent nucleophile, even though the α-effect should assist.

The hydroxyl amine 78 was instead reduced by zinc dust in acetic acid, which resulted in a cleavage of the weak N-O σ-bond. Benzyl alcohol is probably not yet removed from the crude product so the 94% yield of 79 is just an indication (Scheme 62, B).
3.3.3. Alkylation of the primary amine
The amine could be prepared by reductive amination. The aldehyde would in this case be the homoallyl aldehyde. Direct amination is chosen instead.

Following the optimized procedure by Salvatore et al.\textsuperscript{174} the alkylation was performed once at this point. A mass spectrum of the reaction mixture before work-up after 2 days indicates that the monoalkylated product 80 is formed, without any bialkylated product (Scheme 63). Full conversion was not achieved at the time so additional time was allowed. The product 80 was not isolated. Due to time constraints the project was left at this stage, and the reaction has not been further investigated.

Scheme 63
Towards the synthesis of a stable α-galactosidase inhibitor

4. Future work

A suggestion for the synthesis of 2-epi-australine 2 to the steps from the alkylation product will be to use the same sequence describes vide supra (Scheme 64). Lauritsen and Madsen have some selectivity problems, and some of those problems are highly likely to be observed in this strategy too.

![Scheme 64]

The total synthesis could be shortened if D-psicose is employed as the starting material, following the route by Lauritsen and Madsen (Scheme 43). D-Psicose though is much more expensive than D-fructose, and a principle of total synthesis is that the starting material should be cheap and accessible.

![Scheme 65]

For the synthesis of galacto-noeurostegine the synthesis can progress if the cyanide NR can react. Ideally the deprotection/protection steps of the 5-O would be avoided if the protecting group was stable, but none of the groups which can selectively protect primary alcohols are stable enough. The retrosynthetic strategy is depicted in Scheme 66 with the TIPS ether as it was prepared. Other more simple groups like the TMS- or TES-ether will perhaps also work instead of the TIPS-ether and maybe even higher yielding. The number of step would be the same as the detour strategy with the PMB-ether (Scheme 50).
Towards the synthesis of a stable α-galactosidase inhibitor

Scheme 66

Otherwise a completely different strategy is needed.

The search for other novel and stable glycosidase inhibitors continues. Especially inhibitors with L-fucose 81 and D-glucuronic acid 82 configuration are of great interest (Scheme 67).

Scheme 67
5. Conclusion
The aim of this project was to synthesize a novel and stable inhibitor of the enzyme α-galactosidase. An inhibitor was not synthesized but 2 can potentially be prepared in a 6 step synthesis from the synthesized amine 79.

An overview of all the described compounds synthesized is depicted in the Appendix. This displays the maze that the total synthesis of a compound can become, including the dead ends. By the preferred direct route from D-ribose to the amine 79, the synthesis consists of 16 steps with an overall yield of 5.9% (average yield of 84% per step) (Scheme 68).

The synthesis toward galacto-noeurostegine 1 involved an important regioselective ring opening of the benzylidene acetal 43 to prepare the alcohol 44. Great regioselectivity was achieved toward the desired product with the TIPS ether group positioned at 5-O. Attempts to interconvert the alcohol into a better leaving group and subsequently substitute this functionality unfortunately lead to the cyclization product 54 so a detour was needed. A multi step olefination was achieved and optimized, and eventually alcohol 72 was prepared. A multistep substitution procedure was performed from the alcohol 72 to the nitrile 76. This nitrile was unfortunately unwilling to react the desired way; hence the nitrile was a dead end.

2-epi-australine 2 was a new destination and was potentially reachable from the alcohol 72. The α-effect was exploited in the multistep synthesis of the amine 79. Due to insufficient time the synthesis is on a temporary hold, and eventuually time will probably be reinvested in this project.

The ideality percent for the 16 steps towards 2 is 12.5%, as the synthesis consists of 1 strategic redox reaction and 1 construction reaction. In the postulated 22 steps overall total synthesis of 2 the ideality percent is increased to 23%. The low ideality percent is acceptable as the starting material is complex, as it
Towards the synthesis of a stable α-galactosidase inhibitor

consists of a lot of the needed elements and configurations, and the concession steps are necessary to introduce the right chemo-, regio- and stereo-selectivity.

Though the synthesis and evaluation of a novel α-galactosidase inhibitor was not achieved, a lot of knowledge of the syntheses is gained.
6. Experimental procedures

6.1. General Experimental Information

All reagents except otherwise stated were used as purchased without further purification. Glassware used for reactions carried out under nitrogen or argon atmosphere were flame dried or dried from the oven (ca. 120 °C).

CH$_2$Cl$_2$, THF or toluene were dried by MB SPS-800 solvent purification system (Mbraun, Germany) or according to standard procedures prior to use (‘Purification of Laboratory Chemicals, 3rd Edition’ D. D. Perrin, W. L. F. Armarego, 1988, Butterworth-Heinemann Ltd). CH$_2$Cl$_2$ and acetonitrile was dried by distillation over CaH$_2$, and diethyl ether was dried over sodium. THF was dried over sodium and distilled with benzophenone. Toluene was dried by distillation over CaH$_2$. Acetone and methanol were dried over molecular sieves 3Å and pyridine was dried over molecular sieves 4Å.

Flash chromatography was performed with Merck silica 60 (230-400 mesh) as stationary phase. TLC was performed on silica-coated glass plates (Merck 60 F$_{254}$). TLC plates were first observed in UV-light and then visualized with:

1) CeMol (cerium (IV) sulphate (0.2%) and ammonium molybdat (5%) in 10% sulphuric acid)

2) KMnO$_4$-stain (1.5 g KMnO$_4$ in 1.25 mL 10% NaOH and 200 mL H$_2$O)

6.2. Apparatus

$^1$H-, gCOSY-, gHMQC-, DEPT-135 NMR (400 MHz) and $^{13}$C-NMR (100 MHz) were recorded at a Varian Mercury 400 spectrometer. CDC$_3$ (δ 7.26 ppm (CHCl$_3$) for proton and δ 77.16 ppm for carbon resonances) was used as internal references. MS spectra were recorded at a Micromass LC-TOF instrument by using electrospray ionization (ESI). High resolution spectra were recorded with either of the following compounds as internal standard: (Boc-L-alanine: C$_8$H$_{15}$NO$_4$Na m/z 212.0899; BzGlyPheOMe C$_{19}$H$_{20}$N$_2$O$_4$Na m/z 363.3629; BocSer(OBn)SerLeuOMe C$_{25}$H$_{39}$N$_3$O$_8$Na m/z 532.2635; erythromycin: C$_{37}$H$_{67}$NO$_{13}$Na m/z 756.4510). Masses of standards and analytes are calculated and reported in Daltons for un-charged species.

Optical rotation was measured on a PE-214 polarimeter and reported in units of deg*cm$^2$/g. Concentrations are reported in g/100 mL.

Microwave experiments were carried out on a Biotage Initiator (Biotage, Sweden). Reaction times listed refer to ‘hold time’ at the specified temperature.
6.3. Synthesis

Synthesis of 2,3-O-isopropylidene-D-ribofuranose (13)

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With sulphuric acid catalyst and Ca(OH)$_2$ work-up

D-(−)-Ribose 11 (5.014 g, 33.40 mmol) was suspended in dry acetone (50 mL). Sulphuric acid (98.5%, 0.12 mL) was added. The reaction mixture stirred for 90 minutes at room temperature. Calcium hydroxide (69 mg) was added to pH 7. The mixture was filtered through celite and the solvent from the filtrate was removed under reduced pressure to yield the crude product 13 (5.786 g, 30.42 mmol) as colorless oil. The crude product was used for the next step without further purification.

With solid phase sulphuric acid catalyst

To a D-(−)-Ribose 11 (5.032 g, 33.52 mmol) suspension in dry acetone (50 mL) a few beads of Amberlite IR 120 was added and the reaction stirred over night. The mixture was filtered and concentrated under reduced pressure to afford the crude product 13 (7.500 g). The crude product was used for the next step without further purification.
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 2,3-O-isopropylidene-5-O-pivaloyl-D-ribofuranose (32) and pivaloyl 2,3-O-isopropylidene-5-O-pivaloyl-D-ribofuranoside (35)

13 (0.784 g, 4.12 mmol) was dissolved in dry pyridine (2 mL). The solution was cooled on an ice bath and stirred under nitrogen atmosphere. Pivaloyl chloride (0.516 mL, 4.19 mmol) was added over 10 minutes. The reaction was followed by TLC (ethyl acetate). Precipitation of a salt in the reaction mixture was observed. After 60 minutes additional pivaloyl chloride (0.100 mL, 0.813 mmol) was added, and after additional 90 minutes more pivaloyl chloride (0.150 mL, 1.22 mmol) was added. The reaction was quenched with H₂O, and stirred for 10 minutes. The product was extracted with diethyl ether and the organic phase was washed with aqueous hydrochloric acid (1 M), saturated aqueous sodium bicarbonate and H₂O. The organic phase was dried over MgSO₄, filtered, and concentrated under reduced pressure. The product was purified by column chromatography (1:5 ethyl acetate/pentane) to yield the product 32 as an α-β mixture (0.671 g, 2.45 mmol, 59%, α/β 1:4). By-products 35 were also formed as an α-β mixture (0.262 g, 0.73 mmol, 16%).

Data for 2,3-O-isopropylidene-5-O-pivaloyl-D-ribofuranose (32)

$^1$H-NMR (400 MHz, CDCl₃) δH 5.46 (d, $J_{1,H} = 1.5$ Hz, 0.8H, H1), 5.40 (dd, $J_{1a,2a} = 3.8$ Hz, $J_{1a,OH} = 10.8$ Hz, 0.2H, H1a), 4.03-4.69 (m, 5H), 3.92 (d, 0.2H, $-OH_a$), 3.90 (d, 0.8H, $-OH$), 1.57 (s, 0.6H, $-CH_3_a$), 1.48 (s, 2.4H, $CH_3$), 1.39 (s, 0.6H, $-CH'_3a$), 1.33 (s, 2.4H, $CH_3$), 1.21-1.22 (bs, bs, 9H, $-C(CH_3)_3$). The spectroscopic data are in agreement with previously reported data.[85]

Data for pivaloyl 2,3-O-isopropylidene-5-O-pivaloyl-6-D-ribofuranoside (8-35)

$^1$H-NMR (400 MHz, CDCl₃) δH 6.11 (d, $J_{1,2} = 4.5$ Hz, 1H, H1), 4.83 (dd, $J_{2,3} = 7.0$ Hz, 1H, H2), 4.64 (dd, $J_{3,4} = 3.1$ Hz, 1H, H3), 3.40 (m, $J_{4,5} = 3.6$ Hz, $J_{4,5'} = 3.5$ Hz, 1H, H4), 4.24 (dd, $J_{5,5'} = 12.0$ Hz, 1H, H5), 4.16 (dd, 1H, H5'), 1.54 (s, 3H, $CH_3$), 1.34 (s, 3H, $CH_3$), 1.23 (s, 9H, $-C(CH_3)_3$), 1.19 (s, 9H, $-C(CH_3)_3$)

Data for pivaloyl 2,3-O-isopropylidene-5-O-pivaloyl-6-D-ribofuranoside (α-35)

$^1$H-NMR (400 MHz, CDCl₃) inter alia δH 6.19 (s, 1H, H1), 4.68 (d, $J_{2,3} = 6.0$ Hz, 1H, H2), 4.64 (d, 1H, H3), 4.43 (t, $J_{4,5} = 8.2$ Hz, $J_{4,5'} = 6.8$ Hz, 1H, H4), 4.06 (dd, $J_{5,5'} = 11.3$ Hz, 1H, H5), 4.00 (dd, 1H, H5'), 1.47 (s, 3H, $CH_3$), 1.30 (s, 3H, $CH_3$), 1.18 (s, 9H, $-C(CH_3)_3$), 1.16 (s, 9H, $-C(CH_3)_3$)

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Towards the synthesis of a stable α-galactosidase inhibitor

**Synthesis of 2,3-O-isopropylidene-5-O-pivaloyl-d-ribitol (33)**

32 (3.372 g, 12.29 mmol) was dissolved in dry methanol (30 mL). The solution was cooled on an ice bath and stirred under nitrogen atmosphere. Sodium borohydride (0.811 g, 21.4 mmol) was added, and the reaction was stirred overnight. Additional sodium borohydride (0.450 g, 11.9 mmol) and after 60 minutes the reaction was quenched with dry ice, and stirred for 10 minutes. The solvent was removed under reduced pressure. The oil was dissolved in ethyl acetate and washed with brine. The organic phase was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure to yield 33 (3.227 g, 11.68 mmol, 95%) as a colorless oil.

