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Probes and Potential Drugs for Lysosomal Diseases and Alzheimer´s

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Meinen Eltern und Astrid

A spoonful of sugar helps the medicine go down,…

Julie Andrews

Danksagung

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EIDESSTATTLICHE ERKLÄRUNG

Ich erkläre an Eides statt, dass ich die vorliegende Arbeit selbstständig verfasst, andere als die angegebenen Quellen/Hilfsmittel nicht benutzt, und die den benutzten Quellen wörtlich und inhaltlich entnommenen Stellen als solche kenntlich gemacht habe. Das in TUGRAZonline hochgeladene Textdokument ist mit der vorliegenden Dissertation identisch.

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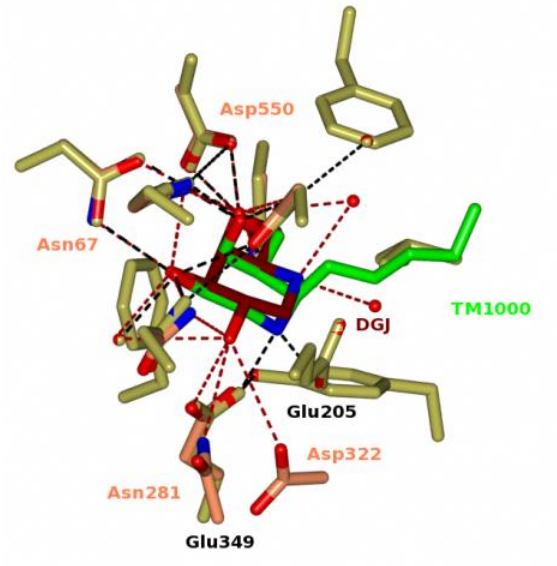
KURZFASSUNG

Lysosomale Speicherkrankheiten sind angeborene Krankheiten die durch Mutationen in spezifischen Genen ausgelöst werden. Diese Mutationen führen zu Fehlern in der Biosynthese von lysosomalen Enzymen, welche ihrerseits für die „Verstoffwechselung“ von Kohlenhydraten in lebenden Organismen verantwortlich sind. Ein neuer Ansatz in der Therapie dieser Krankheiten stellt die sogenannte „Chaperone-Therapie“ dar. Diese basiert auf starken Wechselwirkungen zwischen den beeinträchtigten Enzymen und Active-Site spezifischen Molekülen in der Regel Glycosidase-Inhibitoren. Viele Iminozucker, als auch Isoiminozucker, sind starke Inhibitoren der betreffenden Glycosylhydrolasen. Einige Vertreter aus beiden Stoffgruppen zeigten zusätzlich ein erstaunliches Verhalten als chemische Chaperone. Im Rahmen dieser Arbeit wurde der erste synthetische Zugang zu einer neuen Familie von Glycosidase-Inhibitoren, nämlich zu *C*-5a-derivatisierten 4-*epi*-Isofagominen, als potentielle chemische Chaperone für die Therapie von G_{M1}-Gangliosidose, Morbus Morqiuo B und Morbus Fabry entwickelt. Die erarbeitete Synthese ermöglicht unzählige Modifikationen der neu eingeführten Seitenkette in Bezug auf deren Konfiguration, Länge und Funktionalität. Ein auf Erfahrungen basierender Aufbau dieser Seitenkette schuf den zur Zeit besten Inhibitor von β-Galacosidasen. Dieser fluoreszenzmarkierte Isoiminozucker zeigte zusätzlich ein ausgezeichnetes Verhalten als chemisches Chaperon und konkurriert eindeutig mit dem Benchmark Molekül *N*-octyl-4-*epi*-β-valienamine (NOEV).

ABSTRACT

Lysosomal storage diseases are devastating hereditary diseases caused by single mutations in specific genes, which are responsible for the biosynthesis of lysosomal enzymes that are required for the metabolism of carbohydrates in living organisms. A novel concept for the treatment of such diseases is the so-called “chaperone mediated therapy” which applies specific small active site directed molecules, such as glycosidase inhibitors, that act as folding templates for the respective lysosomal protein mutant. Many iminosugars and isoiminosugars are typical examples of powerful glycosidase inhibitors. Moreover, several *N*-alkylated iminoalditols have gained importance as potential chemical chaperones for the treatment of various lysosomal storage diseases. In this thesis, the first synthetic approach to a new family of glycosidase inhibitors, namely *C*-5a-modified 4-*epi*-isofagomines, as putative chemical chaperones for the D-galactosidase deficiency related diseases G_{M1}-gangliosidosis, Morbus Morqiuo B, and Morbus Fabry is described. The developed synthetic strategy allows various modifications of the newly introduced spacer-arm in terms of configuration, length, and functionality. An experience based composition of the side-chain provided the best inhibitor of β-galactosidases known to date. Moreover, this fluorescently tagged isoiminosugar has been found to exhibit notable chaperoning properties clearly rivalling benchmark molecule *N*-octyl-4-*epi*-β-valienamine (NOEV).



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Abbreviations

$[a]_D^{20}$	specific optical rotation
9-BBN	9-borabicyclo(3.3.1)nonane
Bn	benzyl
Boc	tert-butoxycarbonyl
n-Buli	*n*-butyllithium
t-BuOK	potassium *tert*-butoxide
C	cyclohexane
Cbz	benzyloxycarbonyl
DGJ	1-deoxy-D-galactonojirimycin
DIPA	diisopropylamine
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
DNJ	1-deoxynojirimycin
EA	ethylacetate
ER	endoplasmatic reticulum
ERAD	endoplasmatic reticulum associated degradation
Et	ethyl
GSL	glycosphingolipide
IFG	isofagomine
LAH	lithium aluminium hydride
LSD	lysosomal storage disease
MOM	methoxymethylene
MW	molecular weight
NOEV	*N*-octyl-4-*epi*-β-valienamine
OxCl	oxalyl chloride
PC	pharmacological chaperone
R_f	retardation factor
TBS	*tert*-butyldimethylsilyl
TBDPS	*tert*-butyldiphenylsilyl
THF	tetrahydrofuran
TLC	thin-layer chromatography

1 Introduction

1.1 Carbohydrates

Carbohydrates (sugars or saccharides) are the most widely distributed naturally occurring organic compounds on earth. Although there are approximately two hundred monosaccharides found in nature, they only differ from each other in a few structural features. Thus, the variety of "classical saccharides" relies on permutations regarding fundamental structural conditions (e.g. number of carbon atoms and the relative spatial arrangement of functional groups) of such polyhydroxyaldehydes or –ketones.

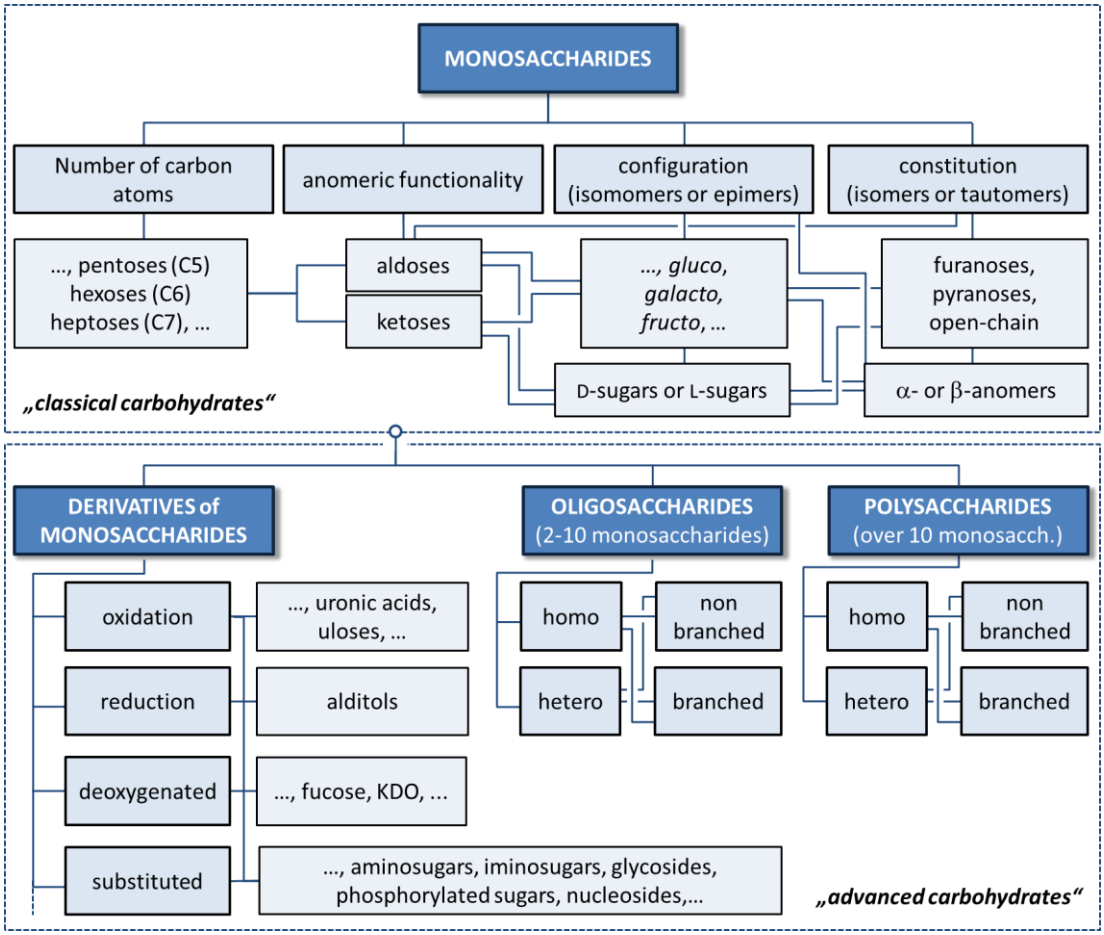


Figure 1: Schematic classification of carbohydrates.

Definitions of carbohydrates include derivatives that can be either be obtained by oxidation or reduction of sugars as well as deoxygenated and/or substituted analogues. Additionally, such sugar units known as "monosaccharides" can be linked together by so called glycosidic linkages providing di-, oligo- or polysaccharides. A schematic overview regarding the diversity and classification of carbohydrates is provided in Figure 1.

Consequently, the large variety of monosaccharides provides a complex diversity of oligo- and polysaccharides. For example, the limited number of eight different monosaccharides, as commonly found in mammals, already enables the formation of over one million of different tetrasaccharides. One of the most prominent and important representatives of such oligosaccharides is Sialyl-Lewis X[1] (Figure 2), which comprises almost all of the above mentioned modifications of "classical carbohydrates".

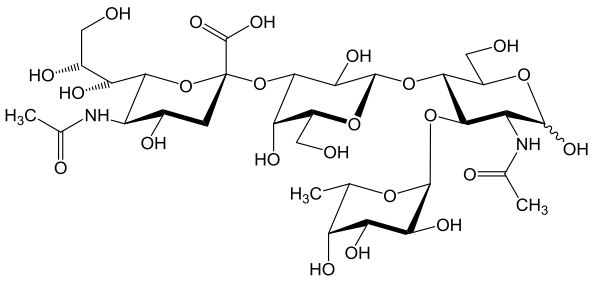


Figure 2: Sialyl-Lewis X:

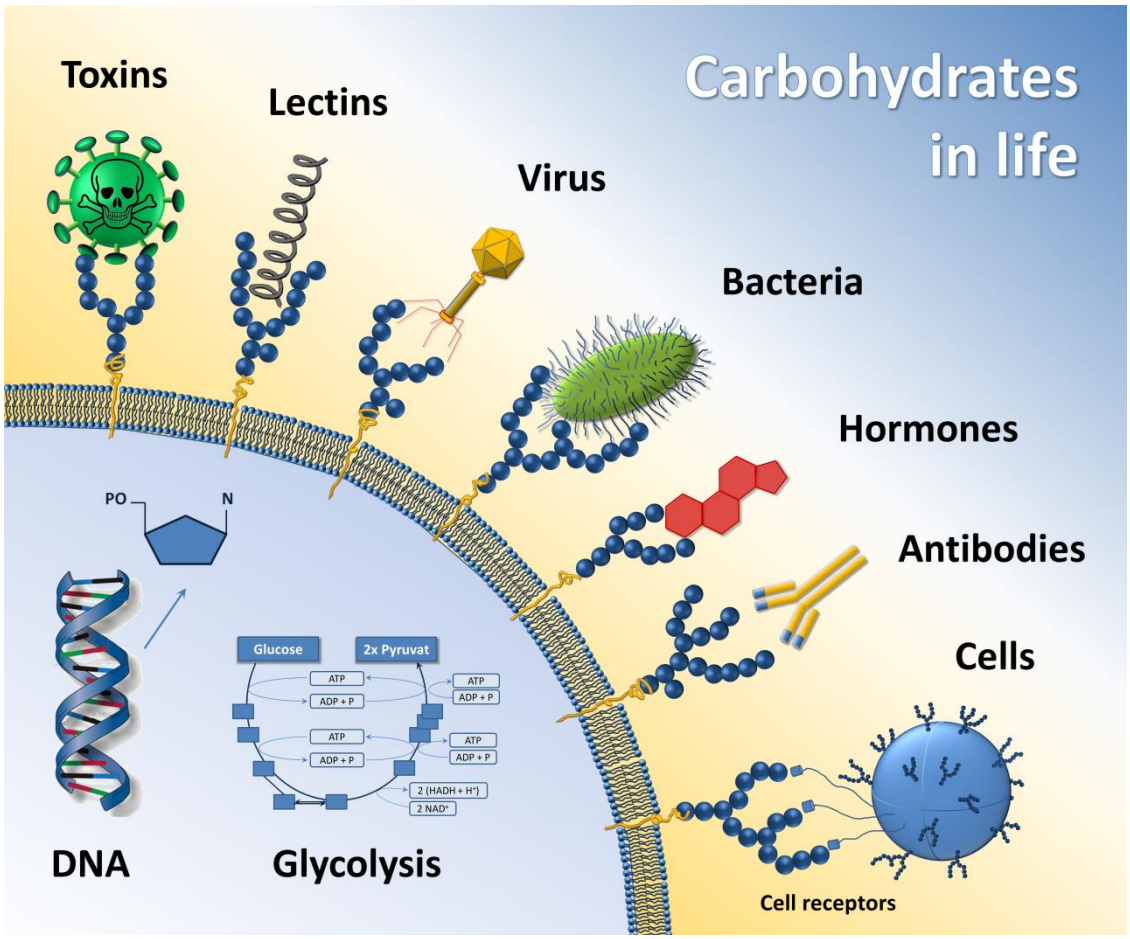
A branched heterotetrasaccharide constructed from different monosaccharides regarding:

- number of carbon atoms
- anomeric functionality
- configurations
- α- and β-anomers
- D- and L-sugars
- conformers
- oxidized moiety
- deoxygenated moieties
- substitued moieties.

Additionally, carbohydrates can be linked to other biomolecules (e.g. lipids and proteins). These so-called glycoconjugates are subdivided into many different categories such as glycolipids, glycoproteins, peptidoglycans, lipopolysaccharides.

Carbohydrates perform numerous roles in living organisms. Interestingly, for a long time they have been considered mainly as an energy source for metabolism. The constant improvement of analytical methods as well as the advanced knowledge of biological processes has provided a new picture regarding carbohydrates. In the broad field of glycobiology, the discovery, isolation, and study of glycoproteins, glycolipids and oligosaccharides has led to a deeper understanding of the importance of carbohydrates in physiological processes other than energy supply and scaffold formation.

Carbohydrates are involved and simultaneously responsible for the regulation and sustainability of various metabolic pathways. For example, the storage of energy relies on polysaccharides and degraded analogues. Moreover, many of such oligosaccharides are involved in cell-cell interactions. Thus, diverse glycoconjugates are implemented in structural components like cell walls and responsible for cell adhesion processes. Likewise, they can function as binding sites for toxins, bacteria, antibodies, hormones or viruses. Furthermore, specific pentoses serve as backbone of the genetic molecules RNA and DNA or act as crucial components of coenzymes (e.g. ATP, NADH, Vitamine C). A schematic overview, regarding selected functions of carbohydrates in living organisms, is given in Picture 1.



Picture 1: Schematic overview of selected functions of carbohydrates in living organisms.[2]

Thus, carbohydrates as well as their conjugates are included in the most important biomolecules that play key roles in various vital processes. Nonetheless, due to a number of reasons, considerably little attention has been drawn to carbohydrate chemistry over the last decades.

The chemical complexity of carbohydrates exacerbates their synthesis as well as their analytical characterization, when compared to other biomolecules. Thus, due to their high level of functionalization, even low molecular weight derivatives may provide difficult problems in terms of synthesis and analytics.[3]

In this context, pharmaceutical industries have been sceptical about carbohydrate based drugs, not only because of the mentioned complexity, but also because of their often non-ideal physicochemical behavior, such as the poor ability of passing biological membranes due to their high polarity as well as the problematic biochemically instability of glycosidic linkages. Thus, these properties promote poor cell-entrance ability or complicate oral applications.

Additionally, such compounds with their receptors often show dissociation constants in the millimolar range, which are relatively high compared to other drug candidates, which typically provide K_i-values in the low nanomolar range.[4]

This situation has changed dramatically with the discovery of the impact of carbohydrates in cancer biology, cell-cell adhesion in metastasis and inflammation as well as diabetes. Today, many other diseases such as Alzheimer´s, HIV, Parkinson´s or lysosomal dysfunctions have been connected to carbohydrate-metabolism.

Thus, investigations into carbohydrate chemistry regarding their synthesis, analytics, biochemical properties or functions as well as possible therapeutic applications are deemed essential.

1.2 Carbohydrate processing enzymes

Carbohydrate processing enzymes manipulate carbohydrates in living organisms. Due to the variety of carbohydrates and as result of diverse metabolic pathways, these essential biocatalysts exhibit highly specific behaviors with their natural substrates in terms reactivity and selectivity.

In general, these proteins are divided into different groups regarding the specific chemical reactions they catalyse (Enzyme commission number, EC).[5] The most common families are glycosyltransferases (EC 2.4) and glycosyl hydrolases (EC 3.2).

Glycosyltransferases are enzymes that establish natural glycosidic linkages. Thus, they transfer sugar moieties from activated glycosyl donors to nucleophilic acceptors which results in the glycosylation of proteins, lipids or growing oligosaccharide chains. Equally important are glycosyl hydrolases, which are responsible for the catalytical cleavage of glycosidic bonds.

Additional types of such enzymes provide the transfer (transferases) or the cleavage (hydrolases) of phosphorus containing moieties. These biocatalysts play important roles in the biosynthesis and catabolism of, e.g., nucleotides such as ATP. Hence they are essential for the generation of energy. These transferases are subdivided into phosphorylases and phosphotransferases. Phosphorylases (members of EC 2.4 and EC 2.7.7) are enzymes that catalyse the addition of a phosphate group from an inorganic phosphate (phosphate + hydrogen) to an acceptor. Phosphotransferases transfer an organic phosphate group from a donor to an acceptor.

The latter group provides the transfer of the phosphorus containing moiety to alcohol groups (EC 2.7.1), carboxyl groups (EC 2.7.2) as well as phosphate groups (EC 2.7.4). In contrast, phosphatases (members of EC 3.1) are responsible for the catalytical hydrolysis of phosphoric esters.

The presented classification only relies on the respective catalysed reaction, but it does not specify these proteins. Thus, different enzymes (e.g. from various sources/organisms) which catalyse the same reaction, receive the same EC number. In other words, such "non-homologous isofunctional enzymes" (NISE) are homologous in function, but they share unique protein sequences or folding. Hence, there may arise confusion regarding the EC-System.

1.2.1 Glycosidases

Glycosyl hydrolases (glycosidases) are vital enzymes that catalyse the hydrolysis of the glycosidic linkages of glycosides. This enzymatic conversion leads to the formation of a sugar hemiacetal or hemiketal and the corresponding liberated aglycon. (Figure 3)

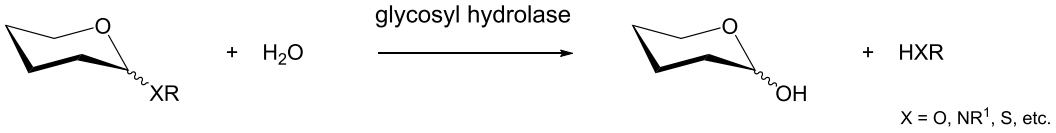


Figure 3: Schematic depiction of an enzyme catalysed hydrolysis of glycosides.

Glycosidases are essential and consequently abundant biocatalysts in all living organisms that rely on the processing of carbohydrates. From the degradation of "structurally simple saccharides" (e.g. starch, chitin, lactose) to the sophisticated deglycosylation-reglycosylation sequence in the functionalisation and maturation of glycoconjugates, a wide range of these vital enzymes catalyse the selective release of a structural variety of aglycons from their corresponding glycon partners.

Their molecular masses vary, between about 20 kDa and well over 100 kDa for the monomeric structures, with the majority of examples being in the range between 40 and 70 kDa. These monomers may form homo- or heterodimers, or higher aggregates such as tetramers (e.g. β-galactosidase from *E. coli*.).[6] Most glycosidases work best around neutral pH-values, although such acid enzymes as lysosomal glycosidases may favor the range between pH 3.5 and 5.0.[7,8]

In general, glycosidases can be distinguished by their configurational as well as anomeric specificities. Furthermore, they are classified in different ways which can be connected to the understanding of their modes of action or structural composition.

Classification by the enzyme commission number:

As mentioned, the classification by EC-numbers is only associated with specific reactions that are catalysed by the respective enzymes. Accordingly, enzymes that hydrolyze (EC 3.x) glycosidic bonds (EC x.2) are summarized as glycosyl hydrolases (EC 3.2). Consequently, these vital biocatalysts can be divided into further subclasses which either hydrolyze *O*-glyosides and thioglycosides (EC 3.2.1) or *N*-glycosides (EC 3.2.2).

Classification as *endo*- or *exo*-glycosidases:

The distinction between *exo*- and *endo*- refers to the ability of the respective glycosidase to cleave a substrate at either the non-reducing end or at some point along a poly- or oligosaccharide chain. Most commonly, *exo*-glycosidases act at the non-reducing end, although a few exceptions are known.[9] A schematic overview is indicated in Figure 4.

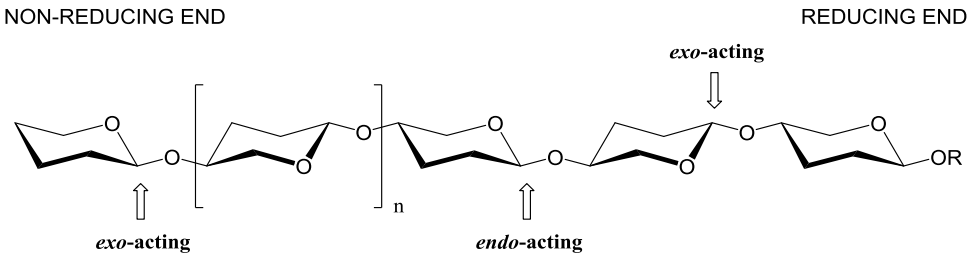


Figure 4: Possible cleavage points of *endo*- and *exo*-glycosidases.

Sequence-based classification:

In general, sequence-based classifications of enzymes are connected with the knowledge of, at least, parts of their amino acid or nucleotide sequence. Algorithmic methods are used to compare and classify these sequences in various families. Thus, each of the resulting groups contains proteins that are related by sequence, and by corollary, three-dimensional fold. The consolidation of families that possess significant similarity in their respective catalytic residues, tertiary structure, and mechanism into super ordinated “clans” has been proposed.

Glycosidases have been currently classified into more than 100 families and 14 clans that are available through the Carbohydrate Active EnZyme (CAZy) database.[10,11] The related “characteristics” of each glycosyl hydrolase family (GH-family) allows various useful predictions to be made since it has been noted that the catalytic machinery and molecular mechanism is conserved for the GH families as well as the geometry around the glycosidic bond. Thus, this classification provides family-typical mechanistic and structural details and has become an indispensable tool in glycosyl hydrolase research.

Classification by reaction mechanism:

Mechanistic classifications of glycosidases are based on the understanding of the catalytical cleavage of their substrates. Depending on the sterical outcome of the hydrolysis (regarding the anomeric carbon), two subgroups, namely inverting and retaining glycosidases, can be distinguished.[12-17]

From early investigations regarding mechanistic principles by pioneers such as Kuhn,[18] Veibel,[19] Pigman,[20,21] and Shafizadeh,[22] the picture has become increasingly more clear with Koshland's contributions,[23-25] which are currently considered as foundation of our modern understanding of glycosidase catalysis. These results were supported by the fist XRD-study of a glycosyl hydrolase investigated by Phillips[26] and co-workers in the early 1960s. The main objectives of glycosidase research have been to understand the detailed mechanisms of the reaction pathways which were considered to progress via "rapid and reversible protonation of the anomeric oxygen, followed by a rate-determining heterolysis giving a carbenium ion,"[27] as also outlined by Vernon.[28]

As mentioned above, the catalycical cleavage of glycosides relies on two most significant reaction mechanisms, which either invert or retain the configuration at the anomeric carbon of the hydrolyzed glycon. It is generally accepted that in both cases an oxocarbenium ion related transition state is formed. The latter exhibits sp^2 hybridization of the anomeric carbon leading to an energy rich half chair conformation of the intermediate. Furthermore, the hydrolysis typically occurs with general acid and general base assistance from two amino acid residues. In general, these acid-base pairs consist of glutamatic or aspartic acids which participate in the conversion from opposite sides of the active site.

Nonetheless, despite these similarities, the respective reaction mechanisms and "structural compositions" of these enzymes are quite different.

The key structural differences between inverting and retaining glycosidases is the spatial distance between the two catalytical carboxyl moieties in the active site. Typically, in inverting enzymes these amino acid residues are located 6-12 Å apart from each other, whereas representatives of the retaining type show distances of approximately 5 Å.[13]

Inverting glycosidases:

The first reaction step of both types of hydrolases is an acid/base catalyzed nucleophilic attack at the anomeric carbon. In the case of inverting glycosides, the attack of an activated water molecule forms transition state **I** which enables a direct substitution of the aglycon. This S_N2 like mechanism is shown in Figure 5.[23,29,30]

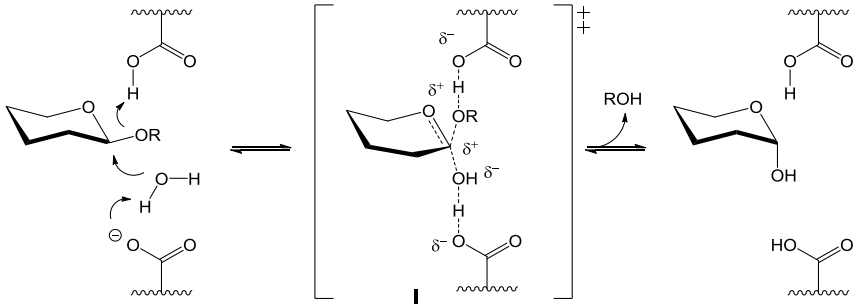


Figure 5: Reaction mechanism of inverting β-glycosidases.[30]

Retaining glycosidases:

The first step in the reaction mechanism of retaining glycosides involves a direct nucleophilic attack of the carboxylate resulting transition state **II**. Due to the influence of the carboxylic acid, a facilitated cleavage of the aglycon is enabled. The generated glycosyl-enzyme intermediate **A** is subsequently attacked by an activated water molecule from the opposite side gaining a new transition state (**III**) which allows the liberation of the hydrolysed carbohydrate. (Figure 6)[30]

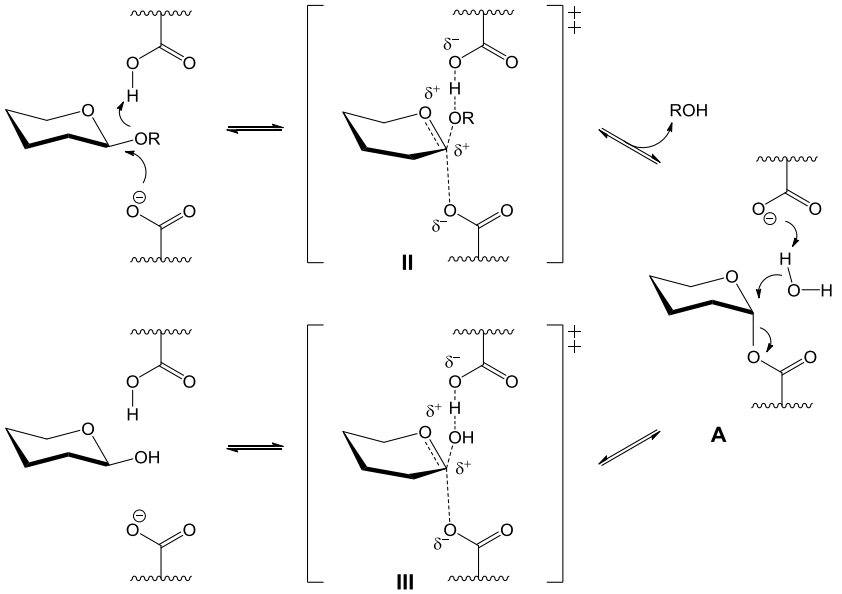


Figure 6: Reaction mechanism of retaining β-glycosidases.[30]

1.3 Glycosidase inhibitors

Due to the vital roles of glycosidases in living organisms, profound interest in substances which are able to influence the activities of these enzymes exists. Compounds which decrease the activities, so called glycosidase inhibitors, have proved to be valuable tools for enzymatic investigation and characterization. Resulting applications can be found in medicine, for example in the treatment of various metabolic diseases.

Many of such compounds come from natural sources such as microbes or plants. Nonetheless, investigations towards suitable synthetic approaches providing selective and powerful inhibitors have become a broad field in chemistry over the last decades.

In general, the biological activities of most glycosidase inhibitors rely on their structural analogy to natural substrates of the respective enzyme. Thus, they are able to bind to the active site, but they cannot be converted due to their chemical nature. Consequently, they compete with the substrate for the active center and are therefore known as competitive or reversible inhibitors. Many glycosidase inhibitors are structurally related to natural substrates, closely resembling carbohydrates. However, various exceptions to this generalization are known. Selected examples of such compounds are shown in Figure 7.[31,32]

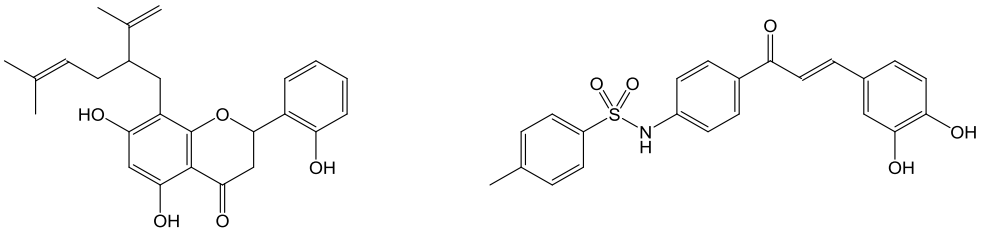


Figure 7: Selected non-carbohydrate based reversible glycosidase inhibitors.

In the case of carbohydrate based structures, modifications of the “natural” acetal or ketal moiety lead to structures such as lactones or lactams (among many others) which are typical reversible inhibitors (Figure 8)[33]. Nonetheless, the most prominent compound classes of these inhibitory substances are “basic sugar analogues” which will be discussed in the next section.

Another type of inhibitors employs functional moieties which undergo chemical reactions (nucleophilic substitution processes, including oxirane- and aziridine-ring opening, addition, Michael addition, or allylic rearrangements) at (or very close to) the active site, resulting in covalent and, hence, catalytically incompetent enzyme–inhibitor adducts. These substances, which are known as “irreversible inhibitors”[34], are obviously not convenient for enzymatic modulations but preferred for mechanistic investigations. (Figure 8)[35]

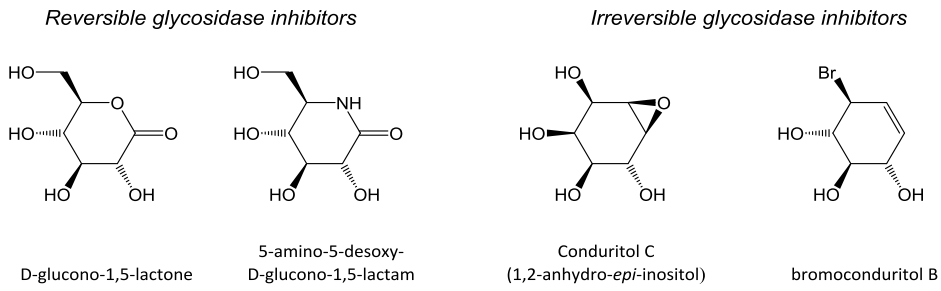


Figure 8: Selected carbohydrate-based reversible and irreversible glycosidase inhibitors.[33,35]

1.4 Basic carbohydrate analogues as glycosidase inhibitors

Most basic carbohydrate analogues are reversible glycosidase inhibitors which are based on the structural analogy to natural substrates. Additionally, they provide a trivalent basic nitrogen which is able to form ionic bonds with the active site moieties of an enzyme resulting in diminished activities of the glycosidase. A schematic mechanism is indicated in Figure 9.

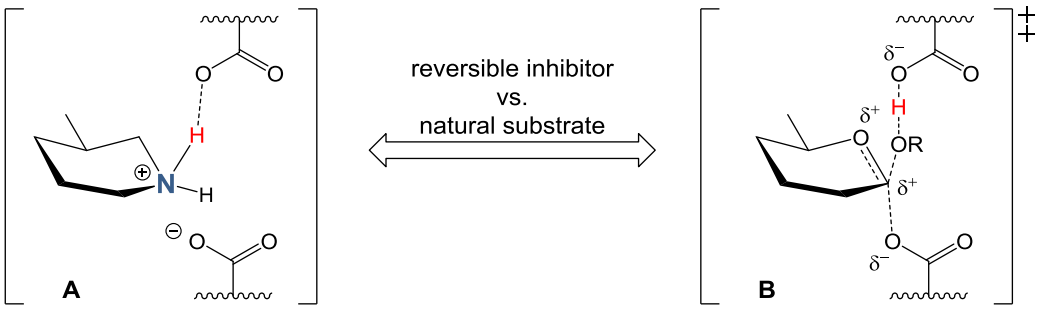


Figure 9: Schematic mechanism of the reversible inhibition of glycosidases by basic sugar analogues (**A**) compared to the transition state **B** during the hydrolysis of the natural substrate.

Thus, primarily, the potency of the inhibitor depends on the ability to fit into the active center and on the strength of the generated ionic bond. Nonetheless, broad investigations in this field indicate the significance of advanced modifications of core molecules, providing additional interactions between the inhibitor and the enzyme, resulting in much more active inhibitors. Consequently, various naturally occurring or synthetic compound classes are part of current research.

The most important representatives of these inhibitors belong to the families of imino- and isoiminosugars as well as carbasugars bearing an amino moiety at the “anomeric carbon”.

1.4.1 Imino-, isoimino- and basic carbasugars

In general, imino- and isoiminosugars as well as basic carbasugars bear the same feature: The naturally occurring acetal or ketal moiety of common carbohydrates (**I**) is modified. Thus, replacement of the ring oxygen by nitrogen or a methylene group leads to either iminosugars (**A**) or, in the latter case, to carbasugars (**C**). In isoiminosugars (**B**), the ring nitrogen is replaced by a methylene group and the anomeric carbon by nitrogen.

Analogously to common carbohydrates, they may differ in ring size, configuration and substitution pattern. Unsaturated derivatives are also available in addition to deoxygenated analogues or bicyclic systems. Selected fundamental structures of proven glycosidase inhibitors are shown in Figure 10.[36]

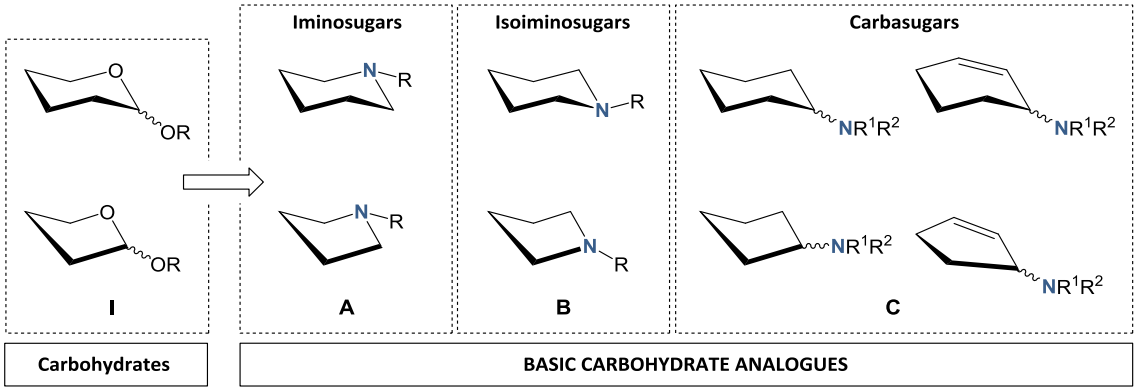


Figure 10: Position of the nitrogen atom in various basic sugar analogues of proven glycosidase activity (disregarding conformational freedom as well as other ring sizes. R = H or organic).[36]

These respective modifications combine several features that are necessary and simultaneously responsible for powerful, selective and reversible inhibition of glycosidases. Due to the undisturbed backbone of the sugar moiety, all naturally occurring hydrophobic and hydrophilic interactions between the enzyme and the inhibitor are retained which serves as foundation for selectivity. In cases, when the new generated moieties are hydrolytically stable, these substances cannot be converted. Additionally, the introduced nitrogen is positioned in regions which are directly involved in the catalytical cleavage, thus the distance to active moieties is minimized which implies a maximized strength of the generated ionic bond.

The area of these irreversible glycosidase inhibitors including their history, synthesis and biological activities as well as applications in medicine has been highlighted in various excellent papers, reviews and books.[36-39] Nonetheless, representative core-structures of basic sugar analogues and selected derivatives as well as their particular biological activities will be discussed in the following.

Imino- and isoiminosugars:

For example, castanospermine (**1**, K_i = 0.015 μM, α-glucosidase from rice)[12,40] and calystegine B_1 (**2**, K_i = 0.1.8 μM, β-glucosidase from sweet almonds)[41] are powerful glucosidase inhibitors and important representatives of naturally occurring bicyclic iminosugars. As representative of furanoid iminosugars, 2,5-dideoxy-2,5-imino-D-mannitol (**3**, K_i = 0.03 μM, α-glucosidase from *bacillus stearothermophilus*)[42], better known as DMDP, is shown in Figure 11.

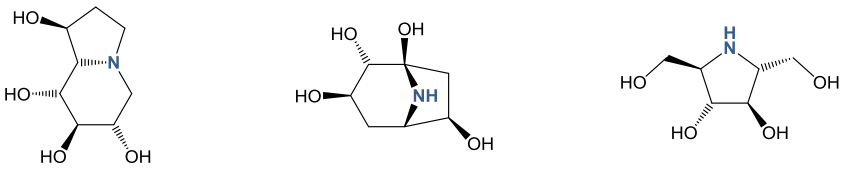


Figure 11: Structures of selected iminosugars.

The most important and best characterized iminosugars are nojirimycins and structural related 1-deoxynojirimycins. They are represented by D-*gluco* configurated parents 5-amino-5-deoxy-D-glucopyranose (**4**)[43-46] and 1,5-dideoxy-1,5-imino-D-glucitol (**5**, DNJ)[47] as well as their D-*galacto*-analogues: 5-amino-5-deoxy-D-galactopyranose (**6**) and 1,5-dideoxy-1,5-imino-D-galactitol (**7**, DGJ). The first synthesis of compound **7** was reported by Paulsen[48] and co-workers.

Isofagomine (**8**) and its D-*galacto* analogue 4-*epi*-isofagomine (**9**) are the most important parent compounds of isoiminosugars. Both structures have been found to be highly potent β-glycosidase inhibitors (e.g. **8**, K_i = 0.11 μM, β-glucosidase from almonds[49]; **9**, IC_{50} = 0.012 μM, β-galactosidase from *Asp. oryzae*[50,51]).