Rf (1:2 ethyl acetate/pentane) 0.23

HRMS (ESI) calculated for C₁₃H₂₄O₆Na m/z 299.1465 found m/z 299.1472

¹H-NMR (400 MHz, CDCl₃) δ_H 4.45 (dd, J₄,5 = 2.1 Hz, J₅,5' = 11.9 Hz, 1H, H5), 4.36 (dt, J₁,₂ 7.6 = Hz, J₁,₁' 5.2 = Hz, J₂,₃ = 5.2 Hz, 1H, H2), 4.19 (dd, J₄,₅' = 5.7 Hz, 1H, H5'), 4.07 (dd, J₃,₄ = 9.7 Hz, 1H, H3), 4.00 (m, 1H, H4), 3.88 (ddd, J₁,₁' = 11.6 Hz, 1H, H1), 3.80 (ddd, 1H, H1'), 3.38 (d, J₆,OH = 3.8 Hz, 1H, -C4OH), 2.77 (dd, J = 4.9 Hz, J = 8.1 Hz, 1H, -C1OH), 1.42 (s, 3H, -C(CH₃)(CH₃)'), 1.34 (s, 3H, -C(CH₃)(CH₃)'), 1.24 (s, 9H, -C(CH₃)₃)

¹³C-NMR (100 MHz, CDCl₃) δ_C 179.8 (1Cw, -C=O), 108.8 (1Cw, -C(CH₃)₂), 77.3 (1C, C2), 76.5 (1C, C3), 68.9 (1C, C4), 66.9 (1C, C5), 60.8 (1C, C1), 39.1 (1Cw, -C(CH₃)₂), 27.9 (1C, -C(CH₃)(CH₃)'), 27.3 (3C, -C(CH₃)₃), 25.3 (1C, -C(CH₃)(CH₃)')
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 1,4-di-O-benzyl-2,3-O-isopropylidene-5-O-pivaloyl-β-d-ribitol (34)

33 (4.269 g, 15.45 mmol) was dissolved in dry DMF (50 mL). The solution was cooled on an ice bath and stirred under nitrogen atmosphere. Benzyl bromide (7.0 mL, 71.44 mmol) was added. Sodium hydride 60% (1.255 g, 31.38 mmol) was added over multiple times. The reaction was allowed to reach room temperature and stirred overnight. Sodium hydride 60% was added while cooled on ice bath. The reaction mixture was diluted with diethyl ether and quenched with H₂O. The organic phase was washed with H₂O and brine, and was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:10 ethyl acetate/pentane) to yield 34 (5.466 g, 11.97 mmol, 77%) as a colorless oil.

Rf (1:6 ethyl acetate/pentane) 0.35

HRMS (ESI) calculated for m/z C_{27}H_{36}O_{6}Na m/z 479.2404 found m/z 479.2412

^1H-NMR (400 MHz, CDCl₃) δH 7.26-7.34 (m, 8H, PhH), 7.16-7.19 (m, 2H, PhH), 4.72 (dd, J_{4,5} = 2.3 Hz, J_{5,5'} = 12.2 Hz, 1H, H5), 4.69 (d, J_{gem} = 10.8 Hz, 1H, PhCH₂), 4.59 (d, J_{gem} = 12.3 Hz, 1H, PhC'H₂), 4.51 (d, 1H, PhC'H₂), 4.42 (dd, J_{1,2} = 3.4 Hz, J_{2,3} = 6.0 Hz, J_{1,2} = 7.4 Hz, 1H, H2), 4.26 (d, J_{gem} = 10.8 Hz, 1H, PhCH₂), 4.18 (dd, J_{3,4} = 8.9 Hz, 1H, H3), 4.10 (dd, J_{4,5'} = 4.0 Hz, 1H H5'), 3.73 (dd, J₁,₁' = 10.3 Hz, 1H, H1), 3.71 (dd, 1H, H4), 3.50 (dd, 1H, H1'), 1.47 (s, 3H, -C(CH₃)₂CH₃), 1.37 (s, 3H, -C(CH₃)₃CH₃), 1.22 (s, 9H, -C(CH₃)₃)

^13C-NMR (100 MHz, CDCl₃) δC 178.3 (1Cw, -C=O), 138.1 (1Cw, Ph), 137.7 (1Cw, Ph), 127.9-128.5 (10C, Pho,m,p), 108.8 (1Cw, -C(CH₃)₂), 76.8 (1C, C2), 75.7 (1C, C4), 75.0 (1C, C3), 73.6 (1C, PhC'H₂), 71.5 (1C, PhCH₂), 68.9 (1C, C1), 62.7 (1C, C5), 39.0 (1Cw, -C(CH₃)₂), 27.9 (1C, -C(CH₃)₂), 27.3 (3C, -C(CH₃)₃), 25.5 (1C, -C(CH₃)₂CH₃)

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Towards the synthesis of a stable $\alpha$-galactosidase inhibitor

Synthesis of 1,4-di-O-benzyl-5-O-pivaloyl-D-ribitol (40)

34 (1.074 g, 2.353 mmol) was dissolved in a 3:1 acetic acid/H$_2$O mixture (40 mL). The reaction mixture was heated on a 60 °C oil bath over night. The solvent was removed under reduced pressure at 50 °C, to yield the product 40 (0.980 g, 2.353 mmol, 100%) as a colorless oil.

$\text{R}_f$ (1:2 ethyl acetate/pentane) 0.40

HRMS (ESI) calculated for C$_{24}$H$_{32}$O$_6$Na m/z 439.2097 found m/z 436.2096

$^1$H-NMR (400 MHz, CDCl$_3$) δ $^1$H 7.23-7.37 (m, 10H, PhH), 4.73 (d, $J_{gem} = 11.4$ Hz, 1H, PhCH$_2$-), 4.60 (dd, $J_{4,5} = 3.0$ Hz, $J_{5,5'} = 12.2$ Hz, 1H, H5), 4.55 (d, $J_{gem} = 11.9$ Hz, 1H, PhC'H$_2$-), 4.48 (d, 1H, PhC'H$_2$-), 4.47 (d, 1H, PhCH$_2$-), 4.24 (dd, $J_{4,5'} = 5.1$ Hz, 1H, H5'), 3.90 (dddd, $J_{1,1'} = 3.9$ Hz, $J_{2,OH} = 4.9$ Hz, $J_{1',2} = 5.1$ Hz, $J_{2,3} = 6.4$ Hz, 1H, H2), 3.85 (dt, $J_{3,OH} = 5.0$ Hz, $J_{3,4} = 6.4$ Hz, 1H, H3), 3.72 (ddd, 1H, H4), 3.64 (dd, $J_{1,2} = 9.8$ Hz, 1H, H1), 3.61 (dd, 1H, H1'), 2.90 (d, 1H, -OH), 2.80 (d, 1H, -OH), 1.22 (s, 9H, -C(CH$_3$)$_3$)

$^{13}$C-NMR (100 MHz, CDCl$_3$) δ $^{13}$C 178.6 (1Cw, -C=O), 137.8 (1Cw, PhH), 137.7 (1Cw, PhH), 127.9-128.5 (10C, Ph$_{o,m,p}$), 78.4 (1C, C4), 73.6 (1C), 72.2 (1C), 71.9 (1C), 71.6 (1C), 70.4 (1C), 63.0 (1C, C5), 38.8 (1Cw, -C(CH$_3$)$_3$), 27.2 (3C, -C(CH$_3$)$_3$)
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 1,4-di-O-benzyl-2,3-O-isopropylidene-5-O-pivaloyl-d-ribitol (42)

40 (0.168 g, 0.404 mmol) and camphorsulphonic acid monohydrate (7 mg, 0.03 mmol) was dissolved in dry toluene, and evaporated under reduced pressure to remove H₂O. The mixture was resolvated in toluene (4 mL) and the solution was stirred under nitrogen atmosphere. Benzaldehyde dimethyl acetal (0.120 mL, 0.800 mmol) was added. The reaction mixture stirred on an oil bath at 40 °C. After 2 hours, the reaction mixture was concentrated under reduced pressure, resolvated in dry toluene and stirred on an oil bath at 40 °C. When finished, the reaction was quenched with ammonium chloride and H₂O, and the product was extracted once with ethyl acetate. The organic phase was dried with MgSO₄, filtered and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:15 ethyl acetate/pentane) to yield the 3:7 diastereomeric mixture of 42 (0.169 g, 0.335 mmol, 83%)

Rᵣ (major diastereomer) (1:15 ethyl acetate/pentane) 0.30
Rᵣ (minor diastereomer) (1:15 ethyl acetate/pentane) 0.28

HRMS (ESI) calculated for C₃₁H₃₆O₆Na m/z 527.2404 found m/z 527.2416

¹H-NMR (400 MHz, CDCl₃) δH 7.17-7.50 (m, 15H, PhH), 6.21 (s, 0.3H, PhCH⁻), 5.83 (s, 0.7H, PhCH⁻), 3.62-4.71 (m, 7H), 1.21 (s, 6.3H, -C(CH₃)₃), 1.18 (s, 2.7H, -C(CH₃)₃)

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Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 1,3,4-tri-O-benzyl-5-O-pivaloyl-D-riboitol (28) and 1,2,4-tri-O-benzyl-5-O-pivaloyl-D-riboitol (45)

42 (2.056 g, 4.074 mmol) was dissolved in dry CH$_2$Cl$_2$. The solution was cooled to –78 °C under nitrogen atmosphere. Triethyl silane (2.7 mL, 17 mmol) was added, and the solution was stirred for 10 minutes, followed by addition of TiCl$_4$ (0.2 mL, 1.8 mmol) over 30 min. The yellow solution was stirred for another 1 hour, and the reaction was quenched with saturated aqueous sodium bicarbonate, extracted with CH$_2$Cl$_2$. The organic phase was washed with brine. The organic phase was dried with MgSO$_4$, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:50 ethyl acetate/CH$_2$Cl$_2$) to yield 28 (1.314 g, 2.590 mmol, 64%) as a colorless oil and 45 (0.491 g, 0.968 mmol, 24%) as a colorless oil.

Data for 1,3,4-tri-O-benzyl-5-O-pivaloyl-D-riboitol

A Reaxys database search result in a hit for this compound. The article was unavailable so no comparison is given.