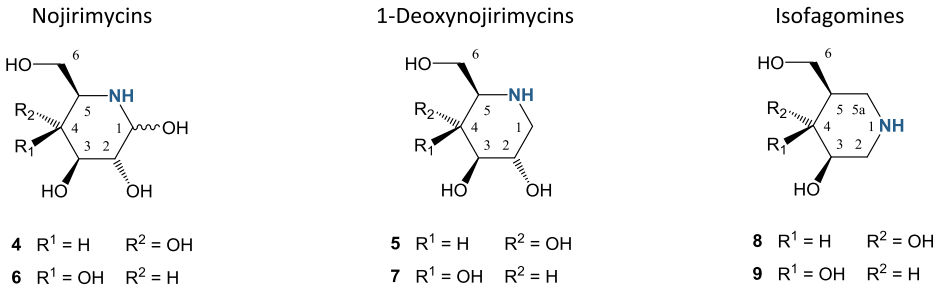


Figure 12: Structures of nojirimycins and isofagomines.

Whereas iminosugars can be found in nature [39,52], isoiminosugars are non-natural products which were introduced by Lundt[53] and Bols[49] and their co-workers.

The aim for this modification was based on the rational that these sugar analogues can bear the positive charge directly at the (formal) anomeric position. Indeed, the first such isoiminosugar, isofagomine (**8**) was found as much more potent β-glucosidase inhibitor (K_i = 0.11 μM, almonds) compared to related iminosugar **5** (K_i = 47 μM, almonds).[54]

Based on the hypothesis that additional interactions between the inhibitor and the enzyme result in much more active substances, diverse elaborations of these core-molecules were investigated. Guiding contributions by several leaders in this field and recent reviews have shown that *N*-modified iminosugars provide distinct enhanced activities against glycosidases compared to their non-substituted parent compounds.[55,56] In general, such modifications lead to potential pharmaceuticals which are intended (or approved) for the treatment of metabolic diseases.

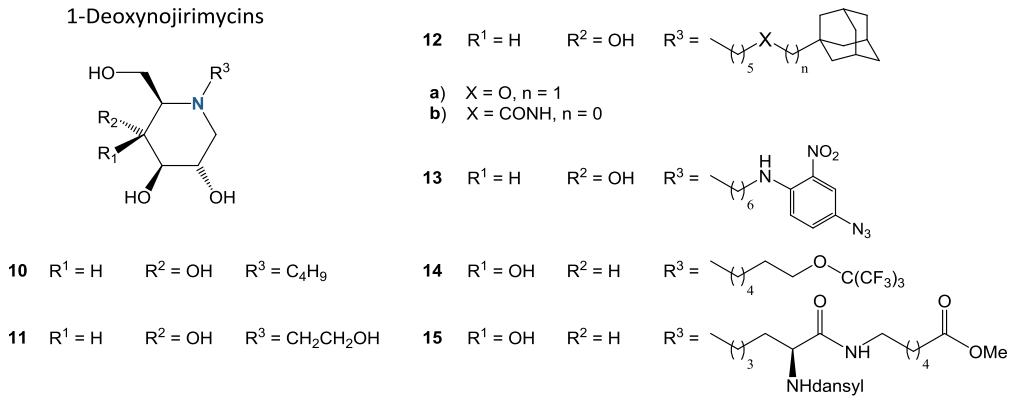


Figure 13: Selected structures of *N*-modified 1-deoxynojirimycins.

For example, *N*-butyl derivative **10** (Miglustat) is used in the therapy of Morbus Gaucher[57] as well as of Morbus Niemann-Pick which both belong to lysosomal storage disorders. *N*-(2-hydroxyethyl) analogue **11** (Miglitol)[58] is a prominent agent for the treatment of type 2 Diabetes.

Overkleeft, Aerts, and co-workers introduced adamantyl-terminated *N*-alkyl substituents which provided noteworthy β-glucosidase inhibitors, for example compound **12a** (K_i = 1 nM, membrane bound β-glucosidase).[59,60] Following these results, Wong and his group found beneficial effects on the activities of several lysosomal glucocerebrosidase mutants related to Gaucher disease employing similar lipophilic derivatives (**12b**).[61,62]

Butters, Fleet and their groups prepared various novel *N*-substituted derivatives of **5** as potential photoaffinity probes. These efforts provided compound **13** (IC_{50} = 17 nM, α-glucosidase from endoplasmatic reticulum)[63], the best α-glucosidases inhibitor known to the best of my knowledge thus far.

Selected representatives of the D-*galacto*-series such as compounds **14**[64,65] and **15**[66,67] have been found to exhibit interesting activities against galactosidases.

For example, screenings have shown a remarkable change in the potency and selectivity by N-alkylation which diminished the activity against α-galactosidases (**14**, K_i = 2.9 μM, green coffee beans) compared to non-substituted parent **7** (K_i = 0.013 μM, green coffee beans), incidentally one of the best α-galactosidase inhibitors thus far. Simultaneously, an increased inhibition of β-galactosidases was observed (from K_i = 13 μM to K_i = 0.39 μM, *E. coli*).

To provide diagnostic tags in this type of biological active compounds, the incorporation of fluorescent moieties such as a dansyl group was investigated by Stütz[67-71] and co-workers. Compound **15** (DLHex-DGJ) serves as powerful representative.

Thus, N-derivatizations of iminosugars commonly improve their activities. In contrast, *N*-substituted isoiminosugars do not fit this generalization. (Figure 14) For example, Fan and his group reported that N-alkylation of powerful human β-glucocerebrosidase (GCase) inhibitor **8** (IC_{50} = 40 nM) significantly decreases activities (**16**, IC_{50} = 44 000 nM; **17**, IC_{50} >100 000 nM)[72]. Furthermore, efforts by Kelly and co-workers provided adamantyl-terminated *N*-alkyl substituted analogues **18**-**20** which have been found moderate GCase inhibitors with pharmacological chaperone activity.[73]

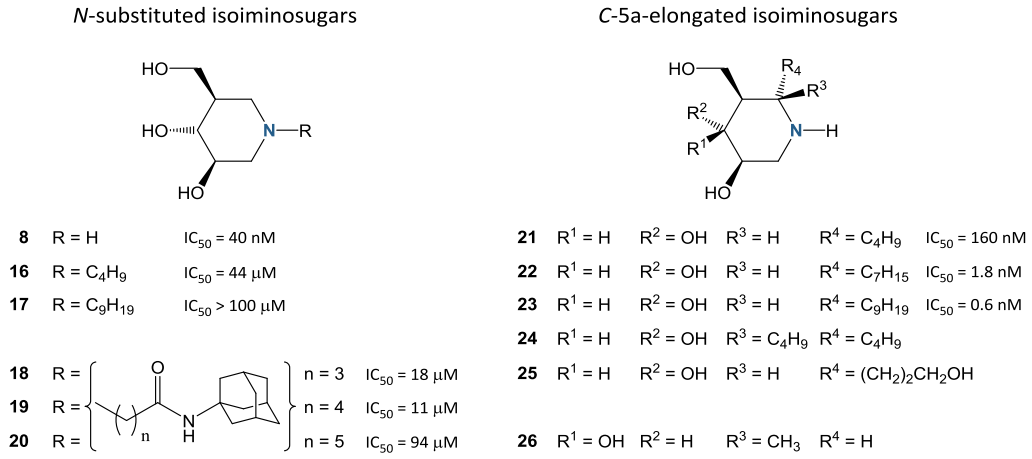


Figure 14: Selected structures of differently substituted isoiminosugars.

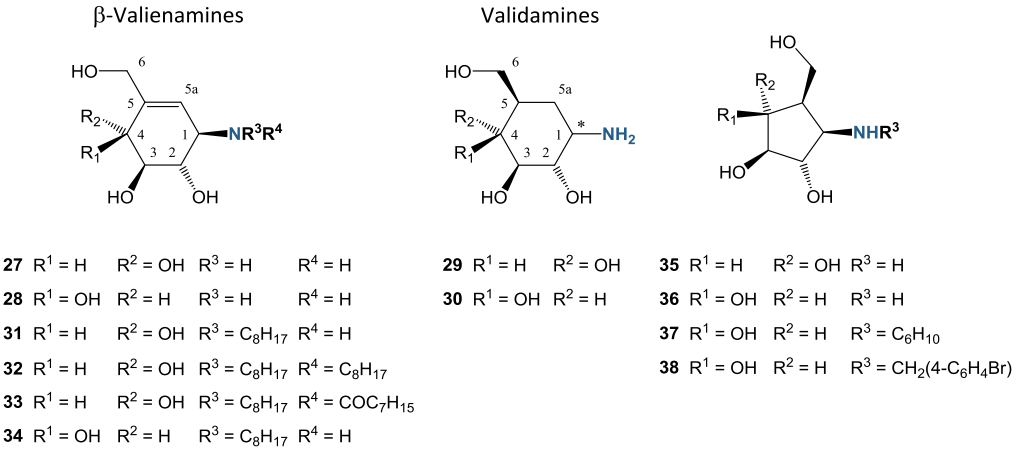
Advanced modifications of IFG (**8**) regarding the introduction of "equally positioned" (at C-5a) spacer-arms compared to *N*-substituted iminosugars, provided substances that exhibit outstanding biological behaviors. Fan[72] and co-workers prepared several *C*-5a-elongated derivatives of parent compound **8** which have been found to be highly potent GCase inhibitors.

Interestingly, (5a*S*)-butyl derivative **21** did not exhibit a better inhibitory activity, whereas (5a*S*)-heptyl derivative **22** was apparently more potent than **8**. Further extensions of the side-chain led to the best GCase inhibitor thus far, 6-*C*-nonyl-isofagomine (**23**[72], actually (5a*S*)-5a-*C*-nonyl-isofagomine according to carbohydrate nomenclature).

Following up these results, other leaders in this field (Withers and Stick, and their co-workers) [74] investigated modified synthetic approaches towards *C*-5a–elongated isofagomines resulting in the preparation of, for example, **24** and **25**. Due to the comparable less benign chemical behavior of the "*galacto*-system", synthetic challenges may have slowed down the development of analogues derivatives of 4-*epi*-isofagomine (**9**) as potentially glycosidase inhibitors. To date, compound **26** has been prepared by a *de-novo* approach.[75] Similar, simple structures analogues to Fan's of the *gluco*-series were claimed only very recently in a patent application.[76]

Carbasugars:

The most important compound class of six-membered carbasugars are valienamines and validamines. β-D-*Gluco* and β-D-*galacto* configurated parent compounds **27** and **28** as well as either the α- or the β-anomer of their related saturated analogues **29** and **30** are shown in Figure 15. *N*-modifications provided various lipophilic derivatives with interesting biological activities.[77,78] For example, N-alkylation of parent **27** gave compound **31** which was found as powerful GCase inhibitor (IC_{50} = 0.502 μM). Introduction of an additional *n*-octyl substituent (compound **32**) provided significantly enhanced activities (IC_{50} = 0.003 μM), whereas N-acylation (**33**, IC_{50} > 100 μM) was not found suitable.



27 R^1 = H R^2 = OH R^3 = H R^4 = H
28 R^1 = OH R^2 = H R^3 = H R^4 = H
31 R^1 = H R^2 = OH R^3 = C_8H_{17} R^4 = H
32 R^1 = H R^2 = OH R^3 = C_8H_{17} R^4 = C_8H_{17}
33 R^1 = H R^2 = OH R^3 = C_8H_{17} R^4 = COC_7H_{15}
34 R^1 = OH R^2 = H R^3 = C_8H_{17} R^4 = H

29 R^1 = H R^2 = OH
30 R^1 = OH R^2 = H

35 R^1 = H R^2 = OH R^3 = H
36 R^1 = OH R^2 = H R^3 = H
37 R^1 = OH R^2 = H R^3 = C_6H_{10}
38 R^1 = OH R^2 = H R^3 = $CH_2(4\text{-}C_6H_4Br)$

Figure 15: Structures of selected *N*-substituted 1-amino-D-carbasugars.

In this context, *N*-octyl-4-*epi*-β-valienamine (**34**, NOEV), introduced by Ogawa[79] and Suzuki[80] and their groups, has to be mentioned. Due to pronounced activities against β-galactosidases[81] (e.g. IC_{50} = 0.125 μM, human lysosomal β-galactosidase)[82] and its outstanding chaperoning behavior[83] (see section 1.7.2), this β-D-*galacto* configurated lipophilic aminocyclitol set the standard to which all biologically active substances concerning β-galactosidase inhibition are compared.

Compounds **35** and **36** represent the β-D-*gluco* and β-D-*galacto* configurated parent compounds of five-membered aminocyclitols. Investigations by Jäger and co-workers showed that N-derivatisation of parent **36** (K_i = 4.5 μM, *E.coli*) is a powerful tool to create more active inhibitors.[84,85] For example, introduction of a cyclohexyl substituent provided twice as active **37** (K_i = 2.4 μM; *E.coli*). Optimized composition of the *N*-substituent (compound **38**) provided the best inhibitor of β-galactosidases (K_i = 0.6 nM, *E.coli*; K_i = 0.7 nM, bovine liver) known thus far.

1.5 Fluorine in carbohydrate chemistry

Carbon-bound fluorine atoms are unique in organic molecules. It is remarkable that, although fluorine is one of the most abundant halogens in the earth crust, it was not involved in the primal evolution of life as a regular component of organic compounds.

Due to its van der Waals radius (1.47 Å), covalently bound fluorine occupies a slightly larger volume than a C-H bond (1.20 Å), but a similar one compared to a C-O bond (1.52 Å).[86] Nonetheless, fluorine is the smallest atom that can be employed for the substitution of a hydrogen or oxygen atom, leading to modifications in which a sterical disturbance is not to be expected. In contrast, due to the powerful inductive and field effects of fluorine, a direct influence on typical properties of neighboring moieties can be achieved. For example, due to their high electronegativity, fluorine substitutes provide a strong electron withdrawing effect, resulting in diminished pK_a-values[87] of functional groups next to them. In general, neighboring amines become less basic. Analogously, fluorine substituted alcohols and carboxylic acids exhibit a stronger acidic behavior.

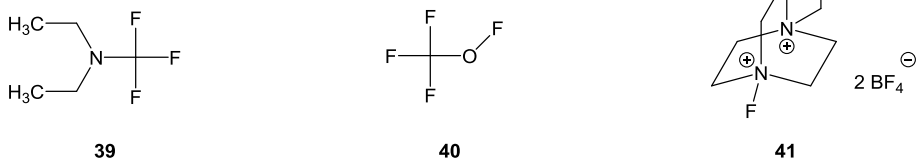
Beyond the inductive effects on neighboring functional groups, the introduction of fluorine provides further alterations of physical properties compared to non-fluorinated analogues. For example, fluorinated alkyl chains will decrease lipophilicity whereas fluorination of an aryl ring

generally increases lipophilicity. Thus, such derivatization(s) cause strong effects on the polarization of the parent molecule.

Thus, the “simple” introduction of a fluorine substituent directly affects typical properties of organic compounds. The resulting important role of fluorine in organic chemistry and applications in medicine is well recognized and part of various current investigations.[88-91]

In this context, biologically active molecules (e.g. low molecular weight inhibitors of various enzymes) serve as interesting starting materials regarding such modifications.

In general, the synthetic introduction of a fluorine substituent has a long history. Numerous reviews[92,93] concerning this modification have been published. In particular, the development of (diethylamino)sulfur trifluoride (**39**, DAST)[94,95] has set the standard by which all alternative nucleophilic fluorination methods are compared. Despite various sources of nucleophilic fluorine, the development of electrophilic fluorination reagents enabled supplementary synthetic applications in this field. Compared to molecular fluorine (the initially sole source of electrophilic fluorine), they ensure an easy handling in terms of toxicity, oxidation potential as well as state of matter. Thus, the development of “safer” electrophilic fluorine sources was essential. The fist type of these reagents, fluoroxytrifluormethane (**40**) was introduced by Barton[96] and co-workers. Recently, general structures featuring R_2N-F or R_3N^+-F gained popularity. Efforts by Umemoto and co-workers led to the first isolated derivatives (*N*-fluoropyridinium salts) which were amenable to commercially production[97]. Recently, Selectfluor® (**41**)[98-100] has been one of the most potent electrophilic fluorination reagents.



39 **40** **41**

Figure 16: Structures of nucleophilic (**39**) and electrophilic (**40**, **41**) fluorination reagents.

Reflecting these developments, considerations raised the question as to whether the introduction of a fluorine substituent would provide additional items of information on the quality as well as the selectivity of glycosidase inhibitors.

In this context, many deoxyfluoro derivatives of carbohydrates have been synthesized in the last decades.

For example, in 1990 the first fluorinated iminosugars were introduced by Stütz[101] (compound **42**) and Vogel[102,103] (compound **43**), and their co-workers. Other selected structures, such as 3-deoxy-3-fluoro L-DMDP (**44**)[104] derivative as well as nojirimycin analogues **45**[105] and **46**[106] are shown in Figure 17. *N*-modified derivatives **47**[64] and **48**[61] serve as representatives of iminosugars bearing poly-fluorinated spacer-arms.

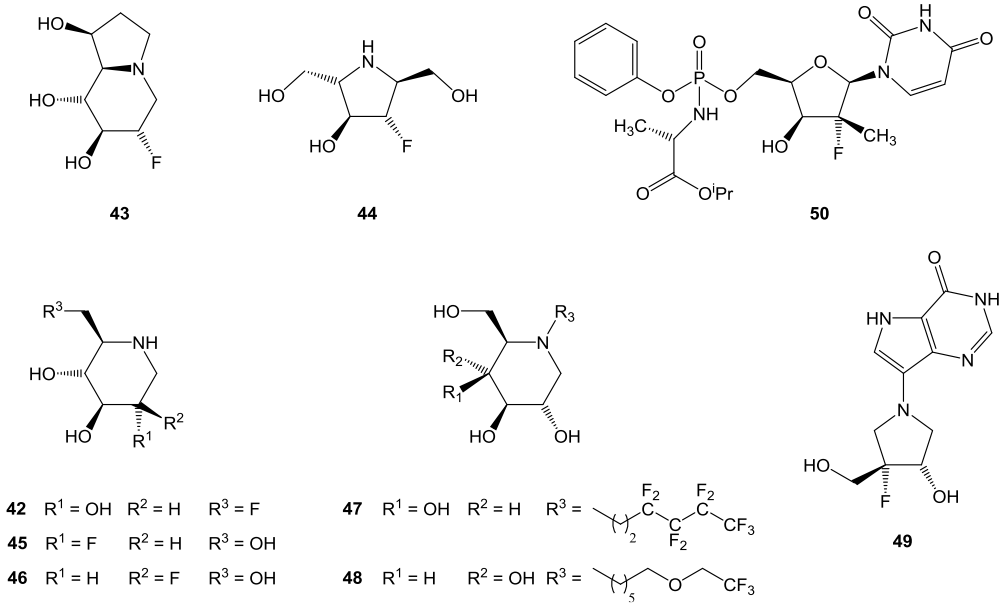


Figure 17: Selected structures of fluorine containing carbohydrates.

Recently, various interesting derivatives featuring fluorine substituents, such as the powerful nucleoside phosphorylase inhibitor "F-DADMe-immucillin-H" (**49**)[107,108] and the anti-hepatitis C drug sofosbuvir (**50**)[109] have been reported.

Thus, investigations towards the synthesis, characterization and biological evaluation of fluorinated carbohydrates are deemed important.

1.6 Glycolipids

Next to other biologically relevant structures such as glycoproteins and glycosylaminoglycans, glycosphingolipids (GSLs) are essential membrane components of living cells. In general, GSLs comprise of various compound classes including carbohydrates (mono- or polysaccharides) which are *O*-glycosidically linked to a hydrophobic moiety such as sphingosine or ceramides. The general structural features of GSLs are shown in Figure 18.[110]

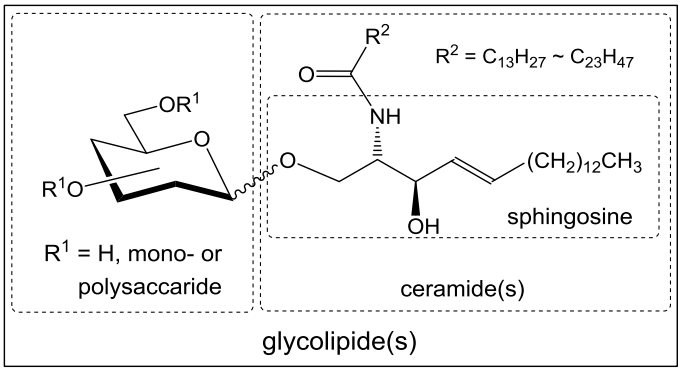


Figure 18: General structure of glycosphingolipids.