R$_f$ (1:40 ethyl acetate/dichloromethane) 0.21

HRMS (ESI) calculated for C$_{33}$H$_{38}$O$_6$Na m/z 529.2566 found m/z 259.2507

$^1$H-NMR (400 MHz, CDCl$_3$) δ$_H$ 7.24-7.36 (m, 15H, PhH), 4.70-4.73 (d, d, $J_{gem} = 11.2$ Hz, $J_{gem} = 11.6$ Hz, PhCH$_2$-; PhC'H$_2$-), 4.48-4.62 (m, 5H, PhCH$_2$-; PhC'H$_2$-; PhC"H$_2$-; PhC"H$_2$-; H5), 4.24 (dd, $J_{4,5} = 6.0$ Hz, $J_{5,5'} = 12.1$ Hz, 1H, H5'), 4.01 (m, 1H, H2), 3.95 (m, 1H, H4), 3.75 (dd, $J = 4.5$ Hz, $J = 6.9$ Hz, 1H, H3), 3.59-3.63 (m, 2H, H1, H1'), 1.21 (s, 9H, -C(CH$_3$)$_3$)

$^{13}$C-NMR (100 MHz, CDCl$_3$) δ$_C$ 178.5 (1Cw, -C=O), 138.1 (2Cw, PhH), 138.0 (1Cw, PhH), 127.7-128.5 (15C, Pho,m,p), 79.0 (1C, C3), 78.2 (1C, C4), 74.0 (1C, PhCH$_2$-), 73.4 (1C, PhC"H$_2$-), 72.4 (1C, PhC"H$_2$-), 71.1 (1C, C1), 70.8 (1C, C2), 63.6 (1C, C5), 38.9 (1Cw, -C(CH$_3$)$_3$), 27.3 (3C, -C(CH$_3$)$_3$)

Data for 1,2,4-tri-O-benzyl-5-O-pivaloyl-D-riboitol

R$_f$ (1:40 ethyl acetate/dichloromethane) 0.13

HRMS (ESI) calculated for C$_{33}$H$_{38}$O$_6$Na m/z 529.2566 found m/z 259.2485

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Towards the synthesis of a stable α-galactosidase inhibitor

$^1$H-NMR (400 MHz, CDCl$_3$) δ$_H$ 7.24-7.33 (m, 15H, PhH), 4.70 (d, $J_{gem} = 11.6$ Hz, 1H, PhCH$_2$-), 4.66 (d, $J_{gem} = 11.4$ Hz, 1H, PhC’H$_2$-), 4.60 (d, 1H, PhCH$_2$-), 4.54 (d, $J_{gem} = 11.9$ Hz, 1H, PhC”H$_2$-), 4.48 (d, 1H, PhC”’H$_2$-), 4.45 (d, 1H, PhC’’H$_2$-), 4.46- 4.56 (1H, H5), 4.20 (dd, $J_{4,5} = 5.7$ Hz, $J_{5,5'} = 12.1$ Hz, 1H, H5’), 4.02 (m, 1H, H3), 3.80 (m, 1H, H2), 3.72-3.81 (m, 2H, H1, H4), 3.65 (dd, $J_{1,2} = 4.7$ Hz, $J_{1,1'} = 10.5$ Hz, 1H, H1’), 1.20 (s, 9H, -C(CH$_3$)$_3$)

$^{13}$C-NMR (100 MHz, CDCl$_3$) δ$_C$ 178.3 (1CW, -C=O), 138.2 (1CW, Ph$_x$), 138.0 (1CW, Ph$_i$), 137.9 (1CW, Ph$_i$), 127.7-128.4 (15C, Ph$_{o,m,p}$), 77.8 (1C, C4), 77.6 (1C, C2), 73.5 (1C, PhCH$_2$-), 72.1 (2C, PhCH$_2$-), 71.2 (1C, C3), 69.7 (1C, C1), 63.6 (1C, C5), 38.8 (1CW, -C(CH$_3$)$_3$), 27.2 (3C, -C(CH$_3$)$_3$)
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 1,3,4-tri-O-benzyl-2-O-mesyI-5-O-pivaloyl-D-riboI (48)

28 (324 mg, 0.640 mmol) was dissolved in dry diethyl ether. The solution was cooled on an ice bath under nitrogen atmosphere. Triethyl amine (0.18 mL, 1.3 mmol) was added followed by addition of mesyl chloride (0.074 mL, 0.95 mmol) was added. After 10 min the solution was diluted in diethyl ether and the organic phase was washed with aqueous hydrochloric acid (1 M), saturated aqueous sodium bicarbonate and H2O. The organic phase was dried with MgSO4, filtered and the solvent was removed under reduced pressure, to yield the crude product 48 (353 mg, 0.604 mmol, 94 %) as a colorless oil. The crude product was used without further purification.

Rf (CH2Cl2) 0.23

LRMS (ESI) calculated for C32H40O8SNa m/z 607.2 found m/z 607.1

1H-NMR (400 MHz, CDCl3) δH 7.25-7.35 (m, 15H PhH), 5.17 (ddd, J1,2 = 8.0 Hz, J1',2 = 3.1 Hz, J2,3 = 2.5 Hz, 1H, H2), 4.79 (d, Jgem 11.0 Hz, 1H, PhCH2-), 4.70 (d, Jgem 11.5 Hz, 1H, PhC'H2-), 4.64 (dd, J4,5 = 2.9 Hz, J5,5' = 12.1 Hz, 1H, H5), 4.58 (d, 1H, PhCH2-), 4.44 (d, 1H, PhC'H2-), 4.44 (d, Jgem 12.0 Hz, 1H, PhC''H2-), 4.42 (d, 1H, PhC''H2-), 4.08 (dd, J4,5' = 4.1 Hz, 1H, H5'), 3.95 (dd, J3,4 = 7.6 Hz, 1H, H3), 3.76 (dd, 1H, H1), 3.67 (m, 1H, H4), 3.61 (dd, 1H, H1'), 2.99 (s, 3H, -SO2CH3), 1.20 (s, 9H, -C(CH3)3)
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 1,3,4-tri-O-benzyl-5-O-pivaloyl-2-O-triflyl-D-ribitol (49)

28 (709 mg, 1.40 mmol) was dissolved in dry CH₂Cl₂ (10 mL). The solution was cooled on an ice bath under nitrogen atmosphere. Dry pyridine (0.30 mL, 3.7 mmol) was added and after 10 minutes, triflic anhydride (0.30 mL, 1.8 mmol) was added. The reaction stirred for 30 minutes. The solution was diluted with DCM, and the organic phase was washed with cold aqueous hydrochloric acid (1 M), sodium bicarbonate and brine. The organic phase was dried with MgSO₄, filtered, and the solvent was removed under reduced pressure to yield the crude product 49 (827 mg). The crude product was used for the next step without further purification.

^1^H-NMR (400 MHz, CDCl₃) δH 7.26-7.38 (m, 15H PhH), 4.49-4.75 (m, 7H), 4.25 (dd, J = 6.0 Hz, J = 12.1 Hz, 1H), 4.02 (m, 1H), 3.96 (m, 1H), 3.76 (dd, J = 4.4 Hz, J = 6.8 Hz, 1H), 3.63 (s, 1H), 3.62 (d, J = 2.5 Hz, 1H), 2.69 (m, 1H), 1.20 (s, 9H, -CH(CH₃)₃)
Towards the synthesis of a stable α-galactosidase inhibitor

**Synthesis of 1,3,4-tri-O-benzyl-2-cyano-2-deoxy-5-O-pivaloyl-d-arabinitol (47) and (4R,3S,E)1,3,4-tri-O-benzylxylo-5-O-pivaloyl-pent-1-ene (50)**

Crude 49 (827 mg) was dissolved in dry THF (10 mL) and cooled on an ice bath. A solution of TBACN (712 mg, 2.65 mmol) in dry THF (5 mL) was added drop wise to the reaction mixture. After 6 hours at 0 °C, the solvent was removed under reduced pressure. The mixture was dissolved in DCM, washed with sodium bicarbonate and brine. The organic phase was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The products were purified by gradient column chromatography (1:20->1:10 ethyl acetate/pentane) to yield the product 47 (350 mg, 0.679 mmol, 49% over 2 steps) and side product 50 (144 mg, 0.295 mmol, 21% over two steps)

**Data for 1,3,4-tri-O-benzyl-2-cyano-2-deoxy-5-O-pivaloyl-D-arabinitol (47)**

Rf (1:15 ethyl acetate/pentane) 0.23

\[ ^1H-NMR \ (400 \ MHz, \ CDCl_3) \delta_{H} \ 7.17-7.27 \ (m, \ 15H, \ PhH), \ 4.65 \ (d, \ gem = 11.0 \ Hz, \ 1H, \ PhCH_2), \ 4.61 \ (dd, \ J_{4,5} = 2.9 \ Hz, \ J_{5,5} = 12.3 \ Hz, \ 1H, \ H5), \ 4.55 \ (d, \ J_{gem} = 11.3 \ Hz, \ 1H, \ PhC'H_2), \ 4.46 \ (d, \ 1H, \ PhC'H_2), \ 4.45 \ (d, \ 1H, \ PhCH_2), \ 4.39 \ (d, \ J_{gem} = 11.9 \ Hz, \ 1H, \ PhC''H_2), \ 4.33 \ (d, \ 1H, \ PhC'''H_2), \ 3.98 \ (dd, \ J_{4,5} = 3.6 \ Hz, \ 1H, \ H5'), \ 3.81 \ (dd, \ J_{2,3} = 2.9 \ Hz, \ J_{3,4} = 7.9 \ Hz, \ 1H, \ H3), \ 3.71 \ (ddd, \ 1H, \ H4), \ 3.58 \ (m, \ J_{1,2} = 6.7, \ 1H, \ H1), \ 3.57 \ (m, \ J_{1',2} = 7.9 \ Hz, \ 1H, \ H1'), \ 3.30 \ (ddd, \ 1H, \ H2), \ 1.13 \ (s, \ 9H, \ -C(CH_3)_3) \]

\[ ^{13}C-NMR \ (100 \ MHz, \ CDCl_3) \delta_{C} \ 178.1 \ (1CW, \ -C=O), \ 137.6 \ (1CW, \ PhH), \ 137.3 \ (1CW, \ PhH), \ 137.2 \ (1CW, \ PhH), \ 127.9-128.6 \ (15C, \ Ph_{o,m,p}), \ 118.1 \ (1C, \ -CN), \ 77.8 \ (1C), \ 74.9 \ (1C), \ 74.7 \ (1C), \ 73.3 \ (1C), \ 72.7 \ (1C), \ 66.7 \ (1C), \ 61.3 \ (1C), \ 39.0 \ (1CW, \ -C(CH_3)_3), \ 35.6 \ (1C, \ C2), \ 27.4 \ (3C, \ -C(CH_3)_3) \]

**Data for (4R,3S,E)1,3,4-tri-O-benzylxylo-5-O-pivaloyl-pent-1-ene (50)**

\[ ^1H-NMR \ (400 \ MHz, \ CDCl_3) \delta_{H} \ 7.25-7.40 \ (m, \ 15H, \ PhH), \ 6.09 \ (d, \ J_{1,2trans} = 12.8 \ Hz, \ 1H, \ H1), \ 4.90 \ (dd, \ J_{2,3} = 9.0 \ Hz, \ 1H, \ H2), \ 4.81 \ (d, \ J_{gem} 12.2 \ Hz, \ 1H, \ PhCH_2), \ 4.78 \ (d, \ 1H, \ PhCH_2), \ 4.69 \ (s, \ 2H, \ PhC'H_2, \ PhC'H_2), \ 4.61 \ (d, \ J_{gem} 12.0 \ Hz, \ 1H, \ PhC''H_2), \ 4.34 \ (d, \ 1H, \ PhC'''H_2), \ 4.30 \ (dd, \ J_{4,5} = 3.9 \ Hz, \ J_{5,5} = 11.5 \ Hz, \ 1H, \ H5), \ 3.74-3.82 \ (m, \ 2H, \ H3, \ H4), \ 1.18 \ (s, \ 9H, \ -C(CH_3)_3) \]
Towards the synthesis of a stable α-galactosidase inhibitor

$^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta_\mathrm{C}$ 178.4 (1C, -C=O), 150.8 (1C, C1), 138.6 (1Cw, Ph$_i$), 138.521 (1Cw, Ph$_i$), 136.7 (1Cw, Ph$_i$), 127.6-128.7 (15C, Ph$_{o,m,p}$), 101.1 (1C, C2), 79.7 (1C), 77.5 (1C), 73.3 (1C, PhCH$_2$), 71.3 (1C, PhCH$_2$), 69.5 (1C, PhCH$_2$), 64.3 (1C, C5), 38.9 (1Cw, -C(CH$_3$)$_3$), 27.3 (3C, -C(CH$_3$)$_3$)
Towards the synthesis of a stable α-galactosidase inhibitor

**Synthesis of 1,3,4-tri-O-benzyl-2-cyano-2-deoxy-D-arabinitol (51)**

47 (101 mg, 0.196 mmol) was dissolved in dry DCM (5 mL) and the solution was cooled to −78 °C under nitrogen atmosphere. DIBALH (0.2 mL, 1M in toluene, 0.2 mmol) was added slowly. The reaction mixture stirred for 90 minutes at −78 °C. Additional DIBALH (0.2 mL, 1M in toluene, 0.2 mmol) was added slowly. The reaction mixture stirred for 2.5 hours at −78 °C. A few drops of methanol were added to quench the reaction. The solution was diluted with DCM, and the organic phase was washed with saturated La Rochelle salt solution, dried over MgSO₄, filtered, and concentrated under reduced pressure. The product was purified by column chromatography (1:40 ethyl acetate/CH₂Cl₂) to yield the product 51 (50 mg, 0.12 mmol, 60%)

**¹H-NMR (400 MHz, CDCl₃)**  δH 7.29-7.41 (m, 15H, PhH), 4.72 (d, J_gem = 11.5 Hz, 1H, PhCH₂⁻), 4.68 (d, J_gem = 11.3 Hz, 1H, PhC’H₂⁻), 4.64 (d, 1H, PhC’H₂), 4.58 (d, 1H, PhCH₂⁻), 4.49 (d, J_gem = 11.9 Hz, 1H, PhC’’H₂), 4.44 (d, 1H, PhC’H₂), 3.97 (dd, J = 2.74 Hz, J = 8.1 Hz, 1H, H3), 3.91 (m, J = 12 Hz, 1H, H5), 3.75 (m, 1H, H5’), 3.60-3.70 (m, 3H, H1, H1’, H4), 3.38 (ddd, J = 2.7 Hz, J = 6.5 Hz, J = 9.2 Hz, 1H, H2), 1.9 (bs, 1H, -OH)
Towards the synthesis of a stable α-galactosidase inhibitor

**Synthesis of 1,3,4-tri-O-benzyl-2-cyano-2-deoxy-1-O-triisopropylsilyl-D-arabinitol (52)**

51 (50 mg, 0.12 mmol) was dissolved in dry CH$_2$Cl$_2$ and cooled on an ice bath under nitrogen atmosphere. 2,4,6-collidine (30 µL, 0.23 mmol) was added followed by addition of TIPSOTf (45 µL, 0.17 mmol). After 10 minutes the reaction mixture was allowed to reach room temperature and was stirred for 5 hours. The solution was diluted with CH$_2$Cl$_2$ and the organic phase was washed with aqueous hydrochloric acid (1 M), sodium bicarbonate and brine. The organic phase was dried over MgSO$_4$, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:40 ethyl acetate/CH$_2$Cl$_2$) to afford the product 52 (22 mg, 0.038 mmol, 31%) as a colorless oil.

R$_f$ (1:15 ethyl acetate/pentane) 0.48

$^1$H-NMR (400 MHz, CDCl$_3$) δ$_H$ 7.26-7.35 (m, 15H, PhH), 4.79 (d, $J_{gem} = 11.3$ Hz, 1H, PhCH$_2$), 4.69 (d, $J_{gem} = 11.6$ Hz, 1H, PhC’H$_2$), 4.63 (d, 1H, PhCH$_2$), 4.54 (d, 1H, PhC’H$_2$), 4.48 (d, $J_{gem} = 11.9$ Hz, 1H, PhC’’H$_2$), 4.42 (d, 1H, PhCH$_2$), 3.99 (dd, $J = 2.9$ Hz, $J = 6.9$ Hz, 1H), 3.97 (dd, $J = 4.0$ Hz, $J = 11.0$ Hz, 1H), 3.84 (dd, $J = 4.4$ Hz, $J = 6.6$ Hz, 1H), 3.60-3.72 (m, 3H), 3.39 (ddd, $J = 2.9$ Hz, $J = 6.5$ Hz, $J = 8.0$ Hz, 1H), 1.03-1.08 (m, 21 H, -CH(CH$_3$)$_2$, -CH(CH$_3$)$_2$)

$^{13}$C-NMR (100 MHz, CDCl$_3$) δ$_C$ 138.2 (1Cw, Ph$_i$), 137.9 (1Cw, Ph$_i$), 137.432 (1Cw, Ph$_i$), 128.0-128.7 (15C, Ph$_o$,$m$,$p$), 118.8 (1C, -CN), 80.3 (1C), 74.9 (1C), 74.1 (1C), 73.5 (1C), 73.4 (1C), 67.2 (1C), 62.3 (1C), 35.3 (1C, C2), 18.2 (6C, -CH(CH$_3$)$_2$), 12.1 (3C, -CH(CH$_3$)$_2$)

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Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 2,3-O-isopropylidene-5-O-triisopropylsilyl-β-D-ribofuranose (36)

13 was dissolved in dry pyridine (1.0 mL). TIPSCI (435 mg, 2.29 mmol) was added and a catalytic amount of 4-dimethylamino pyridine. Precipitation of salt in the reaction mixture was observed. The reaction was stirred over night at 50 °C. The reaction mixture was diluted with DCM and washed with aqueous hydrochloric acid (1 M), saturated sodium bicarbonate and H₂O. The organic phase was dried over MgSO₄, filtered and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:12 ethyl acetate/pentane) to yield the products 36 (349 mg, 1.01 mmol, 44%, α/β ~1:5 (ratio determined by ¹H-NMR))

R₇ (1:15 ethyl acetate/pentane) 0.28

HRMS (ESI) calculated for C₁₇H₃₄O₅SiNa m/z 369.2063 found m/z 369.2073

Data for major anomer 2,3-O-isopropylidene-5-O-triisopropylsilyl-β-D-ribofuranose (36)

¹H-NMR (400 MHz, CDCl₃) inter alia δH 5.29 (d, J₁,₂ = 11.6 Hz, 1H, H1), 4.81 (d, J₃,₄ = 5.9 Hz, 1H, H4), 4.74 (d, 1H, H2), 4.54 (d, 1H, H3), 4.35 (m, 1H, H5), 3.85 (d, J = 2.2 Hz, 1H, H5'), 1.49 (s, 3H, -C(CH₃)(CH₃)'), 1.33 (s, 3H, -C(CH₃)(CH₃)'), 1.06-1.10 (m, 21 H, -CH(CH₃)₂), -CH(CH₃)₆)
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 2,3-O-isopropylidene-5-O-triisopropylsilyl-D-ribitol (38)

36 (302 mg, 0.87 mmol) was dissolved in dry methanol (3.0 mL) and the solution was cooled on an ice bath and stirred under nitrogen atmosphere. Sodium borohydride (60 mg, 1.6 mmol) was added and the reaction stirred overnight at room temperature. Additional sodium borohydride was added and after 60 minutes was the reaction quenches with dry ice, and stirred for 10 minutes. The solvent was removed under reduced pressure. The oil was dissolved in ethyl acetate and washed with brine. The organic phase was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure to yield 38 (304 mg, 0.87 mmol, 100%) as a colorless oil.

Rf (1:6 ethyl acetate/pentane) 0.19

HRMS (ESI) calculated for C₁₇H₃₆O₅SiNa m/z 371.2230 found m/z 371.2232

¹H-NMR (400 MHz, CDCl₃) δH 4.36 (dt, J₁₂,₂ = 5.6 Hz, J₂',₃ = 11.3 Hz, J₂,₃ = 5.6 Hz, 1H, H2), 4.09 (dd, J₃,₄ = 9.3 Hz, 1H, H3), 3.93 (dd, J₄,₅ = 3.2 Hz, J₅,₅' = 9.7 Hz, 1H, H5), 3.89 (m, 1H, H1), 3.72-3.83 (m, 3H, H1', H4, H5'), 3.18 (dd, J₁,OH₁ = 4.9 Hz, J₁',OH₁ = 9.0 Hz, 1H, -OH1), 3.12 (d, J₄,OH₄ = 4.5 Hz, 1H, OH4), 1.39 (s, 3H, -C(CH₃)(CH₃)'), 1.32 (s, 3H, -C(CH₃)(CH₃)'), 1.06-1.12 (m, 21 H, -CH(CH₃)₂, -CH(CH₃)₂)

¹³C-NMR (100 MHz, CDCl₃) δC 108.6 (1Cw, -C(CH₃)₂), 77.8 (1C, C2), 76.7 (1C, C3), 69.7 (1C, C4), 64.7 (1C, C5), 61.0 (1C, C1), 28.0 (1C, -C(CH₃)(CH₃)'), 25.3 (1C, -C(CH₃)(CH₃)'), 18.0 (6C, -CH(CH₃)₂), 12.0 (3C, -CH(CH₃)₂)

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Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 1,4-di-O-benzyl-2,3-O-isopropylidene-5-O-triisopropylsilyl-D-ribitol (39)

38 (279 mg, 0.80 mmol) was dissolved in dry DMF (5.0 mL). The solution was cooled on an ice bath and stirred under nitrogen atmosphere. Benzyl bromide (0.30 mL, 2.5 mmol) was added. Sodium hydride 60% (70 mg, 1.8 mmol) was added over multiple times. The reaction mixture was diluted with diethyl ether and quenched with H₂O. The organic phase was washed with H₂O and brine, and was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:25 ethyl acetate/pentane) to yield 39 (287 mg, 0.543 mmol, 68%) as a colorless oil.