GSLs are important for the rigidization and stabilization of cell membranes. Moreover, due to the large number of naturally occurring GSLs that differ from each other in type, number and linkage of the respective sugar moieties as well as in various lipophilic aglycons, they provide an outstanding scaffold for selective molecular recognition in specific vital processes including cell-cell interactions, receptor modulation and signal transduction. GSLs are involved in adhesion processes resulting in the binding of e.g. viruses, antibodies, hormones, toxins or bacteria.

The catabolism of GSLs predominantly takes place in the lysosomes as well as in the late endosomes, which are the acidic compartments of a cell. This involves the individual cleavage of a terminal carbohydrate unit starting from the non-reducing end of GSLs by lysosomal *exo*-glycosyl hydrolases.[111,112]

In general, these enzymes are non-specific regarding the aglycon, but selective for the hydrolysis of a particular sugar moiety. Finally, glucosylceramide is degraded to sphingosine, long chain fatty acids and D-glucose which are, next to the other released monosaccharides, able to permeate into the cytosol where they are recycled as building blocks in further anabolic pathways. A schematic overview of the degradation pathway of GSLs is shown in Figure 19.[110,113]

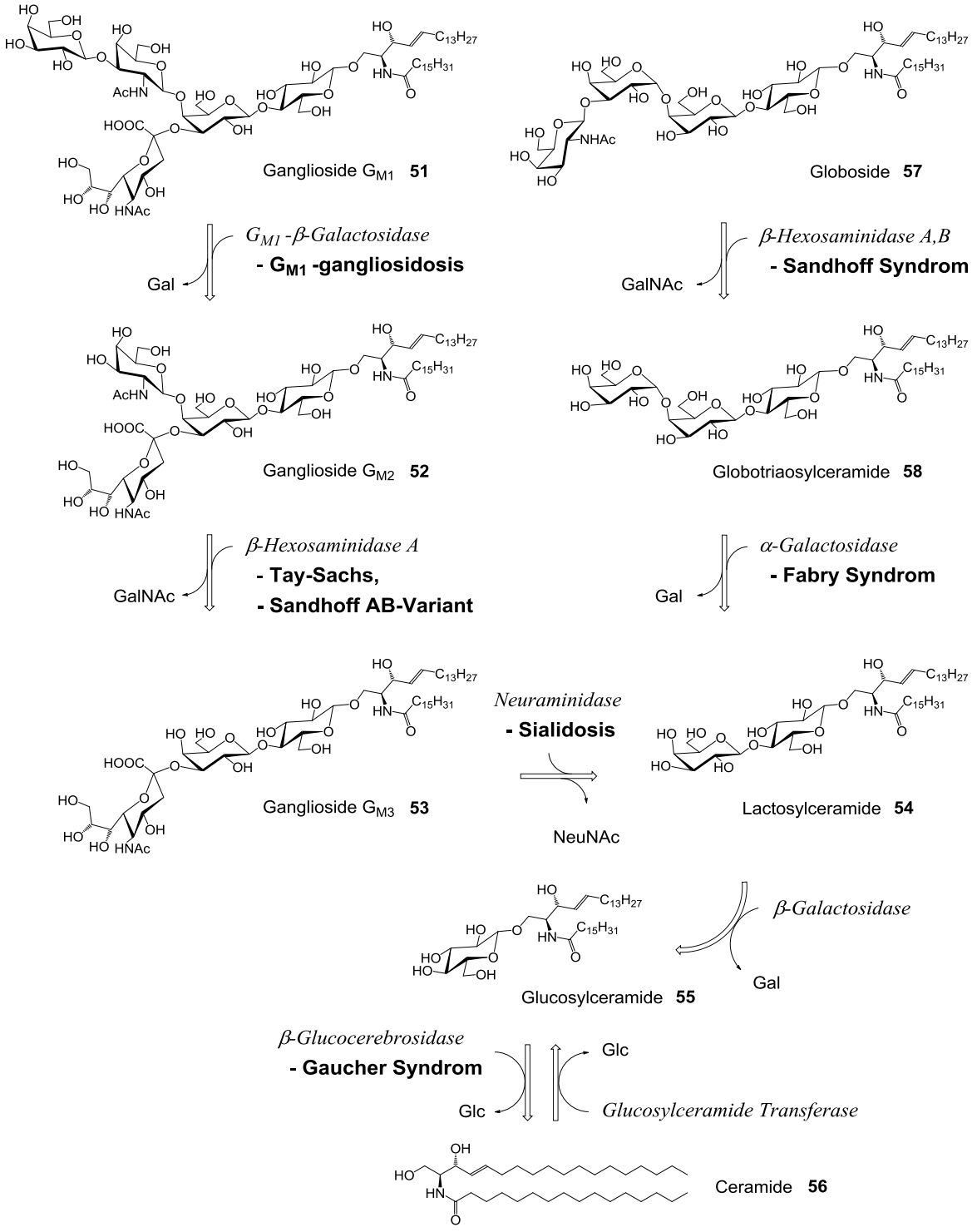


Figure 19: Stepwise degradation pathway of GSLs and related glycosphingolipidoses. The deficiency in one enzyme (italics) results in the accumulation of the undegraded substrate, leading to a specific lysosomal storage disease (bold).[110,113]

The metabolism of GSLs, among other things, is essential for life. In consequence, disorders in the catabolism as well as in the anabolism of these molecules results in various pathologic processes which are associated with e.g. carcinogenesis, HIV-1, and lysosomal storage disorders.

1.7 Lysosomal storage disorders

Lysosomal storage diseases (LSDs) are usually caused by single mutations in specific genes, which are responsible for the biosynthesis of lysosomal enzymes (or non-lysosomal proteins) that are required for the metabolism in living organisms. Thus, LSDs are a group of inherited metabolic disorders resulting from deficient lysosomal biomolecules including acid hydrolases, membrane proteins, enzyme tracking- as well as activator proteins. In consequence, an impaired intracellular substrate turnover leads to the accumulation of unprocessed substances.[114-116]

There are approximately 50 different forms of LSDs known thus far. Primarily, they are characterized by the accumulation of un- or partially degraded substrates within the lysosomal compartment. A summarized overview of the most frequent disorders is shown in Table 1.[129]

The individual incidence is rare (1 : 100 000 birth) but collectively, they form a relevant group of disorders. For example, the incidences of LSDs that are connected to glycosphingolipids has been estimated to occur 1 in 8000 births,[117,118] which makes them to the most frequent cause of pediatric neurodegenerative diseases worldwide.

1.7.1.1 G_{M1}-Gangliosidosis and Morquio B:

G_{M1}-Gangliosidosis and Morquio B (OMIM #253010) are LSDs caused by disorders of human lysosomal β-galacosidase (EC 3.2.1.23) which hydrolyzes the terminal β-galactosidase residue from G_{M1}-ganglioside (**51**), glycoproteins and glycosaminoglycans. (Figure 20)

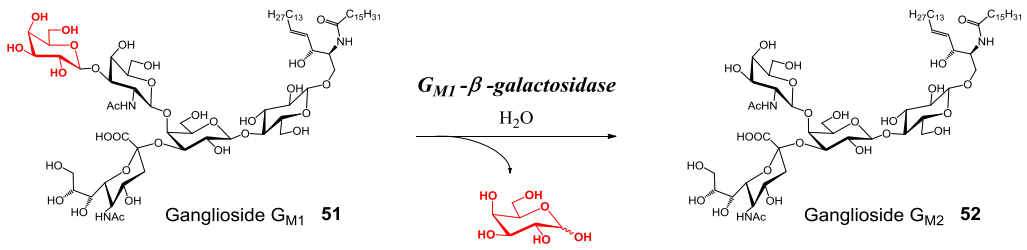


Figure 20: Enzymatic degradation of ganglioside G_{M1}.

These disorders result from different mutations in the human galactosidase beta 1 gene (*GLB1*, Gene ID 2720).[119] This gene encodes the human lysosomal acid β-galactosidase, a member of the glycosyl hydrolase family GH 35. The *GLB1*-gene maps to the short arm of chromosome 3p21.33 and contains 16 exons. Currently, 102 different mutations in *GLB1* have been reported (Figure 21).[120]

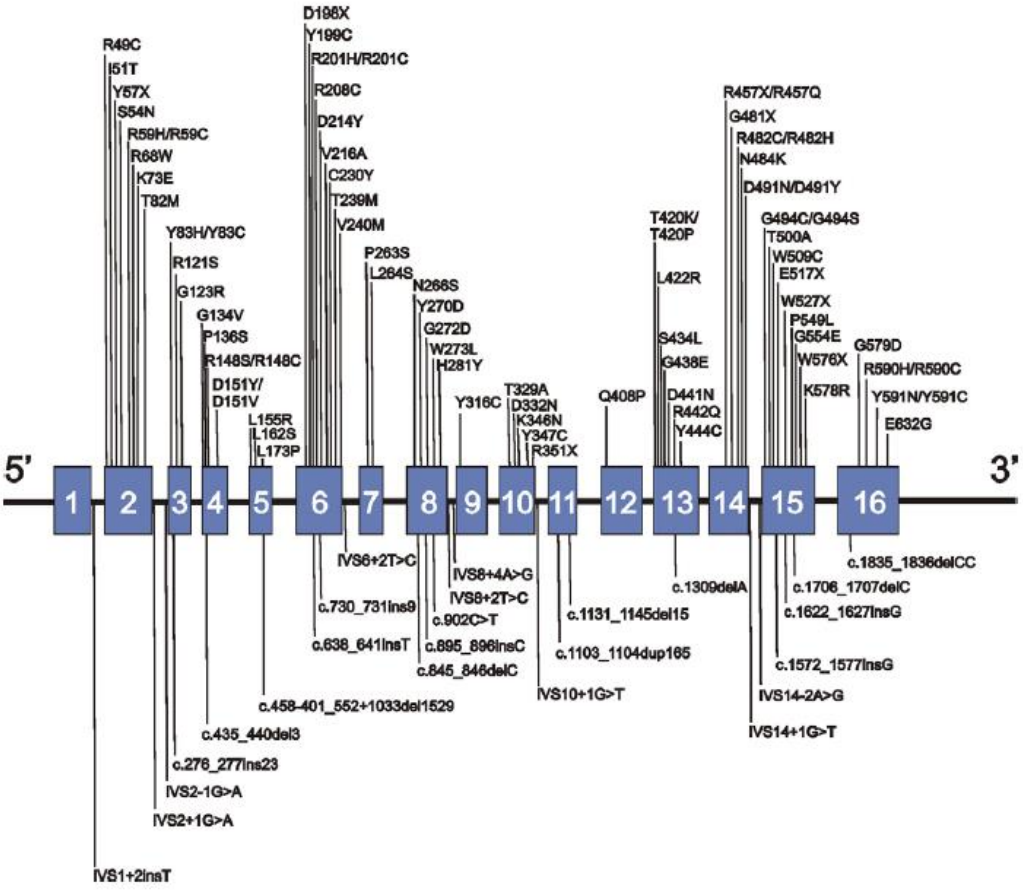


Figure 20: Summary of *GLB1*-mutations causing G_{M1}-gangliosidosis or Morqiuo B.[120]

Due to the enormous molecular heterogeneity in G_{M1}-gangliosidosis, a clear genotype/phenotype correlation is not possible. G_{M1}-Gangliosidosis is predominantly characterized by the accumulation of neuronal ganglioside G_{M1} (**51**), whereas in Morqiuo B syndrome the accumulation of oligosaccharides in inner organs and bones is observed.[121] Patients with the Morquio B syndrome suffer from a generalized skeletal dysplasia and corneal clouding, cardiac involvement, increased urinary excretion of keratan sulfate but usually without affecting the central nervous system (CNS). In contrast, G_{M1}-gangliosidosis is a neurosomatic disorder with derogation of cognitive functions as the result of atrophy of the brain. Additionally, affected patients may suffer from hepatosplenomegalia, osteodysplasia and dysmorphisms. Depending on the age of onset, G_{M1}-gangliosidosis is classified into three types[120]:

- infantile G_{M1}-gangliosidosis or type 1 (OMIM# 230500)
- late infantile or juvenile G_{M1}-gangliosidosis or type 2 (OMIM# 230600)
- adult or chronical G_{M1}-gangliosidosis or type 3 (OMIM# 230650)

In type 1 G_{M1}-gangliosidosis, neurological symptoms such as rapidly progressive hypotonia and severe CNS degeneration occur within the first month of life. The life expectancy is not higher than 1-2 years. With an onset between 7 month and 3 years, type 2 G_{M1}-gangliosidosis progresses slower with a life expectancy of about 10 years. In contrast, the adult or chronical form of G_{M1}-gangliosidosis (type 3) shows a comparatively mild progression of neurological disorders. Usually this variant occurs between 3 and 30 years of life.

Furthermore**,** other "more present" diseases are associated with the same enzymes or substrates involved in LSDs. For example, G_{M1}-ganglioside (**51**) has been found to be connected to the development of Alzheimer's.[122,123] In this context, an enhanced activity of lysosomal G_{M1}-β-gangliosidase, was found in early as well as advanced states of the disease.[124,125] In addition, G_{M1}-ganglioside (**51**) was reported as a regulator of the proteolysis of amyloid precursor protein.[126] Furthermore, **51** influences the neuronal development, the autoimmune suppression and the activity of various enzymes including Ca^{2+}-ATPase. Recently, **51** has been found as a regulator of calcium homeostasis.

In a similar line of pieces of evidence, Parkinsonism has been found to be linked to the presence and activity of lysosomal glucocerebrosidase (EC 3.2.1.45), an acid β-glucosidase which is involved in the last step of the degradation pathway of GSLs.[127,128]

Thus, such malfunctioning processes may very directly be observed and investigated in context with hereditary lysosomal storage diseases in accordance with the enzymatic degradation sequences for complex glycolipids and other glyco-conjugates as well as biologically relevant carbohydrate polymers.

	Disorder	Defective enzyme	Storage material
Mucopolysaccharidoses	MPS I (M. Hurler, M. Scheie, M. Hurler/Scheie)	α-Iduronidase	DS, HS, G_{M2}, G_{M3}, SCMAS
	MPS II (Hunter)	Iduronate-2-sulfatase	DS, HS, G_{M2}, G_{M3}, SCMAS
	MPS IIIA (M. Sanfilippo A)	Heparan *N*-sulfatase (sulfamidase)	HS, G_{M2}, G_{M3}, G_{D2}, SCMAS, ubiquitin
	MPS IIIB (M. Sanfilippo B)	*N*-Acetyl-α-glucosaminidase	HS, G_{M2}, G_{M3}, G_{D2}, SCMAS, unesterified cholesterol
	MPS IV A (M. Morquio A)	*N*-Acetylgalactosamine-6-sulfatesulfatase	KS, chondroitin-6-sulfate
	MPS IV B (M. Morquio B)	β-Galactosidase	KS, oligosaccharides
Mucolipidoses	ML II (I-cell disease)	*N*-Acetylglucosamine-1-phosphotransferase	various lipids, mucopolysaccharides, oligosaccharides
	ML III (Pseudo-Hurler-Polydystrophy)	*N*-Acetylglucosamine-1-phosphotransferase	various lipids, mucopolysaccharides, oligosaccharides
Gangliosidoses	G_{M1}-gangliosidosis	β-Galactosidase	G_{M1}, G_{A1}, G_{M2}, G_{M3}, G_{D1a}, lyso-G_{M1}, glucosylceramide, lactosylceramide, oligosaccharides, keratan sulfate
	G_{M2}-gangliosidosis (M. Tay-Sachs)	β-Hexosaminidase A	G_{M2}, G_{D1a}GalNac, G_{A2}, lyso-G_{M2}
	G_{M2}-gangliosidosis (M. Sandhoff)	β-Hexosaminidase A and B	G_{M2}, G_{D1a}GalNac, globoside, oligosaccharides, lyso-G_{M2}
Lipidoses	M. Gaucher I (chronic), II (neuropathic), III (subacute)	β-Glucosidase	G_{M1}, G_{M2}, G_{M3}, G_{D3}, glucosylceramide, glucosylsphingosine
	Globoid cell leukodystrophy (M. Krabbe)	Galactocerebroside β-galactosidase	Galactosylceramide, psychosine lactosylceramide, globotriaosylceramide, lactosylceramide, globotetraosylceramide, fucosylneolactotetraosylceramide
	M. Niemann-Pick I and II	Sphingomyelinase	Sphingomyelin, cholesterol, bismonoacylglycerophosphate, G_{M2}, G_{M3}, glucosylceramide, lactosylceramide, globotriaosylceramide, globotetraosylceramide
	M. Fabry	α-Galactosidase A	Globotriaosylceramide, galabiosylceramide, globotriaosylsphingosine, blood-group-B glycolipids
	Metachromatic leukodystrophy	Arylsulfatase A	Sulfatide, 3-*O*-sulfolactosylceramide, lysosulfatide, seminolipid, gangliotetraosylceramide-bis-sulfate, G_{M2}

Table 1: Selected examples of lsosomal storage diseases: DS = dermatan sulfate; HS = heparan sulfate; G_{M1}, G_{A1}, G_{A2}, G_{M2}, G_{M3}, G_{D2}, G_{D3}, G_{D1a}, lyso-G_{M1}, lyso-G_{M2}, G_{D1a}GalNac = subtypes of gangliosides; SCMAS =subunit c of mitochondrial ATP synthase.[129]

1.7.2 Therapies for lysosomal storage disorders

Various approaches for the treatment of LSDs have been developed within the last three decades. Nonetheless, a causal therapy of these disorders in currently not possible. Classically, the treatment of such disorders consists in symptomatic care of disease manifestations. The different therapies applied in LSDs can be roughly divided into two subgroups, which either act on the symptoms or those that act on the cause of these diseases.[130,131] (Figure 21)

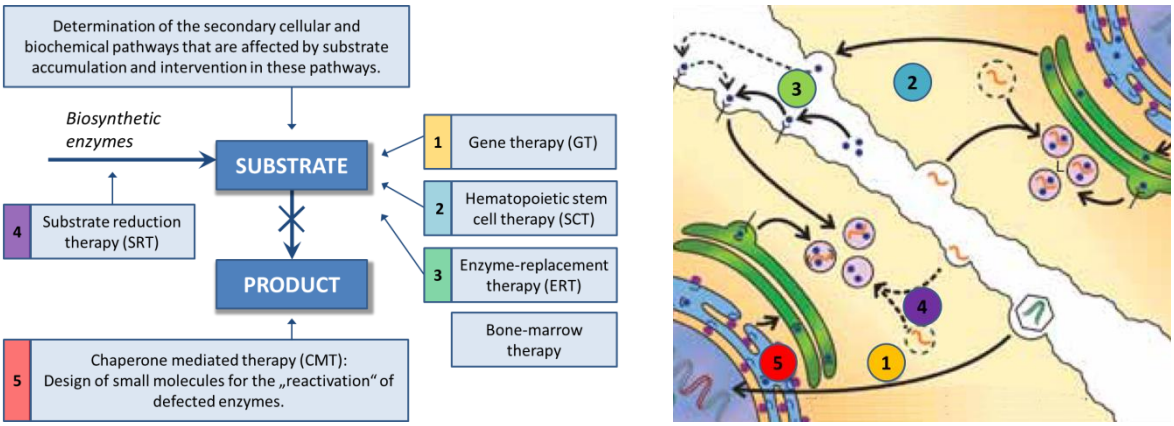


Figure 21: Strategies in the therapy of LSDs: SCT (2), ERT (3), GT (1), SRT (4) and CMT (5).

The object of the most promising approaches in the treatment of LSDs is the restoration of the diminished degradative activities in the lysosomes. Additionally promising therapeutic options rely on the metabolic level and attempt to reduce the influx of the respective substrate to the lysosomes. The different therapy options are summarized in the following:

Gene therapy (1): A viral vector is used to deliver DNA encoding for the missing enzyme. The gene is expressed by the cellular machinery, and provides functional enzyme that can also be secreted and reach adjacent cells by receptor mediated endocytosis.

Stem-cell therapy (2): Healthy donor cells migrate to various tissues. They provide a permanent source of the missing enzyme to host cells via enzyme secretion and receptor mediated uptake.

Enzyme-replacement therapy (3): Administered recombinant enzymes enter the cell by receptor mediated endocytosis and are directed to the lysosomes.

Substrate-reduction therapy (4): The synthesis of storage compounds is partially inhibited, thereby improving the balance between biosynthesis and impaired degradation.

Bone-marrow transplantation therapy (BMT): The aim of BMT is to avoid the obstacle of passing the blood-brain barrier. Bone marrow macrophages can cross the blood-brain barrier to a small extent and can serve as an enzyme source in the brain. Although there have been reports of successful attempts, BMT does not seem to be a suited concept for the treatment of LSDs.