Rₓ (1:30 ethyl acetate/pentane) 0.28

HRMS (ESI) calculated for C₃₁H₄₈O₅SiNa m/z 551.3169 found m/z 551.3169

^1^H-NMR (400 MHz, CDCl₃) δH 7.13-7.26 (m, 10H, PhH), 4.82 (d, Jgem 11.1 Hz, 1H, PhCH₂⁻), 4.52 (d, Jgem 12.4 Hz, 1H, PhC’H₂⁻), 4.495 (d, 1H, PhC’H₂⁻), 4.34 (m, 1H, H2), 4.30 (d, 1H, PhCH₂⁻), 4.11 (dd, J = 6.2 Hz, J = 8.8 Hz, 1H, H3), 4.07 (d, J5,5’ = 11.1 Hz, 1H, H5), 3.79 (dd, J4,5’ = 5.3 Hz, 1H, H5’), 3.67 (dd, J1,2 = 2.9 Hz, J1,1’ = 10.2 Hz, 1H, H1), 3.53 (m, 1H, H4), 3.45 (dd, 1H, H1’), 1.40 (s, 3H, -C(CH₃)(CH₃)’), 1.28 (s, 3H, -C(CH₃)(CH₃)’), 0.99-1.05 (m, 21 H, -CH(CH₃)₂, -CH(CH₃)₃)

^1^C-NMR (100 MHz, CDCl₃) δC 138.4 (1Cw, Ph), 138.2 (1Cw, Ph), 127.9-128.4 (4C, Pho.m), 127.8 (1C, Ph₃), 127.8 (1C, Ph₃), 108.5 (1C, -C(CH₃)₂), 78.5 (1C, C4), 76.9 (1C, C2), 75.0 (1C, C3), 73.5 (1C, PhC’H₂⁻), 72.0 (1C, PhCH₂⁻), 69.2 (1C, C1), 64.2 (1C, C5), 28.0 (1C, -C(CH₃)(CH₃)’), 25.5 (1C, -C(CH₃)(CH₃)’), 18.1 (6C, -CH(CH₃)₂), 12.4 (3C, -CH(CH₃)₃)

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Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 1,4-di-O-benzyl-5-O-triisopropylsilyl-D-ribitol (41)

A solution of 39 (4.301 g, 8.134 mmol) in CH₂Cl₂ (30 mL) was added ZnBr₂ (2.499 g, 11.10 mmol). The reaction was stirred at room temperature for 12 hours. A solution of EDTA (6.630 g, 22.69 mmol) in saturated aqueous sodium bicarbonate was added to the reaction. The organic phase was washed with brine and was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:6 ethyl acetate/pentane) to yield 41 (3.477 g, 7.115 mmol, 87%) as a colorless oil.

Rf (1:6 ethyl acetate/pentane) 0.21

1H-NMR (400 MHz, CDCl₃) δH 7.27-7.38 (m, 10H, PhH), 4.76 (d, Jgem = 11.7 Hz, 1H, PhCH₂), 4.61 (d, 1H, PhCH₂), 4.57 (d, Jgem = 11.7 Hz, 1H, PhCH₂), 4.54 (d, 1H, PhCH₂), 4.11 (dd, J1,1' = 10.7 Hz, J1,2 = 4.8 Hz, 1H, H1), 3.90-3.99 (m, 3H, H2, H3, H4), 3.65-3.73 (m, 3H, H1', H5, H5'), 3.13 (d, J = 4.7 Hz, 1H, -OH), 3.09 (d, J = 4.6 Hz, 1H, -OH), 1.08-1.14 (m, 21 H, -CH(CH₃)₂, -CH(CH₃)₂)

13C-NMR (100 MHz, CDCl₃) δC 138.2 (1Cw, Ph), 137.9 (1Cw, Ph), 127.6-128.3 (10C, Pho,m,p), 79.8 (1C), 73.4 (1C), 72.6 (1C), 72.3 (1C), 71.8 (1C), 70.9 (1C), 63.7 (1C), 17.9 (6C, -CH(CH₃)₂), 11.8 (3C, -CH(CH₃)₂)

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Towards the synthesis of a stable $\alpha$-galactosidase inhibitor

**Synthesis of 1,4-di-O-benzyl-2,3-O-benzylidene-5-O-triisopropylsilyl-d-ribitol (43)**

41 (472 mg, 0.874 mmol) was dissolved in dry toluene (4.0 mL) under nitrogen atmosphere. Benzaldehyde dimethyl acetal (0.14 mL, 0.93 mmol) was added followed by a catalytic amount of dry camphorsulphonic acid. The reaction was stirred at room temperature for 5 hours. The reaction was diluted with CH$_2$Cl$_2$ and quenched with aqueous saturated sodium bicarbonate solution. The phases were separated and the aqueous phase was extracted once with CH$_2$Cl$_2$. The combined organic phases were dried with MgSO$_4$, filtered and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:40 ethyl acetate/pentane) to yield the 3:10 diastereomeric mixture of 43 (279 mg, 0.657 mmol, 75%)

**HRMS (ESI)** calculated for C$_{33}$H$_{48}$O$_5$SiNa$^+$ 599.3169 found 599.3175

**Data for major diastereomer:**

R$_f$ (1:40 ethyl acetate/pentane) 0.14

$^1$H-NMR (400 MHz, CDCl$_3$) \textit{inter alia} \(\delta_H\): 7.48-7.51 (m, 2H, PhH$_o$(PhCH$_-$)), 7.24-7.38 (m, 13H, PhH), 5.82 (s,1H, PhCH$_-$), 4.91 (d, \(J_{gem} = 11.2\) Hz, 1H, PhCH$_2$-$\theta$), 4.56 (d, \(J_{gem} = 12.2\) Hz, 1H, PhC'/$H_2$-$\theta$), 4.51 (d, 1H, PhC'/$H_2$-$\theta$), 4.49 (ddd, \(J_{1,2} = 3.0\) Hz, \(J_{1,2} = 7.4\) Hz, \(J_{2,3} = 6.4\) Hz, 1H, H2), 4.41 (d, 1H, PhCH$_2$-$\theta$), 4.27 (dd, \(J_{3,4} = 8.8\) Hz, 1H, H3), 4.15 (dd, \(J_{4,5} = 11.1\) Hz, \(J_{4,5} = 2.3\) Hz, 1H, H5), 3.90 (dd, \(J_{4,5} = 5.3\) Hz, 1H, H5'), 3.862 (dd, \(J_{1,1'} = 10.5\) Hz 1H, H1'), 3.75 (ddd, 1H, H4), 3.64 (dd, 1H, H1), 1.20-1.24 (m, 21 H, -CH(CH$_3$)$_2$, -CH(CH$_3$)$_2$)

$^{13}$C-NMR (100 MHz, CDCl$_3$) \(\delta_C\): 138.4 (1Cw, Ph$_o$), 138.2 (1Cw, Ph$_o$), 137.6 (1Cw, Ph$_o$), 126.9-129.3 (15C, Ph$_o,m,p$), 103.5 (1C, Ph-$\theta$), 78.5 (1C, C4), 78.1 (1C, C2), 76.2 (1C, C3), 73.6 (1C, Ph-C'/$H_2$-$\theta$), 72.2 (1C, Ph-C'/$H_2$-$\theta$), 69.1 (1C, C1), 64.2 (1C, C5), 18.1 (6C, -CH(CH$_3$)$_2$), 12.0 (3C, -CH(CH$_3$)$_2$)

**Data for minor diastereomer:**

R$_f$ (1:40 ethyl acetate/pentane) 0.19

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Towards the synthesis of a stable α-galactosidase inhibitor

**Synthesis of 1,3,4-tri-O-benzyl-5-O-triisopropylsilyl-d-ribitol (44)**

43 (337 mg, 0.585 mmol) was diluted in dry CH₂Cl₂ (4.0 mL), and the solution was cooled on an ice bath under nitrogen atmosphere. DIBAL (1.0 M solution in toluene, 1.5 mL, 1.5 mmol) was added drop wise. After 90 minutes a few drops of methanol was added. The solution was diluted with DCM, washed with La Rochelle’s Salt solution. The organic phase was dried over MgSO₄, filtered and the solvent was removed under reduced pressure to yield the crude product X16 (318 mg, 0.549, 94%) as colorless oil.

Rᵣ (1:12 ethyl acetate/pentane) 0.33

**HRMS (ESI)** calculated for C₅₃H₇₀O₇SiNa m/z 601.3325 found m/z 601.3331

[α]₀²⁹⁺ = -2.2 (c = 1.0, CHCl₃)

**¹H-NMR (400 MHz, CDCl₃)** δH 7.25-7.36 (m, 15 H, PhH), 4.80 (d, J_gem = 11.7 Hz, 1H, PhCH₂⁻), 4.71 (d, J_gem = 11.4 Hz, 1H, PhC’H⁻), 4.65 (d, 1H, PhCH₂⁻), 4.59 (d, 1H, PhC’’H⁻), 4.54 (d, J_gem = 12.0 Hz, 1H, PhC’’’H⁻), 4.50 (d, 1H, PhC’’’H⁻), 4.05-4.09 (m, 2H, H1, H5), 3.84-3.92 (m, 2H, H5’, H4), 3.80 (m, 1H, H3), 3.63-3.64 (m, 2H, H1’, H2), 2.97 (bs, 1H, -OH), 1.08-1.11 (m, 21 H, -CH(CH₃)₂, -CH(CH₃)₂)

**¹³C-NMR (100 MHz, CDCl₃)** δC 138.5 (1Cw, PhH), 138.5 (1Cw, PhH), 138.1 (1Cw, PhH), 127.5-128.3 (15C, Pho,m,p), 80.9 (1C, C4), 79.1 (1C, C3), 73.5 (1C, PhCH₂⁻), 73.3 (1C, PhCH₂⁻), 72.9 (1C, PhCH₂⁻), 71.3-71.0 (2C, C2, C1), 63.5 (1C, C5), 18.0 (6C, -CH(CH₃)₂), 11.9 (3C, -CH(CH₃)₂)

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Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of \((1R,2R,3R)-2,3\text{-di-benzyl}o\text{xo-1-(benzyl})o\text{xymethyl-tetrahydrofuran (54)}\)

\[ \text{44 (129 mg, 0.22 mmol) was dissolved in dry } \text{CH}_2\text{Cl}_2 \text{ (1.0 mL), under nitrogen atmosphere. Dry pyridine (0.05 mL, 0.6 mmol) was added and after 10 minutes, triflic anhydride (0.05 mL, 0.3 mmol) was added. The reaction was stirred for 10 minutes and the color of the reaction mixture turned pink. The solution was diluted with DCM, and the organic phase was washed with aqueous hydrochloric acid (1 M), saturated aqueous sodium bicarbonate and brine. The organic phase was dried over MgSO}_4 \text{, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:5 ethyl acetate/pentane) to yield the product 54 (72 mg, 0.18 mmol, 82%) as a colorless oil.} \]

\[ \text{R} f \text{ (1:5 ethyl acetate/pentane) 0.24} \]

\[ \text{HRMS (ESI) calculated for } C_{26}H_{28}O_4\text{Na m/z 427.1880 found m/z 427.1887} \]

\[ \text{H-NMR (400 MHz, CDCl}_3 \text{) } \delta \text{ 7.26-7.36 (m, 15H, PhH), 4.76 (d, } \text{J}_\text{gem} = 11.9 \text{ Hz, 1H, PhCH}_2-, \text{) 4.50-4.62 (m, 6H), 4.16-4.19 (m, 1H), 4.07-4.13 (m, 1H), 3.89-3.93 (m, 1H), 3.71-3.80 (m, 1H)} \]

\[ \text{C-NMR (100 MHz, CDCl}_3 \text{) } \delta \text{ 138.4 (1Cw, Ph), 138.4 (1Cw, Ph), 138.1 (1Cw, Ph), 127.6-128.5 (15C, Ph}_o,m,p \text{), 79.1 (1C), 78.6 (1C), 77.9 (1C), 73.5 (1C), 73.3 (1C), 72.4 (1C), 69.5 (1C), 69.3 (1C)} \]
Towards the synthesis of a stable α-galactosidase inhibitor

**Synthesis of 1,3,4-tri-O-benzyl-2-O-(4-methoxy)benzyl-5-O-triisopropylsilyl-D-ribitol (65)**

44 (2.456 g, 4.243 mmol) was dissolved in dry DMF (30 mL) and the solution was cooled on an ice bath and stirred under nitrogen atmosphere. 4-methoxybenzyl chloride (0.63 mL, 4.6 mmol) was added. Sodium hydride 60% (170 mg, 4.3 mmol) was added over multiple times. The reaction was stirred at room temperature over night. The reaction mixture was diluted with diethyl ether and quenched with n-butyl amine (4 mL). The organic phase was washed with aqueous hydrochloric acid (1 M), saturated aqueous sodium bicarbonate in H₂O and brine, and was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:30 ethyl acetate/pentane) affording 65 (2.260 g, 3.233 mmol, 76%) as a colorless oil.

Rf (1:30 ethyl acetate/pentane) 0.28

**HRMS (ESI)** calculated for C₄₃H₆₈O₆SiNa m/z 721.3900 found m/z 721.3885

**¹H-NMR (400 MHz, CDCl₃)** δH 7.25-7.33 (m, 17H, PhH, Ph(PMB)Hₘ), 6.84 (d, J₀,m = 8.6 Hz, 2H, Ph(PMB)Hₒ), 4.80 (d, J_gem = 11.7 Hz, 1H, PhCH₂), 4.69 (d, J_gem = 11.4 Hz, 1H, Ph′CH₂), 4.66 (m, 2H, Ph′CH₂, Ph′′CH₂), 4.62 (d, 1H, PhCH₂), 4.58 (d, J_gem = 11.3 Hz, 1H, Ph′′CH₂), 4.52 (d, J_gem = 12.1 Hz, 1H, Ph′′′CH₂), 4.49 (d, 1H, Ph′′′′CH₂), 3.95-4.02 (m, 2H), 3.84-3.87 (m, 2H), 3.74-3.81 (m, 5H), 3.69 (dd, J = 6.0 Hz, J = 10.5 Hz, 1H), 1.05-1.08 (m, 21H, -CH(CH₃)₂, -CH(CH₃)₂)

**¹³C-NMR (100 MHz, CDCl₃)** δC 159.2 (1Cw, Ph(PMB)), 139.1 (1Cw, Phₘ), 138.8 (1Cw, Phₜ), 138.8 (1Cw, Phₜ), 131.1 (1Cw, Phₜ(PMB)), 127.4-129.5 (1Ct, Phₐ,m,p), 113.8 (2C, Phₐ(PMB)), 80.8 (1C), 79.0 (1C), 78.5 (1C), 73.7 (1C, PhCH₂⁻), 73.4 (1C, PhCH₂⁻), 73.1 (1C, PhCH₂⁻), 72.2 (1C, PhCH₂⁻), 70.7 (1C), 64.6 (1C), 55.4 (1C, -OCH₃), 18.3 (6C, -CH(CH₃)₂), 12.1 (3C, -CH(CH₃)₂)
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 1,3,4-tri-O-benzyl-2-O-(4-methoxy)benzyl-d-ribitol (66)

65 (2.029 g, 2,903 mmol) was dissolved in dry THF (0.5 mL) and the solution was stirred under nitrogen atmosphere. Tetrabutylammonium fluoride (1.0 M solution in THF, 3.0 mL, 3.0 mmol) was added. After 30 minutes the reaction was quenched with a few drops of H₂O, and extracted with ethyl acetate. The organic phase was washed with H₂O and brine, dried over MgSO₄, filtered and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:4 ethyl acetate/pentane) to yield 66 (1.536 g, 2.830 mmol, 97%) as a colorless oil.

Rf (1:4 ethyl acetate/pentane) 0.19

HRMS (ESI) calculated for C₃₄H₃₈O₆Na m/z 565.2566 found m/z 565.2565

[α]D²⁹³K = -0.9 (c = 3.6, CHCl₃)

¹H-NMR (400 MHz, CDCl₃) δH 7.26-7.34 (m, 15H, PhH), 7.25 (d, J₀₋₁ = 8.5 Hz, 2H, Ph(PMB)H₀), 6.85 (d, 2H, Ph(PMB)H₀), 4.71 (d, J₀₋₁ = 11.3 Hz, 1H, PhCH₂), 4.66 (d, J₀₋₁ = 11.3 Hz, 2H, PhCH₂, PhC’H₂), 4.58 (s, 2H, PhC’’H₂, PhC’’H₂), 4.57 (d, 1H, PhC’’H₂), 4.53 (d, J₀₋₁ = 12.1 Hz, 1H, PhC’’’H₂), 4.49 (d, 1H, PhC’’’H₂), 3.93 (t, J₂,₃ = 4.7 Hz, J₂,₄ = 4.7 Hz, H3), 3.87 (q, J = 4.7 Hz, 1H), 3.80 (s, 3H, -OCH₃), 3.65-3.76 (m, 5H), 2.31 (t, J₅,OH = 5.9 Hz, 1H, -OH)

¹³C-NMR (100 MHz, CDCl₃) δC 159.2 (1Cw, Ph(PMB)), 138.4 (1Cw, Ph₀), 138.3 (2Cw, Ph₀), 130.4 (1Cw, Ph₀(PMB)), 127.6-129.6 (17C, Ph₀,m,p), 113.8 (2C, Ph₀(PMB)), 79.1 (1C), 79.0 (1C), 77.8 (1C), 73.9 (1C), 73.3 (1C), 72.1 (1C), 71.9 (1C), 69.8 (1C), 61.4 (1C), 55.2 (1C, -OCH₃)
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of 2,3,5-tri-O-benzyl-4-O-(4-methoxy)benzyl-L-ribose (67)

Oxidation with IBX
66 (140 mg, 0.258 mmol) was dissolved in ethyl acetate (3.0 mL), and was added 2-iodoxybenzoic acid (220 mg, 0.786 mmol). The reaction was stirred at reflux for 90 minutes. The reaction mixture was filtered and the solvent was removed under reduced pressure to afford the crude product 67 (137 mg). The product was used in the next step without further purification.

Oxidation with Parikh-Doering reagent
66 (852 mg, 1.57 mmol) was dissolved in dry CH₂Cl₂ (4.0 mL) and dimethyl sulfoxide (4.0 mL). The solution was cooled on an ice bath under nitrogen atmosphere. Dry triethyl amine (1.5 mL) was added, followed by addition of sulfur trioxide-pyridine complex (1.04 g, 6.53 mmol) over multiple times. The reaction was stirred at 0 °C for 90 minutes and the mixture was diluted with diethyl ether and washed with brine. The organic phase was dried over MgSO₄, filtered and was concentrated under reduced pressure to afford the crude product 67. The crude product was used without further purification.

Rf (1:6 ethyl acetate/pentane) 0.38

HRMS (ESI) calculated for C₃₄H₃₆O₆Na m/z 563.2404 found m/z 563.2412

¹H-NMR (400 MHz, CDCl₃) δ 9.48 (s, 1H, H1), 7.22-7.33 (m, 15H, PhH), 7.20 (d, J₁,m = 8.7 Hz, 2H, Ph(PMB)H₂m), 6.81 (d, 2H, Ph(PMB)H₂o), 4.74 (d, J₅,₆ = 12.0 Hz, 1H, PhCH₂), 4.69 (d, 1H, PhCH₂), 4.63 (d, J₅,₆ = 11.1 Hz, 1H, PhCH₂), 4.58 (d, J₅,₆ = 11.4 Hz, 1H, PhCH₂), 4.46-4.53 (m, 4H, PhCH₂), 4.38 (dd, J₃,₄ = 2.2 Hz, 1H, PhH), 3.99 (ddd, J₃,₄ = 8.6 Hz, 1H, PhH), 3.88 (ddd, J₃,₄ = 2.3 Hz, J₇,₈ = 4.5 Hz, H₄), 3.79 (s, 3H, -OCH₃), 3.68 (dd, 1H, H5), 3.60 (dd, 1H, H5"

¹³C-NMR (100 MHz, CDCl₃) δ 201.1 (1C, C1), 159.2 (1Cw, Ph(PMB)), 138.3 (1Cw, Ph), 137.7 (1Cw, Ph), 137.6 (1Cw, Ph), 130.2 (1Cw, Ph(PMB)), 127.6-129.7 (17C, Ph₁,₂,₃,₄,₅,₆,₇,₈,°,₉,₁₀), 113.7 (2C, Ph₂(PMB)), 82.5 (1C, C2), 80.6 (1C, C3), 76.4 (1C, C4), 73.4 (1C, PhCH₂), 73.1 (1C, PhCH₂), 72.9 (1C, PhCH₂), 72.4 (1C, PhCH₂), 69.2 (1C, C5), 55.3 (1C, -OCH₃)
Towards the synthesis of a stable \( \alpha \)-galactosidase inhibitor

**Synthesis of \((4R,E)\)-2,5-di-benzyloxy-4-(4-methoxy)benzyloxy-pent-2-enal (68)**

Methyl triphenylphosphine bromide (63 mg, 0.18 mmol) in dry THF (2 mL) was cooled on an ice bath under argon atmosphere and n-butyl lithium (0.1 mL, 0.2 mmol) was added drop wise, and the yellow reaction mixture was stirred for 1 hour. Crude IBX oxidized 67 (91 mg, 0.17 mmol) was added at 0 °C. The reaction mixture was allowed to reach room temperature and was stirred over night. The mixture was diluted with diethyl ether, filtered and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:8 ethyl acetate/pentane) to yield the product 68 (yield not determined) as colorless oil.

**LRMS (ESI)** calculated for \( C_{27}H_{28}NO_5Na \) m/z 455.2 found m/z 455.0

\(^1\text{H-NMR (400 MHz, CDCl}_3\) \( \delta \) 9.29 (s, 1H, H5), 7.25-7.34 (m, 13H, PhH), 7.19 (d, \( J_{o.m} = 8.8 \) Hz, 2H, Ph(PMB)Hm), 6.83 (d, 2H, Ph(PMB)Ho), 5.94 (d, \( J_{2,3} = 8.6 \) Hz, 1H, H3), 5.10 (d, \( J_{gem} = 11.5 \) Hz, 1H, PhCH2), 5.03 (d, 1H, PhCH2), 4.59 (dt, \( J_{1,2} = 5.2 \) Hz, 1H, H2), 4.50 (s, 2H, PhC’H2), 4.38 (d, \( J_{gem} = 11.6 \) Hz, 1H, PhC’’H2), 4.29 (d, 1H, PhC’’H2), 3.79 (s, 3H, -OCH3), 3.46 (d, 2H, H1)
Towards the synthesis of a stable α-galactosidase inhibitor

Formation of trimethylsilyl methane magnesiumchloride

Solid Magnesium (829 mg, 34.1 mmol) in dry diethyl ether (12.5mL) was added a catalytic amount of 1,2-dibromoethane under argon atmosphere. Trimethylsilyl methane chloride (2.2 mL, 16 mmol) was added drop wise. The reaction was stirred for 60 minutes warmed at 43 °C (oil bath temperature). The solution was filtered and the concentration of product in diethyl ether was determined by titration of the solution in a menthol solution in diethyl ether to a concentration of 1.2 M.
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of \((2R/S,3R,4S,5S)-3,4,6\text{-tri-O-benzyloxy-5-O-(4-methoxy)benzyloxy-1-trimethylsilylhexan-2-ol (69)}\)

With crude product 68 from IBX oxidation
Freshly prepared trimethylsilyl methane magnesiumchloride (0.41 mL, 1.2 M in diethyl ether, 0.49 mmol) was diluted in dry diethyl ether (3 mL). The solution was cooled to 0 °C under Argon atmosphere. Crude 68 (237 mg) from the IBX oxidation was dissolved in diethyl ether (3 mL) and added to the cooled reaction mixture. After 10 minutes of stirring, the reaction was quenched with saturated ammonium chloride solution in \(\text{H}_2\text{O}\), and the organic phase was separated. The organic phase was washed twice with saturated brine, dried over \(\text{MgSO}_4\), filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:12 ethyl acetate/pentane) to yield the diastereomeric mixture of 69 (162 mg, 0.258 mmol, 59% over 2 steps) as a colorless oil.