Chaperone therapy (5): This novel concept for the treatment of LSDs relies on the application of stabilizing which bind to misfolded enzymes in the ER and induce proper folding, thereby preventing ER-associated degradation and stimulating transport to the lysosomes.

Each of the mentioned therapeutic options has been elucidated in several excellent reviews, including their therapeutic basement, advantages as well as drawbacks.[110,130,132-138]

1.7.2.1 Chaperone mediated therapy (CMT)

In most LSDs, residual enzyme activities can be observed. Thus, a small amount of impaired protein is transported to the lysosomes. This may serve as basis for the therapeutic employment of pharmacological chaperones.

In general, disease-causing mutations in genes result in the biosynthesis of un- or misfolded proteins. These impaired enzymes are recognized and consequently degraded by one of the cells internal "quality-control" mechanisms, the endoplasmatic-reticulum-associated-degradation pathway (ERAD).[139,140] Thus, an externally supported stabilization of the protein during its biosynthesis may induce a "better" folding and prevent the recognition and degradation in the quality control mechanism.

In general, CMT is based on the concept that small molecules such as active site specific reversible inhibitors can already associate with enzymes during the folding process. Due to their properties, these substances are called "active site specific chaperones" (ASSCs) or "pharmacological chaperones" (PCs).

The interactions between the inhibitors and the active center assist the proper folding of the proteins, providing "chaperone-enzyme-complexes" which are further stabilized during trafficking. This may result in enhanced concentrations of the properly folded enzyme-complexes at their cellular destination. There, the PCs are either replaced by the natural substrates or dissociate from the active site (different pH-values in the cell) releasing employable enzymes.

A schematic overview of ER protein folding, quality control and putative action of a PC in CMT is indicated in Figure 22.[110]

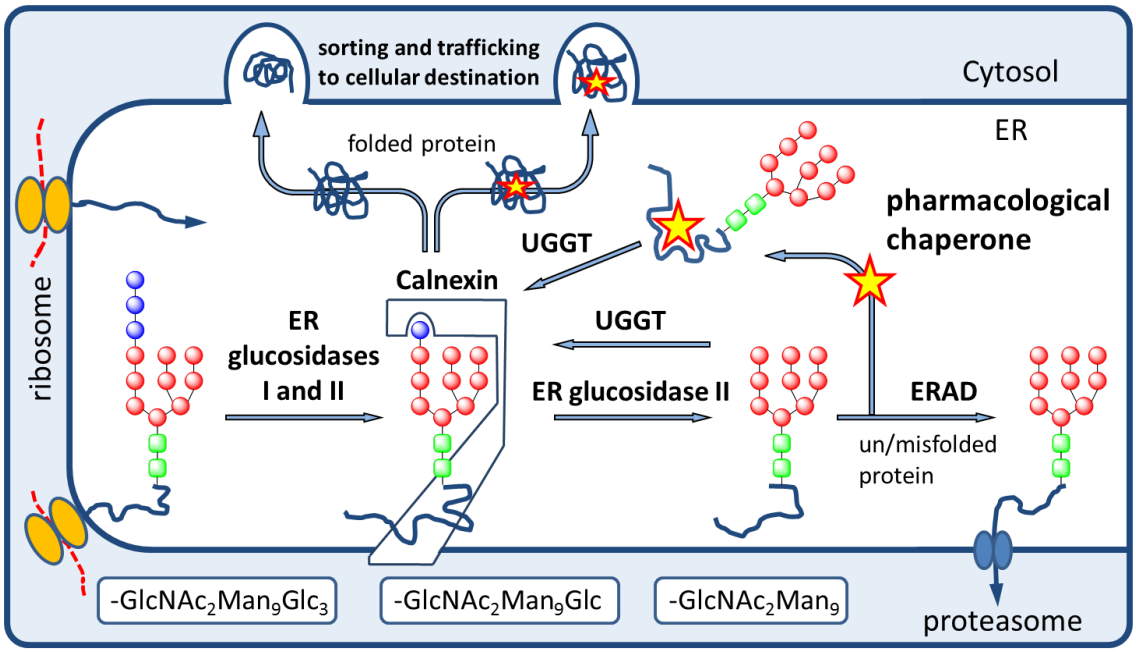


Figure 22: Overview of ER protein folding, quality control and putative action of a PC in CMT. ER glucosidase I and II remove two glucose residues, after which the enzymatic chaperones calnexin or calreticulin recognize the trimmed structure and assist in protein folding. ER glucosidase II cleaves the final glucose residue. Correct folded proteins are sorted and released to their cellular destination. Un- or misfolded proteins are recognized by UDP-glucose glycoprotein glucosly transferase (UGGT). The reglucosylated protein is either reassisted in folding or rejected to the ERAD.[110]

The first example of a PC associated stabilization of a lysosomal glycosidase was reported by Fan and co-workers in 1999.[141] Enhanced concentrations of various mutants of α-galactosidase A were observed in the cells from patients with Fabry disease as a result of the application with galactostatin (**7**) when administrated in concentrations lower than those required for inhibition.

Since then, the general CMT concept has also been demonstrated for several other common LSDs, such as Gaucher (β-glucocerebrosidase), Fabry (α-galactosidase), G_{M1}-gangliosidosis and Morquio B (β-galactosidase).

In the case of human lysosomal β-galactosidase associated with G_{M1}-gangliosidoses and Morquio B disease, only two types of glycosidase inhibitors have been investigated as potential PCs. The most examined inhibitor of lysosomal β-galactosidases is NOEV (**34**). Assays on 50 different cell lines expressing various mutant β-galactosidases showed a highly potent chaperoning behavior even at sub-micromolar concentrations. These encouraging results were confirmed in mouse models carrying human R201C. In this context, **34** became the benchmark-molecule for CMT of G_{M1}-gangliosidoses.[81,83,142] Recently, various unbranched analogues (e.g. compound **59**) lacking the hydroxymethyl moiety have been presented and their activities with patient´s skin fibroblasts carrying β-galactosidase mutations (R201C) were reported.[143]

The second group of chaperones was structurally based on DGJ (**7**) which by several modifications could be transformed into two subgroups, namely *N*-substituted and bicyclic analogues. As already indicated in section 1.4.1, various *N*-modified analogues of DGJ (**7**) exhibit excellent inhibition behaviors against β-galactosidases. Despite this generalization, only a small number of typical derivatives (e.g. **14**, **15**, **60**-**63**; in case of several *N*-substituted-variations in the same publication, only the most potent is described)[64,66,83,144-146] provide interesting properties as experimental PCs for the treatment of G_{M1}-gangliosidoses. Additionally, modifications at C-6 and C-2 (compounds **64** and **65**) have led to noteworthy pharmaceutical chaperones.[147,148]

The most recent sub-group of modified 1-deoxygalactonoririmycins was introduced by Fernandez and his group, namely the so called sp^2-iminosugars (**66**). [149,150] Despite higher IC_{50}-value compared to benchmark molecule NOEV (**34**), 6S-NBI-DGJ (**67**) turned out a highly potent PC for a wide range of mutants of human β-galactosidases.[151,152] Moreover, assays on 88 mutants provided 24 positive results in terms of increased activities, of which 16 mutants were not responding to NOEV (**34**).

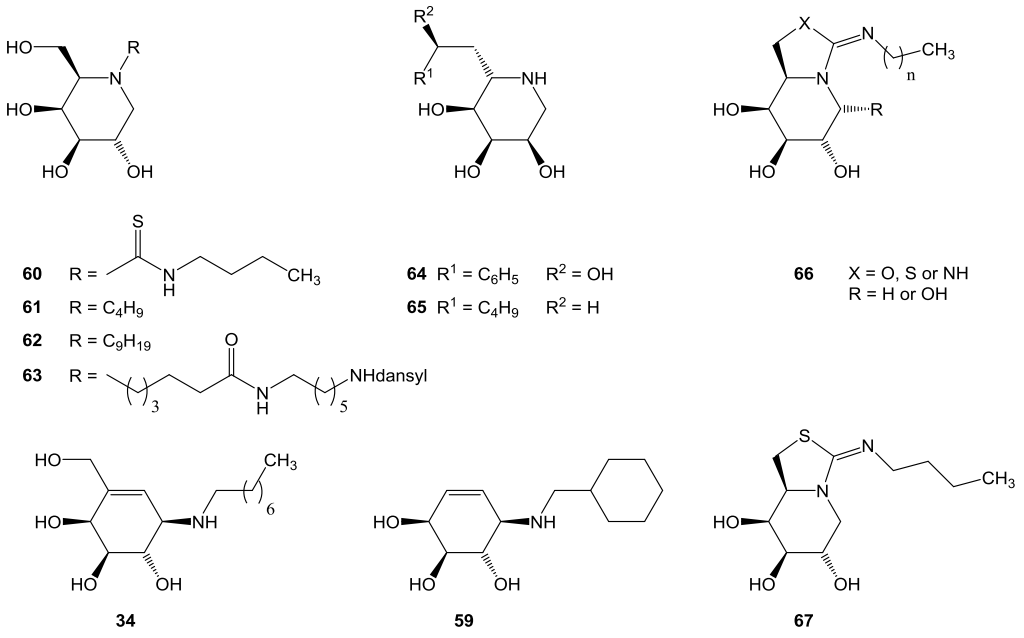


Figure 23: Selected structures of β-galactosidase inhibitors of proven chaperoning behavior.

In conclusion, all noteworthy pharmaceutical chaperones reported thus far showed inhibition activities against their respective glycosidases probed. Inhibition potency is essential but not a warranty for chaperoning behavior. For example, the best GCase inhibitor (**23**) thus far, did not show appreciable chaperoning activities against human β-glucocerebrosidase (Gaucher disease).

2 General Aims and Synthetic Targets

Many iminosugars (**I**) have been shown to be powerful glycosidase inhibitors. Broad investigations in this field indicate an impressively enhanced biological activity of the corresponding *N*-substituted analogs (**II**) when compared to the parent compounds, based on the auxiliary interactions between the spacer-arms and hydrophobic pockets within the glycosidases. Moreover, *N*-alkylated iminoalditols gained importance as potential chemical chaperones for the treatment of various lysosomal storage diseases.

Furthermore, synthetic structural modifications in terms of the position of the ring-nitrogen have provided a new compound class, namely isoiminosugars (**III**), which exhibit outstanding inhibition behaviors with β-selective enzymes compared to iminosugars. For example, in conjugation with the mentioned improved activity of systems bearing lipophilic spacers (**IV**), 6-*C*-nonyl-isofagomine (**23**, actually (5a*S*)-5a-*C*-nonyl-isofagomine according to carbohydrate nomenclature) has to be highlighted as the best β-glucocerebrosidase inhibitor (IC_{50} 0.9 nM) thus far.

It is also noteworthy to mention that, despite all efforts, only a few examples of the (5a*S*) D-*gluco*-series are present in the literature. Thus, three out of the four most interesting stereo isomers, namely the (5a*S*)- and the (5a*R*)-derivatives of the D-*galcto*-series as well as the D-*gluco*-(5a*R*)-series, serve as highly desirable targets in carbohydrate chemistry worldwide.

Reflecting these developments, investigations towards the synthesis and biological evaluation of *C*-5a elongated isoiminosugars (**IV**) are deemed important. A summarized description is shown in Figure 24.

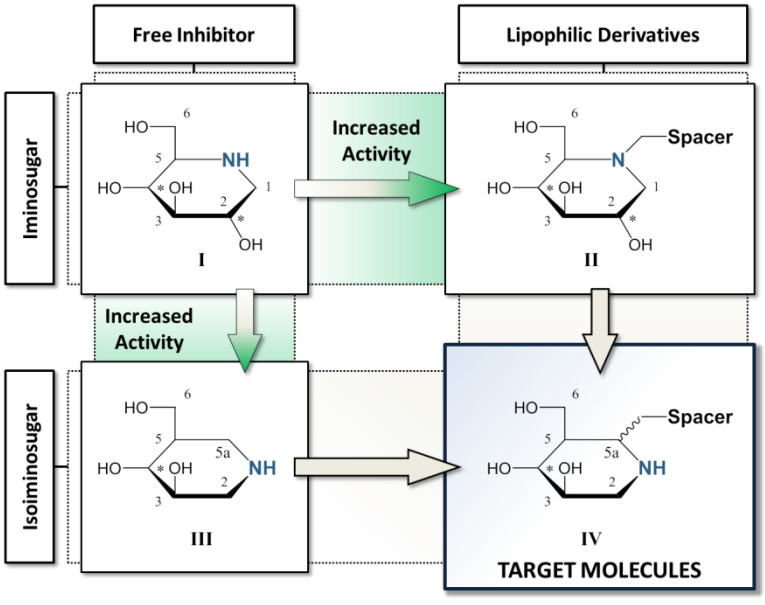


Figure 24: General considerations leading to the development of *C*-5a-elongated isoiminosugars.

Following our main interests in powerful β-galactosidase inhibitors, which are also potential chaperones for the treatment of G_{M1}-gangliosidosis, the development of a synthetic route towards D-*galacto* configurated *C*-5a-elongated 4-*epi*-isofagomines has been the aim of this thesis.

Thus, a synthetic approach starting from a simple and commercially available carbohydrate, involving the introduction of nitrogen and a side-chain as well as a cyclization reaction, leading to both isomeric series of the new compound class had to be developed. (Figure 25)

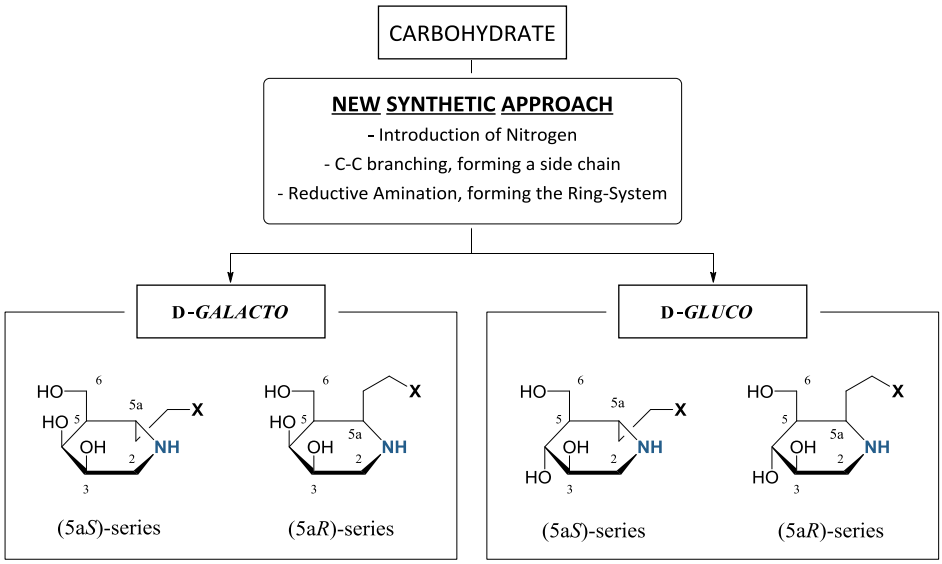


Figure 25: Intended approach towards *C*-5a-elongated isofagomines. (X = Spacer of choice)

Furthermore, it was important to establish a protecting group strategy for gaining selective access to the *C*-5a side chain for advanced modifications. Along with this, all functional groups at the sugar moiety were intended to be protected in a similar manner (e.g. employing acid labile groups), whereas the in Figure 25 as "**X**" indicated moiety is suggested as an orthogonally protected alcohol (OSilyl, OBn, OAc, etc.). Simultaneously, the benefit of releasing the final inhibitor in one deprotection step is provided.

Furthermore, extension of the approach to the D-*gluco*-series has also been part of the investigations, in particular for the development of (5a*R*)-analogues.

Apart from the mentioned derivatizations effecting interactions between the introduced spacer-arms and hydrophobic pockets within the glycosidases, other modifications of parent compound 4-*epi*-isofagomine (**9**) were intended to influence the binding affinity to the active site of the respective enzymes.

For example, the introduction of a fluorine substituent, due to the powerful inductive and field effects of this atom, was planned as interesting modification, directly affecting typical properties, especially the basicity of the nitrogen as one of the biologically relevant features. Furthermore, due to its isosteric properties with hydrogen, sterical negative and conformational consequences were not to be expected. (Figure 26)

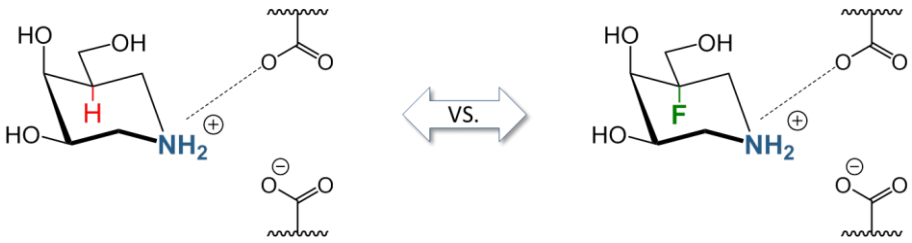


Figure 26: Intended introduction of a fluorine substituent.

Following these considerations, the synthesis of 5-fluoro derivatives of 4-*epi*-isofagomine (**9**) has been an additional aim of this thesis.

3 Results and Discussion

3.1 Preliminary studies

3.1.1 General approach to 4-*epi*-isofagomine and *C*-5a-elongated derivatives

In this section, the originally scheduled synthetic route to 4-*epi*-isofagomine (**9**, 4-*epi*-IFG) and *C*-5a-elongated derivatives will be presented. Related issues and consequences will be discussed. As indicated in Figure 27, a general synthetic sequence starting from commercially available D-glucose, employing C-C branching, introduction of nitrogen and final cyclisation was intended to serve as an elegant approach to a set of new isoiminosugars.

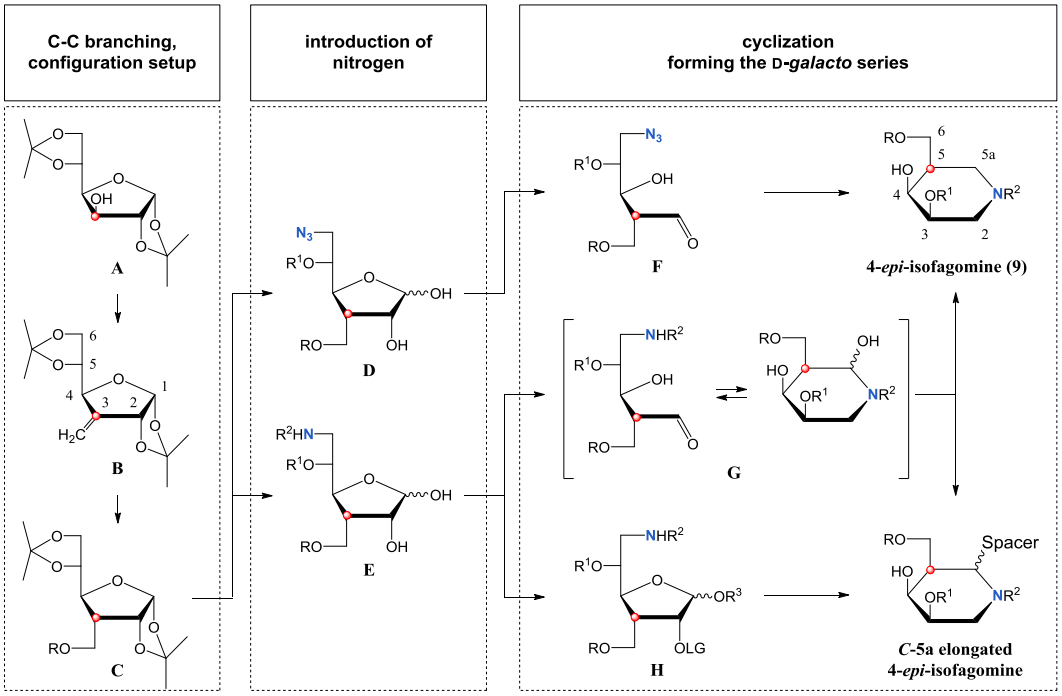
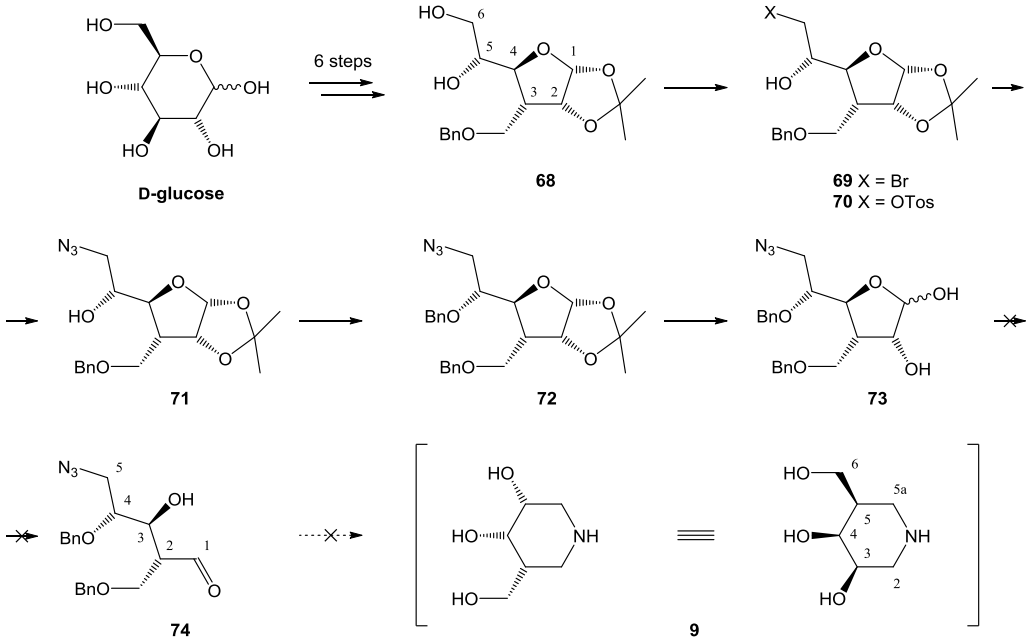


Figure 27: Intended approach to 4-*epi*-isofagomine (**9**) and *C*-5a-elongated derivatives.