With crude product from Parikh Doering oxidation
Freshly trimethylsilyl methane magnesiumchloride (0.41 mL, 1.2 M in diethyl ether, 0.49 mmol) was diluted in dry diethyl ether (5 mL). The solution was cooled to 0 °C under Argon atmosphere. Crude 68 from the Parikh Doering oxidation was dissolved in diethyl ether (3 mL) and added to the cooled reaction mixture. After 10 minutes of stirring the reaction was quenched with saturated ammonium chloride solution in \(\text{H}_2\text{O}\), and the organic phase was separated. The organic phase was washed twice with brine, dried over \(\text{MgSO}_4\), filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:12 ethyl acetate/pentane) to yield the diastereomeric mixture of 69 (849 mg, 1.35 mmol, 86 % over 2 steps) as a colorless oil.

\(R_f\) (major) (1:10 ethyl acetate/pentane) 0.33

\(R_f\) (minor) (1:10 ethyl acetate/pentane) 0.27

\(^1\text{H-NMR (400 MHz, CDCl}_3\) \(\delta \) (m, 15H, Ph\(\text{H}\)), 6.83 (m, 2H, Ph(PMB)\(\text{H}_3\)), 4.48-4.71 (m, 8H), 3.91-4.03 (m, 3H), 3.77-3.81 (m, 4H), 3.70 (dd, \(J = 5.4 \text{ Hz}, J = 10.4 \text{ Hz}, 1\text{H}\)), 3.51 (dd, \(J = 3.4 \text{ Hz}, J = 5.6 \text{ Hz}, 1\text{H}\)), 2.78 (d, \(J = 6.7 \text{ Hz}, 1\text{H}, -\text{OH}\)), 0.79 (dd, \(J = 10.8 \text{ Hz}, J_{1,1'} = 14.3 \text{ Hz}, 1\text{H}, \text{H1}\)), 0.68 (dd, \(J = 3.7 \text{ Hz}, 1\text{H}, \text{H1'}\)), 0.00 (s, 9H, -Si(CH\(3\))\(3\))

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Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of (2S,3R,4R)-1,3,4-tri-benzyloxy-2-(4-methoxy)benzoyloxy-hex-5-ene (70) and (2S,3R,4R,E/Z)-1,3,4-tri-benzyloxy-(4-methoxy)-1-trimethylsilyl-benzoyloxy-hex-5-ene (71)

![Chemical Structures 70 and 71]

**Peterson olefination with thionyl chloride**

69 (244 mg, 0.388 mmol) was dissolved in dry pyridine (2.0 mL) and cooled to 0 °C under nitrogen atmosphere and was thionyl chloride (85 μL, 1.2 mmol). After 30 minutes of stirring the reaction mixture was diluted with ethyl acetate and the organic phase was washed with aqueous hydrochloric acid (1 M), saturated aqueous sodium bicarbonate and brine, and was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The products were purified by column chromatography (1:25 ethyl acetate/pentane) to yield a mixture of products 70 and 71 (156 mg) as a colorless oil.

**Peterson olefination with sodium hydride**

69 (4.116 mg, 6.544 mmol) was dissolved in DMF (35 mL) under nitrogen atmosphere and was added sodium hydride 60% (340 mg, 8.50 mmol). The reaction was stirred for 30 minutes at room temperature, and was diluted with ethyl acetate and washed with brine. The organic phase was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:10 ethyl acetate/pentane) to yield the product 70 (2.417 g, 4.487 mmol, 69%)

**Data for (2S,3R,4R)-1,3,4-tri-benzyloxy-(4-methoxy)benzoyloxy-hex-5-ene (70)**

\[ \text{Rf} (1:6 \text{ethyl acetate/pentane}) 0.43 \]

\[ \text{HRMS (ESI) calculated for C}_{35}\text{H}_{38}\text{O}_{5}\text{Na m/z 561.2611 found m/z 561.2608} \]

\[ [\alpha]_{D}^{293K} = -3.0 \text{ (c = 1.0, CHCl}_3) \]

\[ ^1\text{H-NMR (400 MHz, CDCl}_3) \delta_H: \text{7.20-7.34 (m, 15H, PhH), 7.21 (d, J_{6,7} = 8.7 Hz, 2H, Ph(PMB)H}_m), 6.84 (d, 2H, Ph(PMB)H}_o), 5.92 (ddd, J_{5,6} = 17.4 Hz, J_{5,6'} = 10.4 Hz, J_{4,5} = 8.0 Hz, 1H, H5), 5.33 (dd, J_{6,6'} = 1.9 Hz, 1H, H6), 5.20 (dd, 1H, H6'), 4.83 (d, J_{gem} = 11.4 Hz, 1H, PhCH}_2), 4.65 (d, J_{gem} = 11.8 Hz, 1H, PhC'H}_2), 4.64 (d, 1H, PhCH}_2), 4.62 (d, 1H, PhC''H}_2), 4.52 (s, 2H, PhC''''H}_2), 4.47 (d, 1H, PhC'H}_2), 4.38 (d, 1H, PhC'H}_2), 4.13 (dd, J_{3,4} = 4.0 Hz, 1H, H4), 3.89 (dd, J_{2,3} = 6.3 Hz, 1H, H3), 3.82 (s, 3H, -OCH}_3), 3.78 (dd, J_{1,1'} = 9.6 Hz, J_{1,2} = 2.0 Hz, 1H, H1), 3.70 (m, J_{1',2} = 5.2 Hz, 2H, H1', H2) \]

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Towards the synthesis of a stable α-galactosidase inhibitor

$^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$C 159.2 (1Cw, Ph$_i$(PMB)), 138.8 (1Cw, Ph$_i$), 138.8 (1Cw, Ph$_i$), 138.6 (1Cw, Ph$_i$), 135.5 (1C, C5), 130.8 (2C, Ph$_m$(PMB)), 127.4-129.5 (16C, Ph$_o,m,p$(PMB)), 119.4 (1C, C6), 113.8 (2C, Ph$_o$(PMB)), 81.4 (1C, C4), 80.7 (1C, C3), 78.0 (1C, C2), 74.2 (1C, PhCH$_2$-), 73.4 (1C, PhCH$_2$-), 71.9 (1C, PhCH$_2$-), 70.4 (1C, PhCH$_2$-), 69.9 (1C, C1), 55.3 (1C, -OCH$_3$)
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of (2S,3R,4R)-1,3,4-tri-benzyloxy-2-hydroxy-hex-5-ene (72) and (4S,5R,E/Z)-3,4,6-tri-benzyloxy-5-hydroxy-1-trimethyl-silyl-hex-2-ene (74)

Removal of PMB-ether with TFA

70 (2.417 g, 4.487 mmol) was dissolved in dry CH₂Cl₂ (36.0 mL) under nitrogen atmosphere, and added triflouroacetic acid (720 µL). The reaction stirred for 30 minutes and was poured into saturated aqueous sodium bicarbonate and extracted three times with CH₂Cl₂. The combined organic phases were dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:10 ethyl acetate/pentane) to yield product 72 (1.737 g, 4.150 mmol, 85%) as a colorless oil.

Peterson olefination and removal of PMB-ether with BF₃·Et₂O

69 (47 mg, 0.075 mmol) was dissolved in dry dichloromethane (2.5 mL), cooled to 0 °C under nitrogen atmosphere and added boron triflouride-diethyl ether complex (10 µL, 0.080 mmol). The reaction was allowed to reach room temperature, and the solution appeared as a pink colour. After 2 hour the reaction was quenched with saturated aqueous sodium bicarbonate, and diluted with dichloromethane. The organic phase was washed with additional saturated aqueous sodium bicarbonate. The organic phase was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:10 ethyl acetate/pentane) to afford the product 72 (24 mg, 0.056 mmol, 75%) as a colorless oil.

Peterson olefination and removal of PMB-ether with HCl

69 (101 mg, 161 mmol) was added HCl (0.90 mL, 4 M in dioxane, 3.6 mmol) under nitrogen atmosphere. The reaction was stirred at room temperature for 5 hours and was quenched with saturated aqueous sodium bicarbonate. The mixture was extracted with diethyl ether and washed with brine. The organic phase was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:10 ethyl acetate/pentane) to yield the product 72 (44 mg, 0.11 mmol, 69%) and the product 74 (22 mg, 0.045 mmol, 28%) both as colorless oils.
**Peterson olefination with NaH followed by removal of PMB-ether with TFA**

69 (33 mg, 0.052 mmol) was dissolved in DMF (1 mL) under nitrogen atmosphere and was added sodium hydride 60% (10 mg, 0.25 mmol). The reaction was stirred for 60 minutes at room temperature, and was diluted with ethyl acetate and washed with brine. The organic phase was dried over MgSO$_4$, filtered, and the solvent was removed under reduced pressure. The crude product was dissolved in dry CH$_2$Cl$_2$ (2.0 mL) under nitrogen atmosphere. Trifluoroacetic acid (40 μL) was added and the reaction stirred for 30 minutes. The reaction mixture was poured into saturated aqueous sodium bicarbonate and extracted three times with dichloromethane, and the combined organic phases were dried over MgSO$_4$, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:9 ethyl acetate/pentane) to afford product as 72 (19 mg, 0.045 mmol, 87% over 2 steps) as a colorless oil.

**Data for (25,3R,4R)-1,3,4-tri-benzylxy-2-hydroxy-hex-5-ene (72)**

$R_f$ (1:8 ethyl acetate/pentane) 0.35

HRMS (ESI) calculated for C$_{27} $H$_{30} $O$_4 $Na m/z 441.2036 found m/z 441.2041

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta$H 7.25-7.36 (m, 15H, PhH), 5.97 (ddd, J$_{5,6}$ 17.3 Hz, J$_{5,6'}$ 10.3 Hz, J$_{4,5}$ 7.7 Hz, 1H, H5), 5.37 (d, d, 2H, H6, H6'), 4.80 (d, J$_{gem}$ = 11.2 Hz, 1H, PhCH$_2$), 4.66 (d, J$_{gem}$ = 11.9 Hz, 1H, PhC'H$_2$), 4.59 (d, 1H, PhCH$_2$), 4.54 (d, J$_{gem}$ = 11.9 Hz, 1H, PhC''H$_2$), 4.50 (d, 1H, PhC'H$_2$), 4.40 (d, 1H, PhC''H$_2$), 4.17 (dd, J$_{3,4}$ 3.5 Hz, 1H, H4), 3.86 (m, 1H, H2), 3.72 (dd, J$_{2,3}$ 7.5 Hz, 1H, H3), 3.65 (dd, J$_{1,1'}$ 9.5 Hz, J$_{1,1'}$ 2.9 Hz, 1H, H1), 3.61 (dd, J$_{1,1'}$ 5.7 Hz, 1H, H1'), 2.66 (bs, 1H, -OH)

$^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta$C 138.590 (1Cw, Ph), 138.549 (1Cw, Ph), 138.137 (1Cw, Ph), 135.282 (1C, C5), 127.571-128.508 (15C, Ph$_{o,m,p}$), 119.770 (1C, C6), 82.166 (1C, C4), 81.109 (1C, C3), 74.168 (1C, PhCH$_2$), 73.484 (1C, PhC''H$_2$), 71.196 (1C, C1), 70.903 (1C, C2), 70.543 (1C, PhC''H$_2$)

**Data for (4S,5R,E/Z)-3,4,6-tri-benzylxy-5-hydroxy-1-trimethyl-silyl-hex-2-ene (74)**

$R_f$ (1:15 ethyl acetate/pentane) 0.48

HRMS (ESI) calculated for C$_{30} $H$_{38} $O$_4 $SiNa m/z 513.2432 found m/z 513.2437

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Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of (2S,3R,4R)-1,3,4-tri-benzyloxy-2-triflate-hex-5-ene (75)

72 (444 mg, 1.06 mmol) was dissolved in dry CH2Cl2 (17 mL). The solution was cooled on an ice bath, under nitrogen atmosphere. Dry pyridine (0.40 mL, 5.0 mmol) was added and after 10 minutes, triflic anhydride (0.25 mL, 1.5 mmol) was added. The reaction stirred in 30 minutes. The solution was diluted with DCM, and the organic phase was washed with cold aqueous hydrochloric acid (1 M), saturated aqueous sodium bicarbonate and brine. The organic phase was dried with MgSO4, filtered, and the solvent was removed under reduced pressure to yield the crude product 75 (577 mg). The crude product was used for the next step without further purification.

1H-NMR (400 MHz, CDCl3) δH 7.26-7.36 (m, 15H, PhH), 5.79 (ddd, J5,6 = 17.3 Hz, J6,6' = 10.3 Hz, J4,5 = 6.9 Hz, 1H, H5), 5.36-5.45 (m, 3H, H6, H6', H2), 4.66 (d, Jgem = 11.4 Hz, 1H, PhCH2), 4.63 (d, Jgem = 11.9 Hz, 1H, PhC'H3), 4.60 (d, 1H, PhCH2), 4.50 (d, Jgem = 12.5 Hz, 1H, PhC''H2), 4.47 (d, 1H, PhCH2), 4.32 (d, 1H, PhC'H2), 3.81-3.89 (m, H3, H1, H1', H4), 3.69 (dd, J = 2.4 Hz, J = 9.4 Hz, H3)
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of (2R,3R,4R)-1,3,4-tri-benzyloxy-2-cyano-hex-5-ene (76) and unknown

Crude 75 (577 mg) was dissolved in dry THF (3.0 mL). The solution was cooled on an ice bath. A solution of TBACN (200 mg, 3.07 mmol) in dry THF (2.0 mL), was added drop wise to the reaction mixture. After 6 hours, the solvent was removed under reduced pressure. The mixture was dissolved in CH$_2$Cl$_2$, washed with sodium bicarbonate and brine. The organic phase was dried over MgSO$_4$, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:10 ethyl acetate/pentane) to yield the product X24 (235 mg, 0.550 mmol, 52% over 2 steps) and an unidentified product 76 (101 mg, 0.252 mmol, 24%)

Data for (2R,3R,4R)-1,3,4-tri-benzyloxy-2-cyano-hex-5-ene (76)

$R_f$ (1:15 ethyl acetate/pentane) 0.25

HRMS (ESI) calculated for C$_{28}$H$_{29}$N$_{3}$O$_3$Na m/z 450.2040 found m/z 550.2049

$^1$H-NMR (400 MHz, CDCl$_3$) $\delta_H$ 7.17-7.29 (m, 15H, PhH), 5.77 (ddd, $J_{5,6} = 17.3$ Hz, $J_{5,6'} = 10.3$ Hz, $J_{4,5} = 7.5$ Hz, 1H, H5'), 5.37 (d, d, 2H, H6, H6'), 4.61 (d, $J_{gem} = 11.4$ Hz, 1H, PhCH$_2$), 4.55 (d, $J_{gem} = 11.4$ Hz, 1H, PhC’H$_2$), 4.40 (d, 1H, PhCH$_2$), 4.39 (d, $J_{gem} = 11.9$ Hz, 1H, PhC’’H$_2$), 4.34 (d, 1H, PhC’’’H$_2$), 4.33 (d, 1H, PhC’H$_2$), 3.93 (t, $J_{3,4} = 7.4$ Hz, 1H, H4), 3.65 (dd, $J_{2,3} = 3.2$ Hz, 1H, H3), 3.56 (dd, $J_{1,2} = 6.5$ Hz, $J_{1,1'} = 9.2$ Hz, 1H, H1), 3.52 (dd, 1H, H1’), 3.31 (ddd, 1H, H2)

$^{13}$C-NMR (100 MHz, CDCl$_3$) $\delta_C$ 137.9 (1Cw, Ph), 137.7 (1Cw, Ph), 137.3 (1Cw, Ph), 135.4 (1C, C5), 127.9-128.7 (15C, Ph$_{o,m,p}$), 120.8 (1C, C6), 118.3 (1Cw, -CN), 81.0 (1C, C4), 77.5 (1C, C3), 74.5 (1C, PhCH$_2$), 73.4 (1C, PhC’’’H$_2$), 71.0 (1C, PhC’’H$_2$), 66.8 (1C, C1), 35.5 (1C, C2)

Data for unknown

LRMS (ESI) calculated for C$_{27}$H$_{26}$O$_3$Na m/z 423.2 found m/z 423.4
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of (3R,4R)-1,3,4-tri-benzylxoy-hex-5-ene-2-one (77)

72 (209 mg, 0.499 mmol) was dissolved in ethyl acetate (5.0 mL), and added 2-iodoxybenzoic acid (423 mg, 1.51 mmol). The reaction was stirred at 80 °C for 2 hours. The reaction mixture was filtered and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:12 ethyl acetate/pentane) to yield 77 (134 mg, 0.322 mmol, 64%) as a colorless oil.

Rf (1:10 ethyl acetate/pentane) 0.41

HRMS (ESI) calculated for C_{27}H_{28}O_{4}Na m/z 439.1880 found m/z 439.1884

^1H-NMR (400 MHz, CDCl$_3$) δ$_H$ 7.23-7.33 (m, 15H, PhH), 5.84 (ddd, $J_{5,5'}$ = 17.2 Hz, $J_{5,6'}$ = 10.4 Hz, $J_{4,5}$ = 7.8 Hz, 1H, H5), 5.31 (d, d, 2H, H6, H6'), 4.61 (d, $J_{gem}$ = 11.7 Hz, 1H, PhCH$_2$), 4.58 (d, $J_{gem}$ = 11.7 Hz, 1H, PhC’’H$_2$), 4.55 (d, 1H, PhCH$_2$), 4.51 (d, $J_{gem}$ = 11.9 Hz, 1H, PhC’H$_2$), 4.46 (d, 1H, PhC’’H$_2$), 4.37 (d, $J_{1,1'}$ = 18.4 Hz, 1H, H1), 4.34 (d, 1H, PhC’’H$_3$), 4.24 (d, 1H, H1’), 4.15 (dd, $J_{3,4}$ 4.6 Hz, 1H, H4), 4.08 (d, 1H, H3)

^13C-NMR (100 MHz, CDCl$_3$) δ$_C$ 206.9 (1C, C2), 137.9 (1Cw, Ph$_1$), 137.5 (1Cw, Ph$_2$), 137.3 (1Cw, Ph$_3$), 134.3 (1C, C5), 127.7-128.6 (15C, Ph$_{o,m,p}$), 120.2 (1C, C6), 84.9 (1C, C2), 81.4 (1C, C3), 74.3 (1C, C1), 73.4 (1C, PhCH$_2$), 73.3 (1C, PhCH$_2$), 70.7 (1C, PhCH$_2$)
Towards the synthesis of a stable α-galactosidase inhibitor

**Preparation of benzylhydroxylamine**

\[ \text{H}_2\text{N–OBn} \]

Benzylhydroxyl amine hydrochloride (248 mg, 1.55 mmol) was dissolved in dry methanol (2.0 mL). Dry triethylamine (0.21 mL, 1.5 mmol) was added at room temperature and the reaction stirred for 3 hours. The solvent was removed under reduced pressure, and the mixture was dissolved in ethyl acetate and was washed with aqueous saturated sodium bicarbonate. The organic phase was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure to yield the crude product benzylhydroxyl amine (172 mg). The product was used without further purification.
Towards the synthesis of a stable α-galactosidase inhibitor

Synthesis of
O-benzyl-N-[(2R,3R,4R)-1,3,4-tri-benzyloxy-hex-5-en-2-yl]-hydroxyamine (78)

Crude benzylhydroxyl amine (172 mg) was dissolved in dry diethyl ether (4 mL) and mixed with crude 75 (148 mg) under nitrogen atmosphere. The diethyl ether was evaporated off the mixture with nitrogen flow. The reaction stirred for 28 hours at room temperature and was dissolved in ethyl acetate and washed with aqueous saturated sodium bicarbonate. The organic phase was dried over MgSO₄, filtered, and the solvent was removed under reduced pressure. The product was purified by column chromatography (1:25 ethyl acetate/pentane) to yield the product 78 (92 mg, 0.18 mmol, 62% over 2 steps) as a colorless oil.

Rf (1:20 ethyl acetate/pentane) 0.29

HRMS (ESI) calculated for C₃₄H₃₇NO₄Na m/z 546.2620 found m/z 546.2615

[α]D²⁹K = -3.0 (c = 1.0, CHCl₃)

¹H-NMR (400 MHz, CDCl₃) δH 7.19-7.29 (m, 20H, PhH), 5.86 (ddd, J₅.₆ = 17.3 Hz, J₅.₆’ = 10.4 Hz, J₄.₅ = 7.8 Hz, 1H, H5), 5.20-5.29 (d, d, 2H, H6, H6’), 4.70 (d, J₅.₆’ = 11.2 Hz, 1H, PhCH₂), 4.60 (s, 2H, PhCH₂), 4.53 (d, J₅.₆ = 11.8 Hz, 1H, PhC’H₂), 4.41 (d, 1H, PhCH₂), 4.42 (d, J₅.₆’ = 12.0 Hz, 1H, PhC’’H₂), 4.39 (d, 1H, PhC’’’H₂), 4.26 (d, 1H, PhC’H₂), 4.02 (dd, J₄.₅ = 5.2 Hz, 1H, H4), 3.81 (t, J₃.₄ = 5.2 Hz, 1H, H3), 3.51-3.60 (m, 2H, H1, H1’), 3.33 (m, 1H, H2)

¹³C-NMR (100 MHz, CDCl₃) δC 138.7 (1Cw, PhH), 138.7 (1Cw, PhH), 138.3 (1Cw, PhH), 138.1 (1Cw, PhH), 135.7 (1C, C5), 127.5-128.5 (20C, Phω,m,p), 119.5 (1C, C6), 81.2 (1C, C4), 79.5 (1C, C3), 76.5 (1C, PhC’H₂), 74.5 (1C, PhCH₂), 73.3 (1C, PhC’’H₂), 70.4 (1C, PhC’’’H₂), 68.0 (1C, C1), 60.9 (1C, C2)
Towards the synthesis of a stable α-galactosidase inhibitor

Appendix
Towards the synthesis of a stable α-galactosidase inhibitor
Towards the synthesis of a stable α-galactosidase inhibitor

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Towards the synthesis of a stable $\alpha$-galactosidase inhibitor
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