A major topic was the introduction of the hydroxymethyl group and the associated desired configuration at C-5 of the isoiminosugars. The broadly investigated stereoselective hydroboration of alkene **B**, formally a C-3 derivative of D-glucose, provided a suitable access to the mentioned moiety. Furthermore, conventional introduction of nitrogen at C-6 should give N-derivatives **D** and **E** which were supposed to cyclize to the respective ring systems. Thereby, nucleophilic attack at C-2 of intermediate **H** enables access to (5a*R*) configurated *C*-5a-elongated isofagomines. They and the corresponding (5a*S*)-series can also be accessed via aminal **G**, as is easily prepared by oxidative cleavage of the anomeric carbon of intermediates **D**. Reduction of aldehydes **F** and **G** was expected to give parent compound 4-*epi*-IFG (**9**).

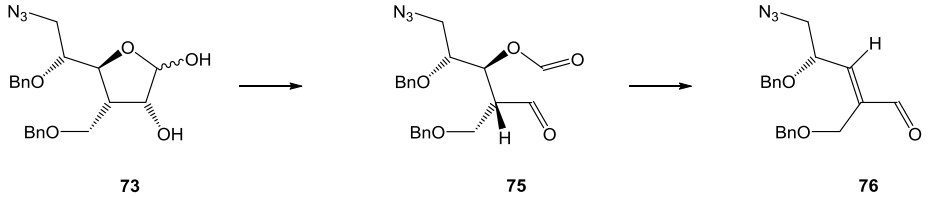
3.1.2 Synthesis starting from D-glucose

Starting from D-glucose, 3-deoxy-3-*C*-benzyloxymethyl-5,6-dihydroxy-1,2-*O*-isopropylidine-α-D-allofuranose (**68**) [153] was synthesised in 6 steps. Activation at C-6 by conventional bromination (**69**) or tosylation (**70**) and consecutive treatment with NaN_3 in DMF in the presence of Na_2CO_3 provided azide **71**. *O*-Benzylation under standard conditions afforded **72** as a key intermediate.



Scheme 1: Intended synthesis of 4-*epi*-isofagomine (**9**).

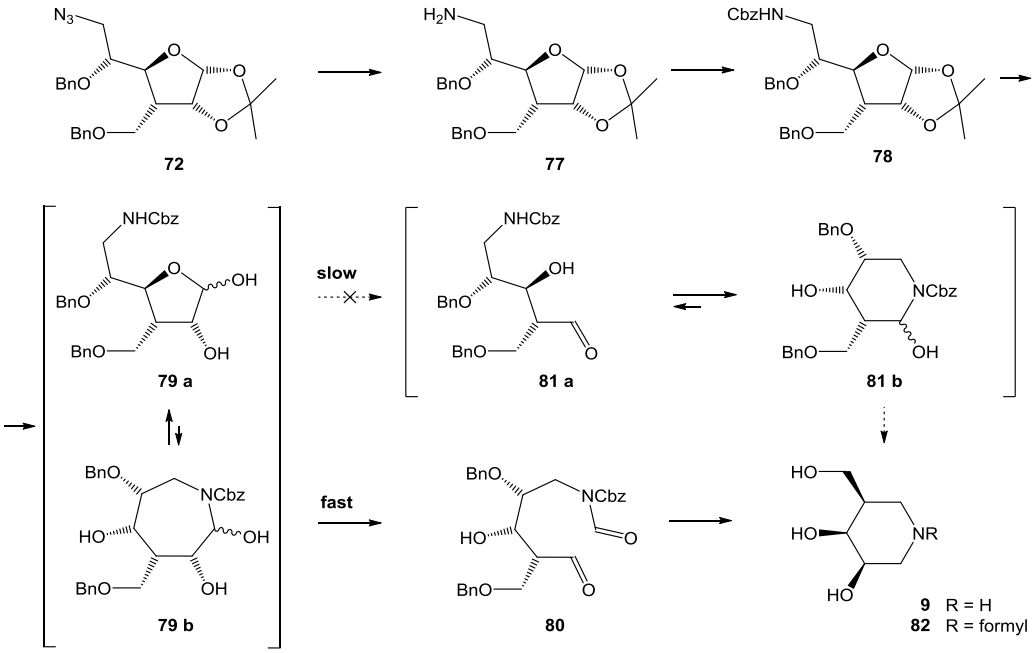
Acidic hydrolysis of the isopropylidene group employing aqueous HCl in MeCN gave diol **73**, which was treated with $NaIO_4$ in Et_2O/H_2O to give **74**. The resulting product was immediately used in the next step. Reduction of the azide moiety employing hydrogen and Pd/C (5%) in MeOH and concomitant intramolecular reductive amination was supposed to give 4-*epi*-IFG (**9**). Disappointingly, formation of **9** was not observed. (Scheme 28)



Scheme 2: Formation of α,β-unsaturated aldehyde **76**.

Further investigations pointed to the formation of the much more stable α,β-unsaturated aldehyde **76** during the oxidative cleavage via elimination of the 3-*O*-formyl group in intermediate **75**. In a series of approaches, other solvent mixtures (THF/H_2O; CH_2Cl_2/H_2O and MeOH/H_2O) were probed. Despite all efforts, aldehyde **74** could not be isolated. (Scheme 29)

Based on the need to create a more stable system, “removal” or “deactivation” of the carbonyl functionality seemed to be necessary. This should be realized by an intramolecular, nucleophilic attack of secondary amine **81 a** in order to gain the corresponding hemiaminal **81 b**. (Scheme 3)



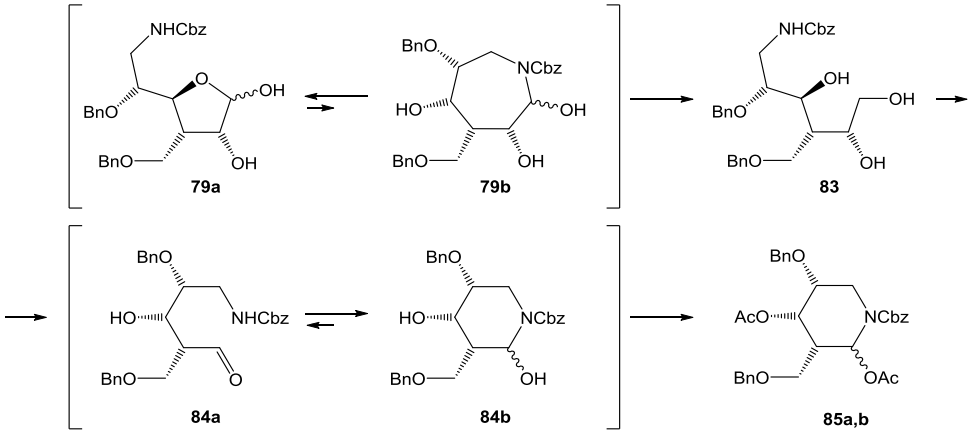
Scheme 3: Alternative approach to 4-*epi*-isofagomine (**9**) and side reactions.

Thus, azide **72** was reduced under Staudinger´s conditions. Free amine **77** was protected with CbzCl in MeOH employing Na_2CO_3 as base to give **78**. Cleavage of the isopropylidene group provided the anomeric mixture of compound **79 a**. It is necessary to mention, that the formation of the corresponding seven-membered ring **79 b** was not verified in NMR studies. Only an equilibration of the ratio of the furanoic α/β epimers (**79 a**) was observed.

Nonetheless, treatment with $NaIO_4$ and subsequent hydrogenolysis did not afford 4-*epi*-IFG (**9**). The reason behind this was, that, following the principal of Curtin and Hammett, the formation of **80** (despite the insignificant concentration of isomer **79 b**) was much faster than the formation of **81 a**,**b**. Consequently, the unexpected intermediate **80** reacted under reductive conditions to give the *N*-formyl analogue **82**. (Scheme 3)

To prevent the formation of **80**, it was necessary to convert aldehyde **79 a**,**b** to alcohol **83**. For this purpose, $NaBH_4$ in THF at 0°C was found most convenient. Other hydride sources and solvents failed due to the undesired reduction of the *N*-Cbz group.

Starting from diol **83**, treatment with $NaIO_4$ in a mixture of CH_2Cl_2 and H_2O afforded **84a** which spontaneously equilibrated to the six-membered ring **84 b**. *O*-Acetylation under standard conditions gave **85 a**,**b**, which could be confirmed by NMR-spectroscopy.



Scheme 4: Synthesis of compound **85 a**,**b**.

As indicated in Figure 28, aminal **85 a**,**b** acts as a potential starting material in the synthesis of *C*-5a-elongated derivatives of 4-*epi*-IFG (**A**). Furthermore, due to the orthogonally protected position C-3, **85 a**,**b** enables the entry into the corresponding D-*gluco*-series (**B**).

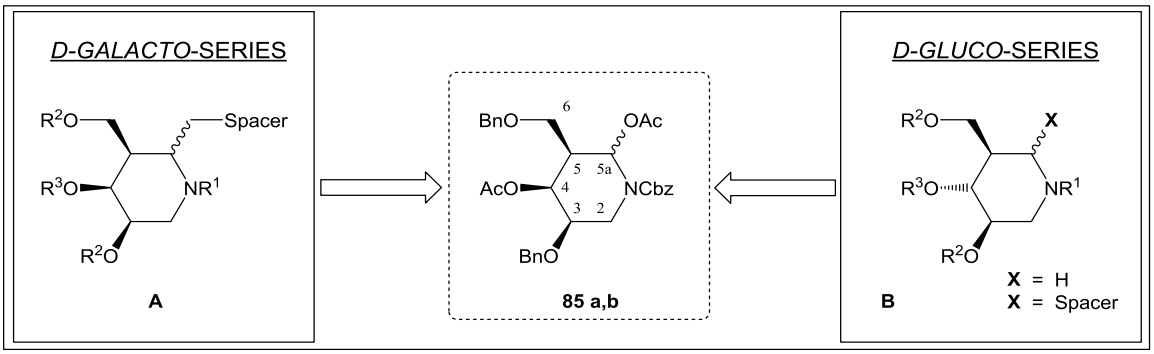
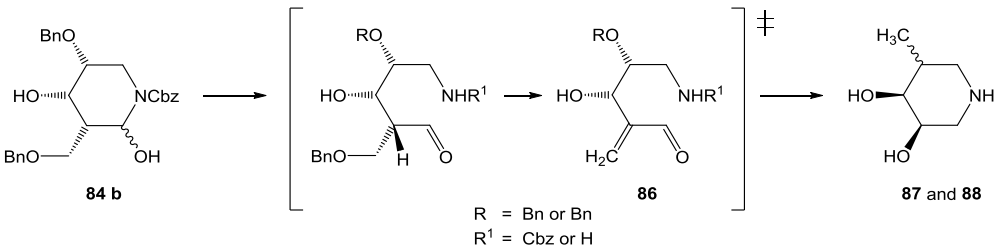


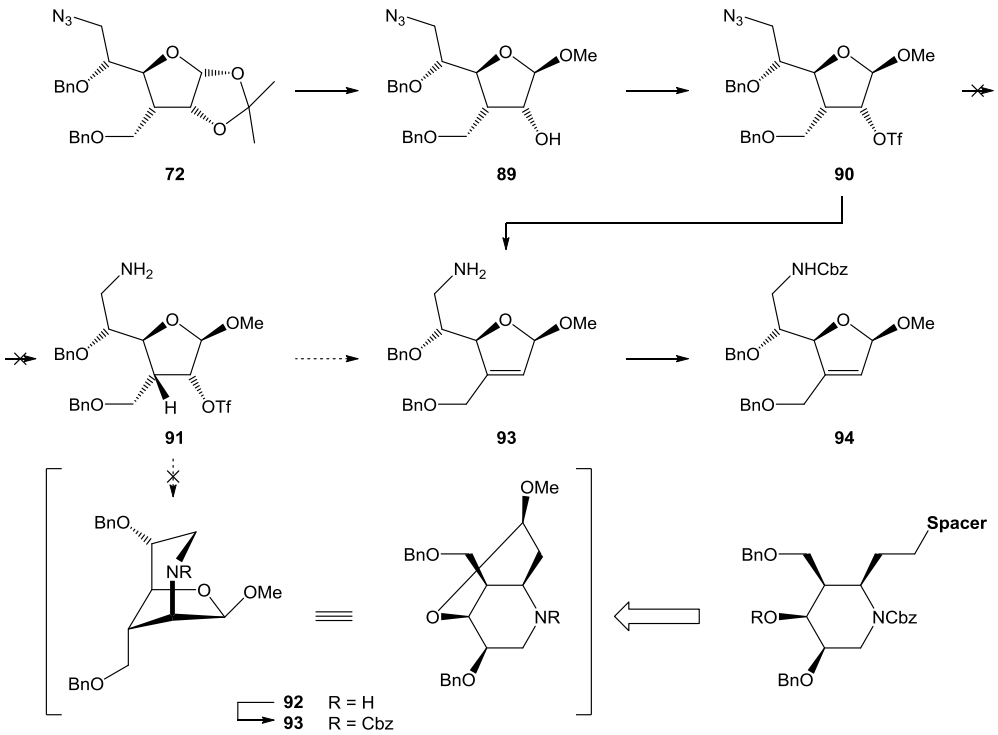
Figure 28: Optional follow up chemistry starting from compound **85 a**,**b**.

In the next step, according to the scheduled synthesis of 4-*epi*-isofagomine (**9**), hemiaminal **84 b** was treated with hydrogen in MeOH, employing Pd/C (5%) as catalyst. Again, because of the basic character of the liberated free amine, elimination of BnOH formed unsaturated system **86**, which reacted further to the corresponding 6-deoxy derivatives **87** and **88** (ration 1:1). (Scheme 5)



Scheme 5: Formation of 6-deoxy derivatives **87** and **88**.

Another promising sequence to *C*-5a-elongated 4-*epi*-isofagomines also started from azide **72**. Transacetalation in methanolic HCl afforded compound **89**. Next, activation at C-2 (compound **90**) and subsequent intramolecular nucleophilic attack[154] of liberated primary amine **91**, generated by standard Staudinger methodology, was expected to form spontaneously bicyclic system **92**. Due to a simplified purification procedure, *N*-Cbz-protection of the resulting reaction mixture was employed. Unrewardingly, NMR-analysis showed that the highly unstable intermediate **90** degraded to alkene **93**, which was consecutively converted into **94**. (Scheme 6)



Scheme 6: Alternative synthesis of *C*-5a-elongated 4-*epi*-isofagomines.

Concluding, the originally favored synthesis of 4-*epi*-IFG (**9**) failed due to the abstraction of a single proton in a tertiary position. Adjusted approaches, targeting **9** as well as *C*-5a-elongated derivatives confirmed this observation.

Despite all advantages (defined stereochemistry of generated chiral centers, potential entry into the *gluco*-series) employing the initial approach of C-C branching and hydroboration, followed by introduction of the nitrogen and final cyclisation had to be rearranged, avoiding the presence of the mentioned reactive proton as long as possible. This led to the plan to switch the sequence of the crucial synthetic steps towards the introduction of the hydroxylmethyl moiety at the end of the sequence. The resulting synthetic strategy will be discussed in the next section.

3.2 Advanced studies: Synthesis of the D-*galacto*-series

3.2.1 General approach to 4-*epi*-isofagomine and *C*-5a-elongated derivatives

In this chapter, another synthetic approach to 4-*epi*-isofagomine (**9**) and *C*-5a-elongated derivatives will be presented. Experiences thus far indicated the benefit of an existing ring system, thus an iminosugar, which is intended as starting material for the introduction of a hydroxymethyl group providing the corresponding isoiminosugar. The general concept of the introduction of nitrogen, formation of the cyclic system and the final installation of the side-chain in position C-5 is shown in Figure 29.

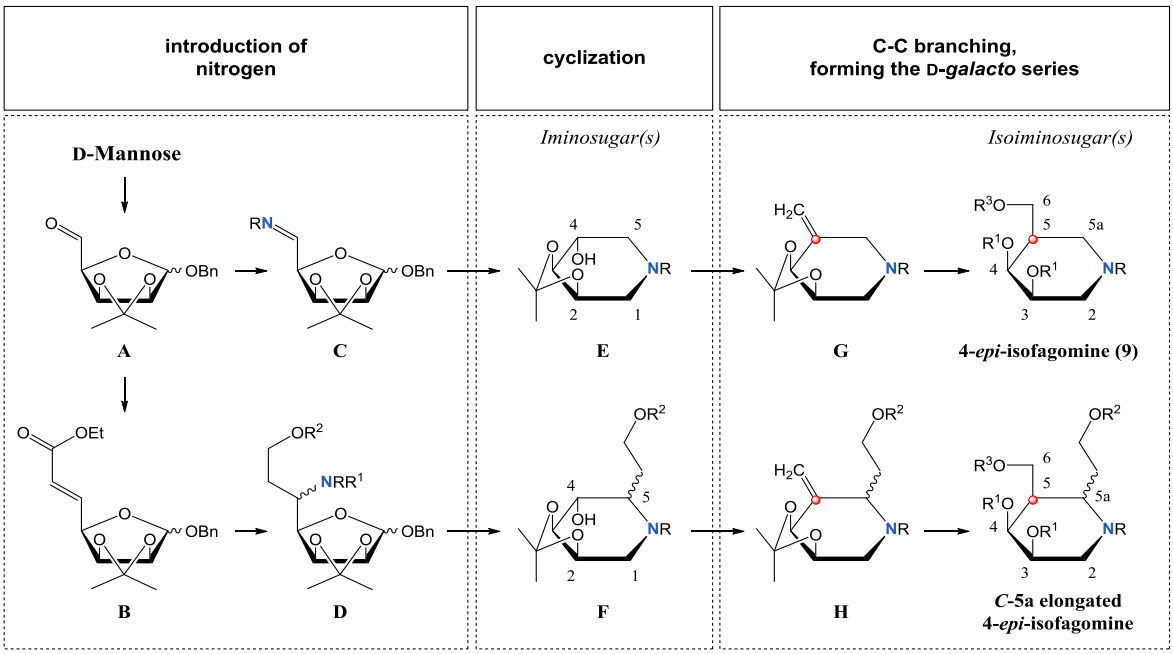


Figure 29: General approach to 4-*epi*-isofagomine (**9**) and *C*-5a-elongated derivatives.

Aldehyde **A**, easily available from D-mannose, serves as starting material in the synthesis of 4-*epi*-isofagomine (**9**) as well as in the approach towards *C*-5a-elongated derivatives. For the latter families, elongation of **A** at C-5 was performed to form Michael acceptor **B**.

Introduction of nitrogen furnishing **A** and/or **B** was supposed to provide N-substituded derivatives **C** and **D** which were intended to provide iminoalditols **E** and **F** under reductive conditions. Finally, C-C branching and subsequent hydroboration was expected to yield isoiminosugars. Starting from **H**, entry to *C*-5a-elongated derivatives of 4-*epi*-isofagomine (**9**) should be feasible which itself was intended to be formed starting from **G**.

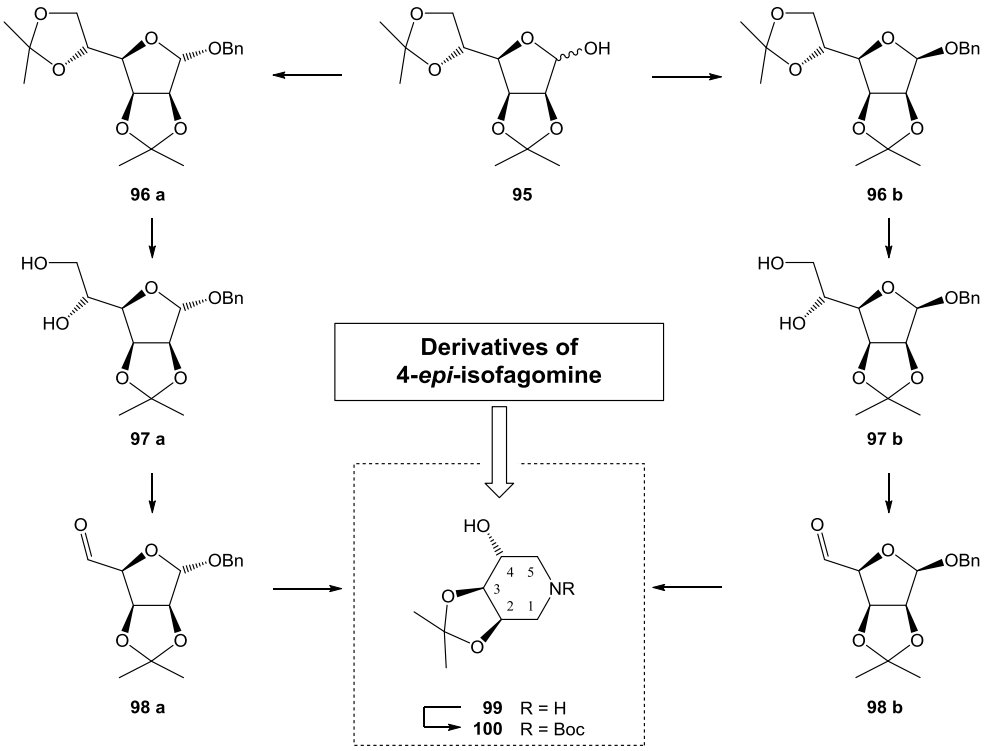
3.2.2 Synthesis of iminoalditols as potential precursors to isoiminosugars

3.2.2.1 Synthesis of *N*-Boc-1,2-*O*-isopropylidene-1,5-dideoxy-1,5-imino-D-lyxitol[155]

Following the presented general approach to 4-*epi*-isofagomine (**9**), both anomers of broadly investigated benzyl-isopropylidene D-lyxaric aldehyde **98 a**,**b**[156] serve as suitable intermediates.

Starting from commercially available 2,3-4,5-di-*O*-isopropylidene-D-mannofuranoside (**95**), different conditions for the formation of the benzyl glycoside enabled access to either the α-series or the β-series. In our hands, *O*-benzylation employing NaH and benzyl bromide in THF/DMF (3:1 v/v) provided a mixture of anomers (**96 a** : **96 b** ca. 3:1). In contrast, according to the procedure of Chen[157], treatment of **95** with KOH and benzyl bromide in the presence of catalytically amounts of PEG-600, inverted the ratio of **96 a** and **96 b**.

Selective cleavage of the 5,6-*O*-isopropylidene group, starting from β-anomer **96 b**, was found a protracted reaction. Due to the simultaneous hydrolysis of the 2,3-*O*-acetonide, only long reaction times (10-14 days), vigorously diluted solvent mixtures (2-5% in MeCN/H_2O) and traces of AcOH provided diol **97 b**. Conversely, diol **97 a** was easy available furnishing **96 a** with a mixture of AcOH and H_2O (1:1 v/v) for 12h.

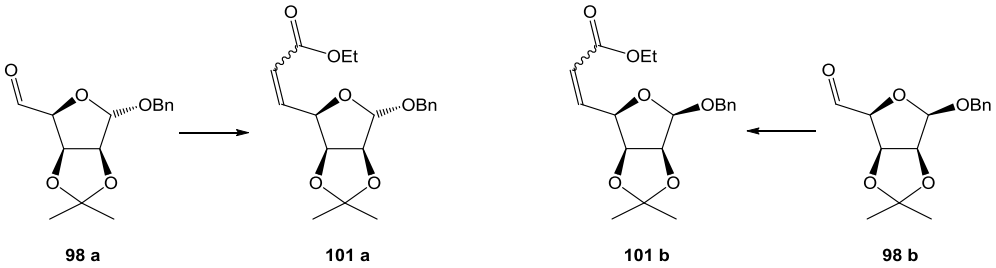


Scheme 7: Synthesis of iminoalditol **100**.

Oxidative cleavage of diols **97 a** and **97 b** using $NaIO_4$ in CH_2Cl_2 and H_2O provided aldehydes **98 a** and **98 b**. Subsequent, double reductive amination employing $BnNH_2$ and Perlman´s catalyst under an atmosphere of hydrogen gave iminoalditol **99**. Standard N-protection (Boc_2O) furnished known *N*-Boc-1,2-*O*-isopropylidene-1,5-dideoxy-1,5-imino-D-lyxitol (**100**). (Scheme 7)

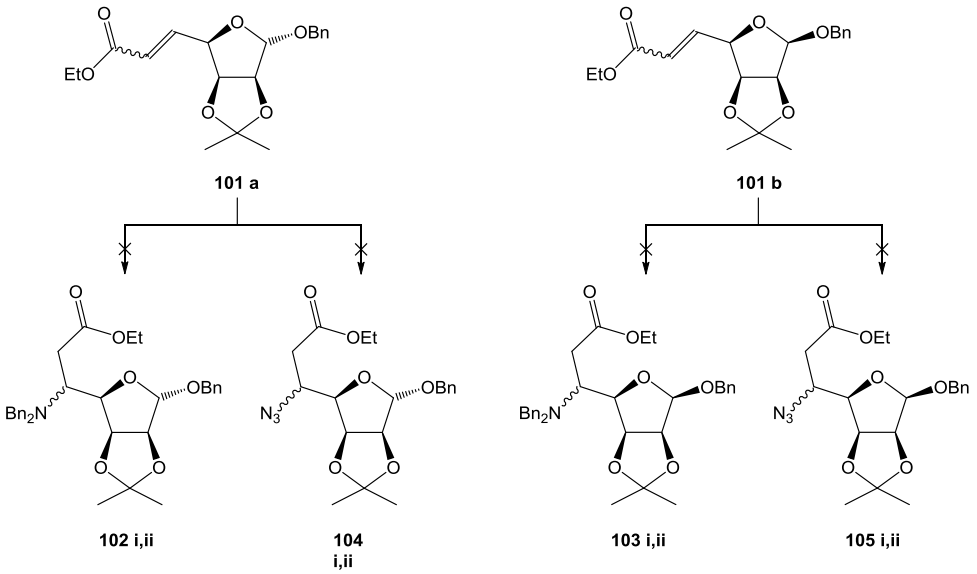
3.2.2.2 Synthesis of iminoalditols leading to *C*-5a-elongated derivatives of 4-*epi*-IFG

In context with our interest in the properties of *C*-5a-elongated isofagomines, C-C branching was necessary. For this purpose, benzyl-isopropylidene D-lyxaric aldehydes **98 a** and **98 b** (**A** in Figure 29) serve as suitable starting materials in a conventional Horner-Wadsworth-Emmons-reaction. Unsaturated ester **101 a**[158] was easily available furnishing aldehyde **98 a** with triethyl phosphonoacetate, activated with *t*-BuOK in dry THF. As expected, formation of the *E*-isomer was favored. In the same manner, aldehyde **98 b** was converted into ester **101 b**[159]. (Scheme 8)



Scheme 8: Synthesis of α,β unsaturated esters **101 a** and **101 b**.

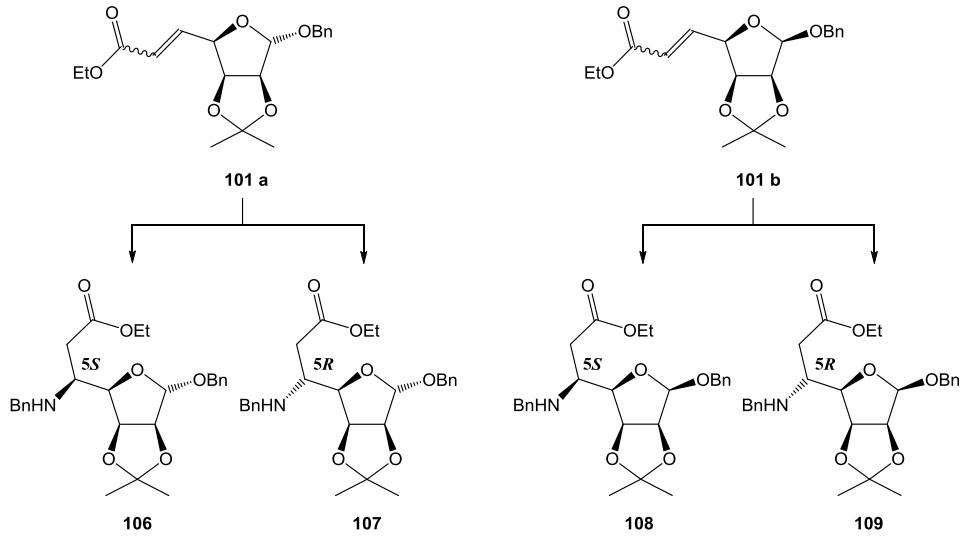
In the next step, introduction of nitrogen should be realized by a conventional aza-Michael addition. In early studies, employing sodium azide and dibenzyl amine in THF respectively MeCN, formation of the desired products (**102 i**,**ii** – **105 i**,**ii**) was not observed, neither in the α-series nor in the β-series (Scheme 8).



Scheme 9: Unsuccessful aza-Michael addition employing Bn_2NH and NaN_3.

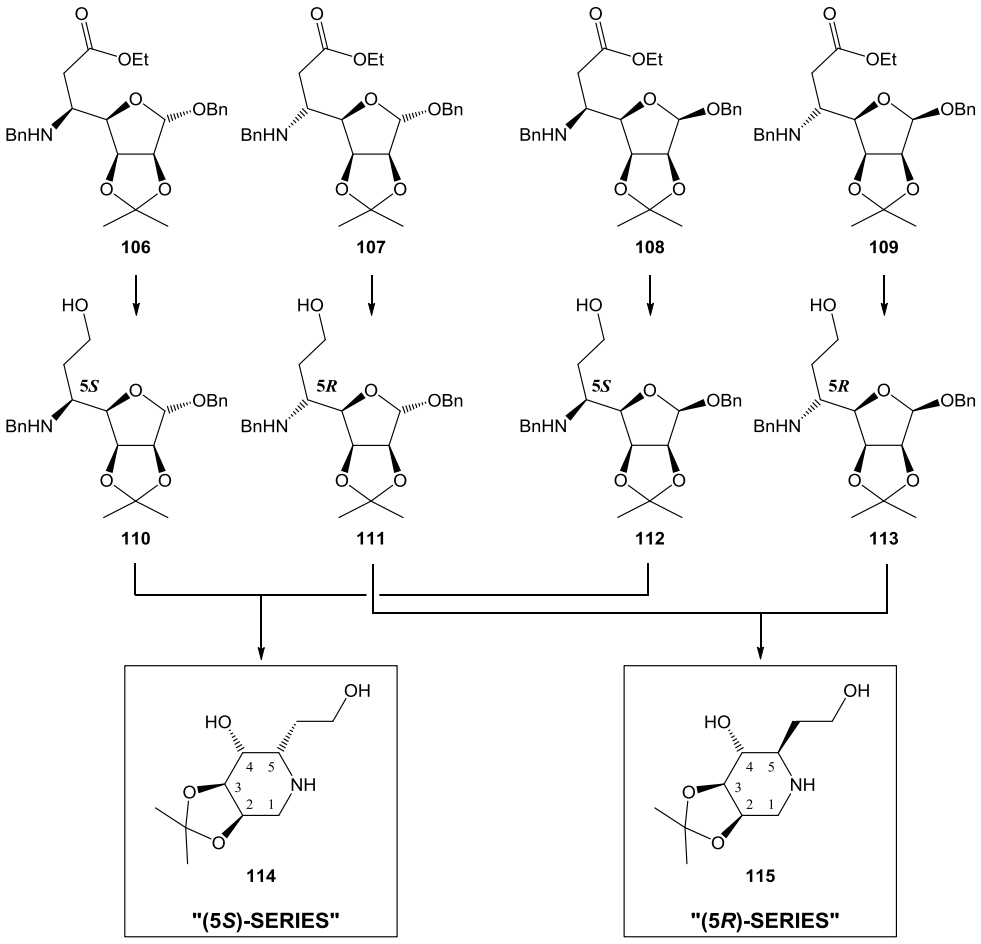
Addition of benzyl amine to α,β-unsaturated ester **101 b** was reported by Sharma[158] and co-workers in 2002. Based on the use of TBAF·$3H_2O$ as catalyst[160], they describe a nearly quantitative formation of (5*S*)-diastereomer **106** (d.r. 91.45 : 4.89; 75% yield).

Due to the need for both isomers[161], we decided to run the reaction without the use of TBAF·$3H_2O$. Treatment of **101 b** with $BnNH_2$ in MeCN or THF (1:100 v/v) provided a mixture of **106** and **107** (ratio 2:1) within 48h. The formation of the corresponding α-anomers required harsher conditions. Temperatures up to 60°C and reaction times of 2 weeks in a mixture of MeCN/$BnNH_2$ (1:1 v/v) were necessary to convert **101 a** into **108** and **109**.



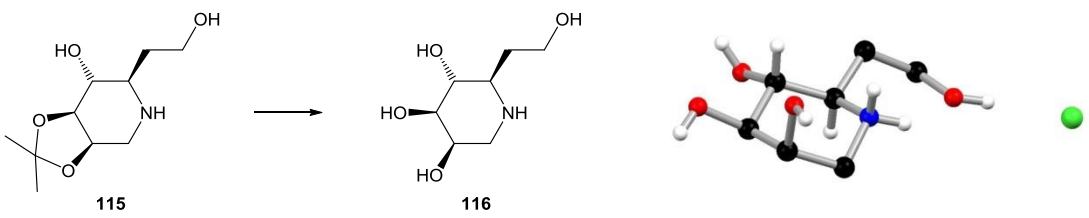
Scheme 10: Aza-Michael Addition furnishing α,β-unsaturated esters **101 a** and **101 b**.

Reduction of the ester moiety employing LAH in THF afforded the corresponding alcohols **110**-**113**. Catalytical hydrogenation over perlman´s catalyst and concomitant intramolecular reductive amination of the (5*S*)-isomers **110** and **112** provided iminoalditiol **114**, formally a derivative of 1-deoxy-L-*gulo*-nojirimycin. Analogous, (5*R*)-isomers **111** and **113** were converted into **115**.



Scheme 11: Synthesis of iminoalditols **114** and **115**.

Deprotection of **115** in methanolic HCl gave 6-*homo*-1-deoxymannonojirimycine (**116**) as hydrochloride of which a crystal structure could be obtained confirming (*R*)-configuration at C-5.

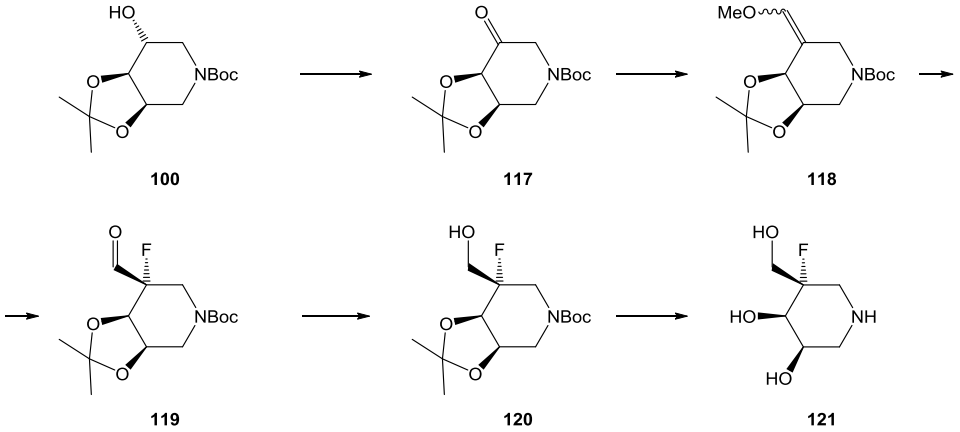


Scheme 12: Synthesis of compound **116** and XRD of **116**·HCl.

3.2.3 Synthesis of 5-fluoro derivatives of 4-*epi*-isofagomine

Following our interests in the properties of galactosidases, structural modifications of relevant parent compounds in terms of new interactions between the inhibitor and the respective enzyme turned out a suitable approach. Accordingly, introduction of a fluorine substituent, due to the powerful inductive and field effects, was intended as potent modification, directly affecting typical substance properties, especially the basicity of the ring-nitrogen as one biologically relevant feature.

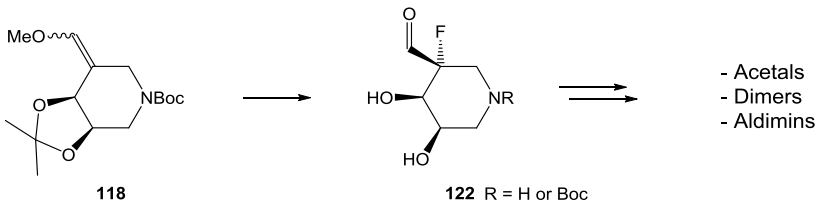
For the synthesis of 5-fluoro-4-*epi*-isofagomine (**117**) and its N-alkyl derivatives, partially protected iminoalditol **100**[155], easily prepared as shown in section 3.2.2.1, was intended as versatile key-intermediate en route to C-C branching as well as fluorine introduction.



Scheme 13: Synthesis of 5-fluoro-4-*epi*-isofagomine (**121**).

As indicated in Scheme 13, Dess-Martin or Swern oxidation of compound **100** afforded ulose **117**[50] in nearly quantitative yields. Formation of exocyclic vinyl ether **118** (mixture of E/Z ca. 1:1) was performed by a conventional Wittig reaction with methoxymethylene triphenylphosphorane in dry THF (-60°C to 25°). This highly unstable product had to be immediately used in the next step. Electrophilic fluorination employing Selectfluor® provided sensitive α-fluoroaldehyde **119** which was directly reduced to α-fluoroalcohol **120**.

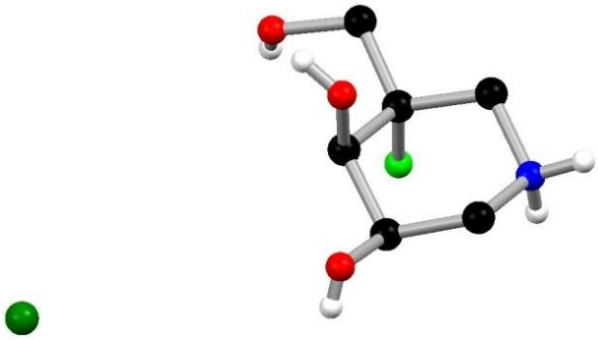
Based on the work of Dax[162] and Wong[163] and their co-workers, in preliminary experiments $MeNO_2/H_2O$ (6:1 v/v) was selected as most promising solvent system. This resulted, due to the slow fluoronium addition and the need of high reaction temperatures (ca. 65°C) in the partially cleavage of the acid labile protecting groups. These intermediates (compound(s) **122**) subsequently formed acetals, dimers and other side products which were not further investigated. (Scheme 14)



Scheme 14: Side reactions in the synthesis of compound **121**.

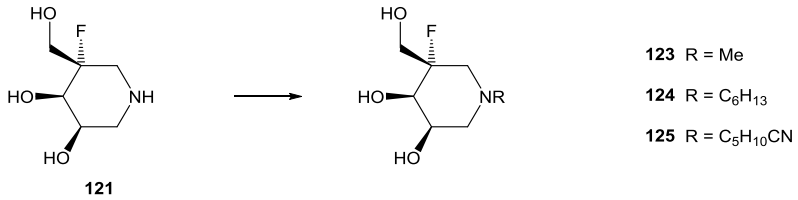
To circumvent this problem, other solvent mixtures were probed. The best results could be obtained using a mixture of MeCN and H_2O (5:1 v/v). Enabled by the faster conversion of the starting material, it was possible to run the reaction at ambient temperature. For the reduction of α-fluoroaldehyde **119**, $NaBH_4$ in THF was found most convenient. Finally, deprotection of **120** in methanolic HCl provided free inhibitor **121** as hydrochloride.

This first 5-fluoro derivative of 4-*epi*-IFG (compound **121**) precipitated nicely upon addition of Et_2O to the reaction mixture. It is worthwhile to mention that the fluoronium addition only occurred from the less hindered *exo*-face, *trans* to the bulky isopropylidene group. The desired stereochemistry in position C-5 was unambiguously confirmed by XRD (Picture 2).

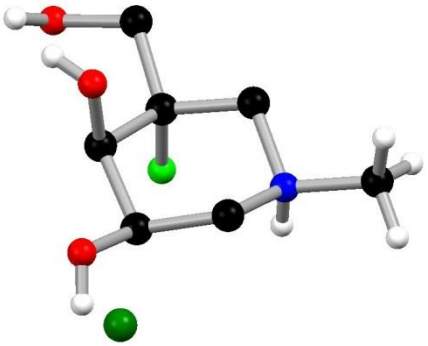


Picture 2: Crystal structure of compound **121**·HCl.

In further studies a set of *N*-alkyl derivatives as potential inhibitors of α/β-galactosidases was prepared. Thus, **121** ·HCl was treated with selected halocarbons in DMF (T = 50°C) employing Na_2CO_3 as base. Following this procedure, *N*-methyl-5-F-4-*epi*-isofagomine (**123**) was obtained by addition of iodomethane. Recrystallization of **123** in MeOH/Et_2O containing HCl (g) enabled reanalysis of the structure by XRD. Reaction of **121** with 1-bromohexane gave the corresponding *N*-hexyl analogue **124**. (Scheme 15)

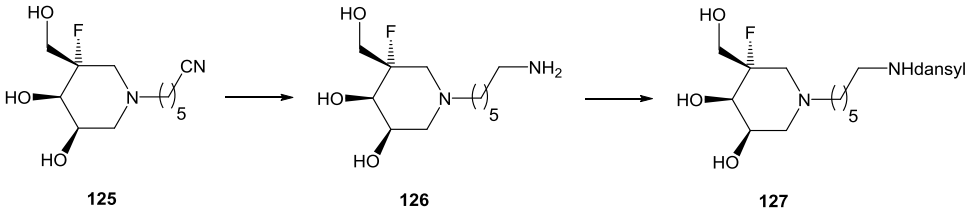


Scheme 15: Synthesis of N-alkylated derivatives of compound **121**.



Picture 3: Crystal structure of compound **123**·HCl.

To facilitate the introduction of additional tags, nitrile **125** was a suitable intermediate. Prepared as presented above, employing 6-bromohexanoic nitrile, it was subsequently reduced to the corresponding amine **126**. This nearly quantitative reduction was performed in MeOH under an atmosphere of hydrogen at ambient pressure using Raney-Ni as catalyst. Reaction of amine **126** with dansyl chloride in MeOH in the presence of Na_2CO_3 led to the fluorescently tagged *N*-(6-aminodansyl)hexyl-5-fluoro-4-*epi*-isofagomine (**127**). (Scheme 16)

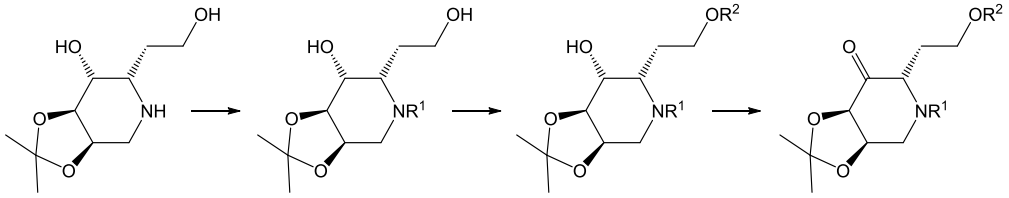


Scheme 16: Synthesis of compound **127**.

3.2.4 Synthesis of *C*-5a-elongated isoiminosugars

3.2.4.1 Synthesis of the (5a*S*)-Series

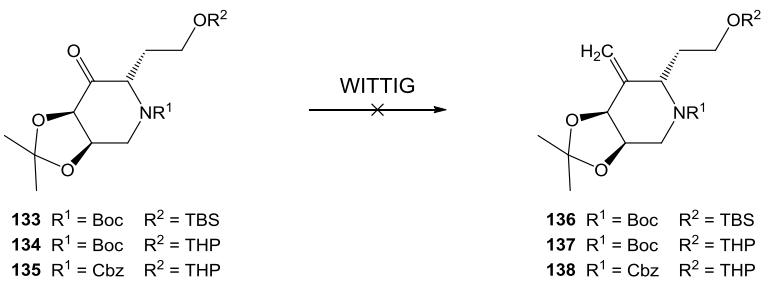
Starting from 1,5,6-trideoxy-1,5-imino-2,3-*O*-isopropylidene-L-*gulo*-heptitol (**114**), prepared as shown in chapter 3.2.2.2, chemoselective access to position C-4 was necessary (see section 3.2.1). Thus, conventional N-protection, followed by regioselective O-protection afforded a set of alcohols which served, after conversion into the corresponding ulose, as starting materials for several C-C branching methods. Various protecting groups had to be probed in order to find a viable approach for the introduction of the *exo*-methylene group.



Scheme 17: Synthesis of different protected uloses **133**, **134** and **135**.

Starting with **114**, treatment with Boc_2O or CbzCl in MeOH in the presence of Et_3N provided **128** and **129**. Conventional silylation of compound **128** afforded **130**. **131** and **132** were prepared employing 3,4-dihydro-2*H*-pyran in CH_2Cl_2 furnishing compounds **128** and **129**. Dess-Martin oxidation of each alcohol gave the corresponding uloses **133**-**135**.

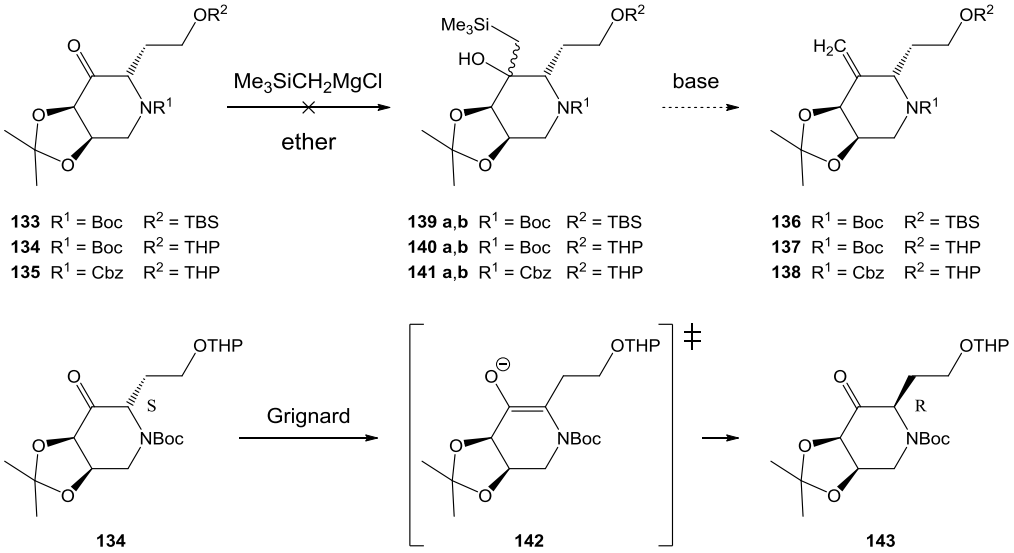
In preliminary studies, conversion of **133** to **136**, employing methylene triphenylphosphorane in dry THF, failed. Due to the basic reaction conditions, replacement of the silyl ether by the base stable THP group seemed to be useful. Nonetheless, starting from **134**, the formation of **137** could not be observed. The reaction with the *N*-Cbz protected analogue **135** also failed.



Scheme 18: Unsuccessful conversion of **133**, **134** and **134** employing a Wittig reaction.

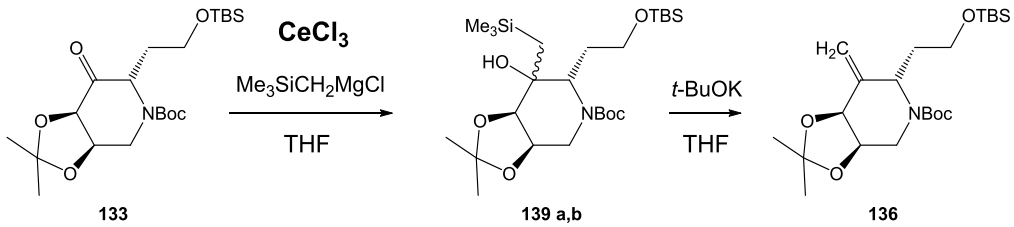
In another series, a two-step synthesis of alkenes **136**-**138** involving the addition of an α-silyl carbanion to the respective carbonyl compounds (**133**-**135**) followed by the elimination of β-hydroxysilanes **139 a**,**b** - **141 a**,**b** was investigated. Unfortunately, this Peterson olefination was neither successful. (Scheme 19)

In some reactions, isomer **143** was observed as a result of the formation of enolate **142**. This indicated the need of less basic conditions during the C-C bond-formation. Thus, transmetalation of the grignard reagent, employing anhydrous cerium (III) chloride[164] was investigated.



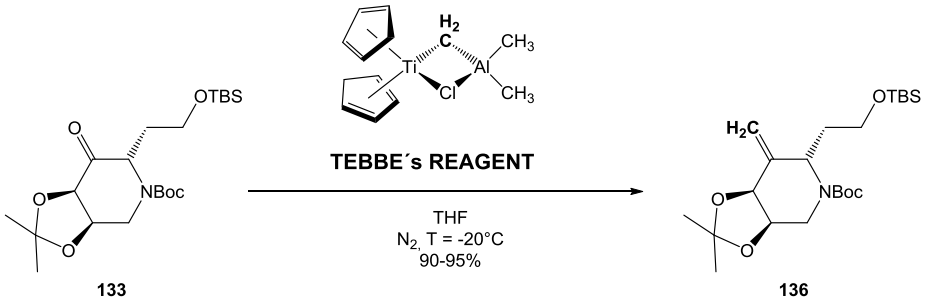
Scheme 19: Unsuccessful conversion of **133**, **134** and **134** employing a Grignard reaction.

Following the procedure of Li[165] and co-workers, (chloromethyl)trimethylsilane was treated with magnesium in dry THF (N_2, T = -20°C) to provide the corresponding Grignard reagent which was subsequently added to a suspension of anhydrous cerium (III) chloride in dry THF. It is important that the cerium (III) chloride suspension had to be stirred for 24h at ambient temperature before addition. After further stirring for 12h, ulose **133** was added dropwise, providing both isomers of β-hydroxysilane **139 a**,**b**. Elimination in dry THF employing *t*-BuOK as base afforded alkene **136** in about 20% starting from alcohol **133**. (Scheme 20)



Scheme 20: Synthesis of compound **136** employing a transmetalized Grignard reagent.

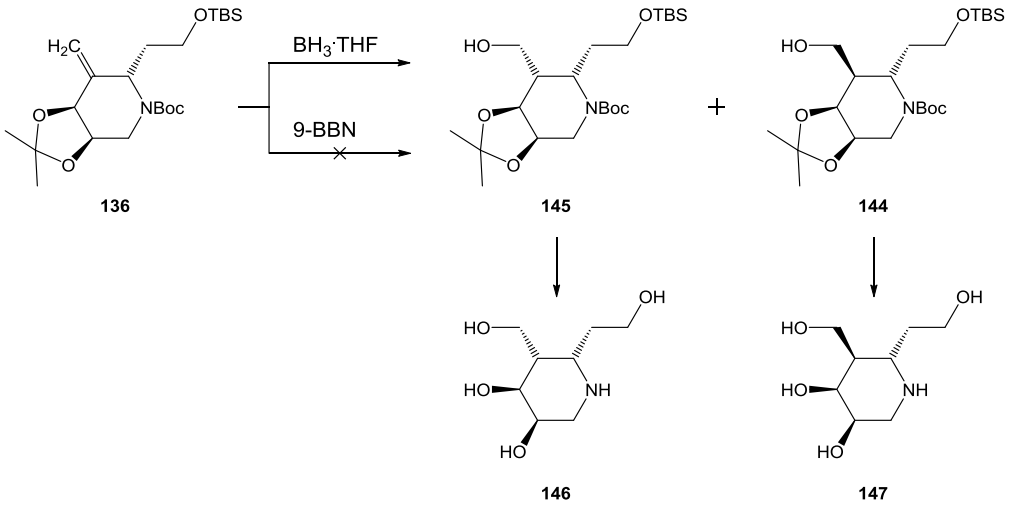
These unsatisfying results required another approach to the *exo*-methylene group. Finally, despite all other efforts made, in our hands only the Tebbe reaction[166-170] allowed convenient access to the desired crucial intermediate **136**.



Scheme 21: Synthesis of compound **136** employing Tebbe´s reagent.

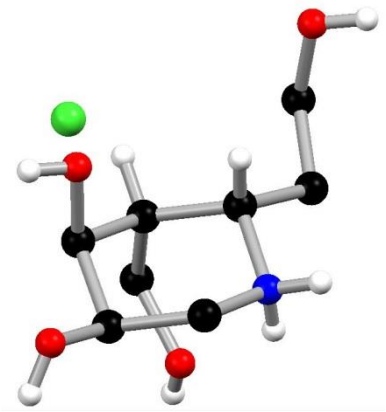
Ulose **133** was treated with Tebbe´s reagent in dry THF. The need of an external base such as pyridine, to facilitate a carbenoid behavior of the titanocene species was not observed. After 12h, completed conversion of the starting material was detected. By addition of NaOH and stirring for additional 12h, titanium salts were precipitated which could be removed by filtration. Subsequent purification afforded alkene **136** in yields up to 95%. (Scheme 21)

Hydroboration of alkene **136** employing BH_3·THF led to 4-*epi* isofagomine derivative **144** in acceptable yields. Due to steric interference of the neighboring silyloxyethyl moiety with the approach of the borane, formation of the diastereomeric side product **145** was also observed. Other attempts such as using the steric demanding 9-BBN, failed in our hands.



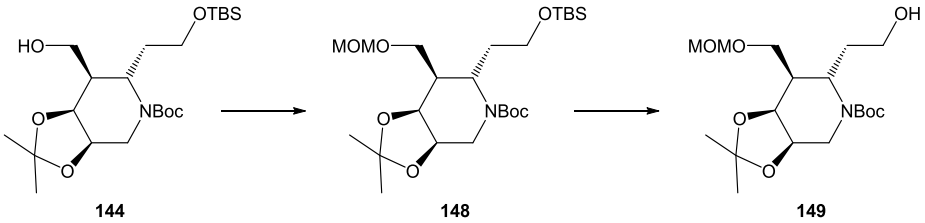
Scheme 22: Synthesis of polyols **146** and **147**.

For unambiguous structural assignment, compound **145** was deprotected to free polyol **146** which could be crystallized for X-ray crystallography (CCDC 1465280). Deprotection of **144** in methanolic HCl gave the inhibitor (5a*S*)-5a-*C*-(2-hydroxy)ethyl-4-*epi*-isofagomine (**147**). (Picture 4)

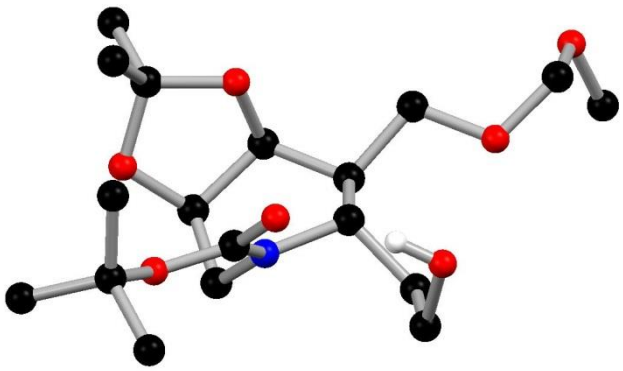


Picture 4: Crystal structure of compound **146**·HCl (CCDC 1465280).

To gain access to a variety of 4-*epi*-isofagomine derivatives in terms of functionality and chemical character of the spacer-arm at *C*-5a, alcohol **144** was prepared as a suitable branching moiety. Starting form **144,** standard O-protection (Boc_2O) gave **148**. Cleavage of the silylether employing TBAF·$3H_2O$ in THF provided alcohol **149** which nicely crystallized from EA. Renewed XRD studies revealed the identity of this key intermediate (CCDC 1465279). (Scheme 23; Picture 5)



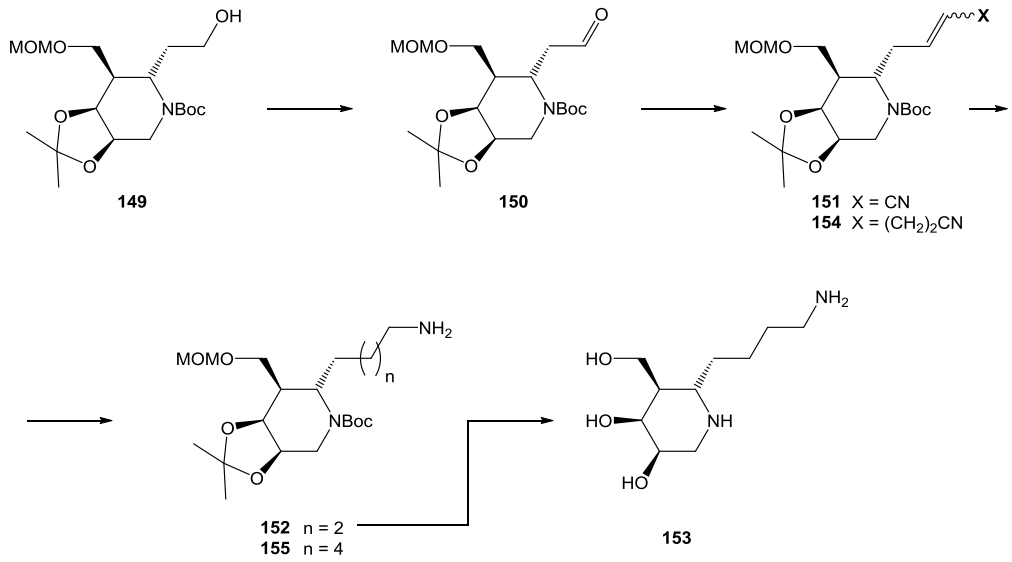
Scheme 23: Synthesis of compound **149** as starting material for *C*-5a side chain elongation.



Picture 5: Crystal structure of compound **149** (CCDC 1465279).

Due to problems vide infra (section 3.2.4.2), direct elongation of the side chain, for instance ether formation or the conversion into the corresponding amine were not investigated. However, in context with our interests in elongated spacer-arms, alcohol **149** was treated with Dess Martin periodinane in CH_2Cl_2 providing aldehyde **150** which served as starting material in C-C bond-forming reactions.

For example, a conventional Horner-Wadsworth-Emmons reaction employing diethyl cyanomethyl phosphonate and *t*-BuOK in THF gave unsaturated nitrile **151** (E/Z isomers ca. 3:1). Subsequent hydrogenolysis (H_2, Raney-Ni) at ambient pressure liberated amine **152**. Deprotection in methanolic HCl provided (5a*S*)-5a-*C*-(4-amino)butyl-4-*epi*-isofagomine (**153**). Elongated analogue **154** was prepared by a Wittig reaction with (3-cyanopropyl)triphenyl phosphorane in THF furnishing aldehyde **150**. Similarly, nitrile **154** was converted into amine **155**. (Scheme 24)

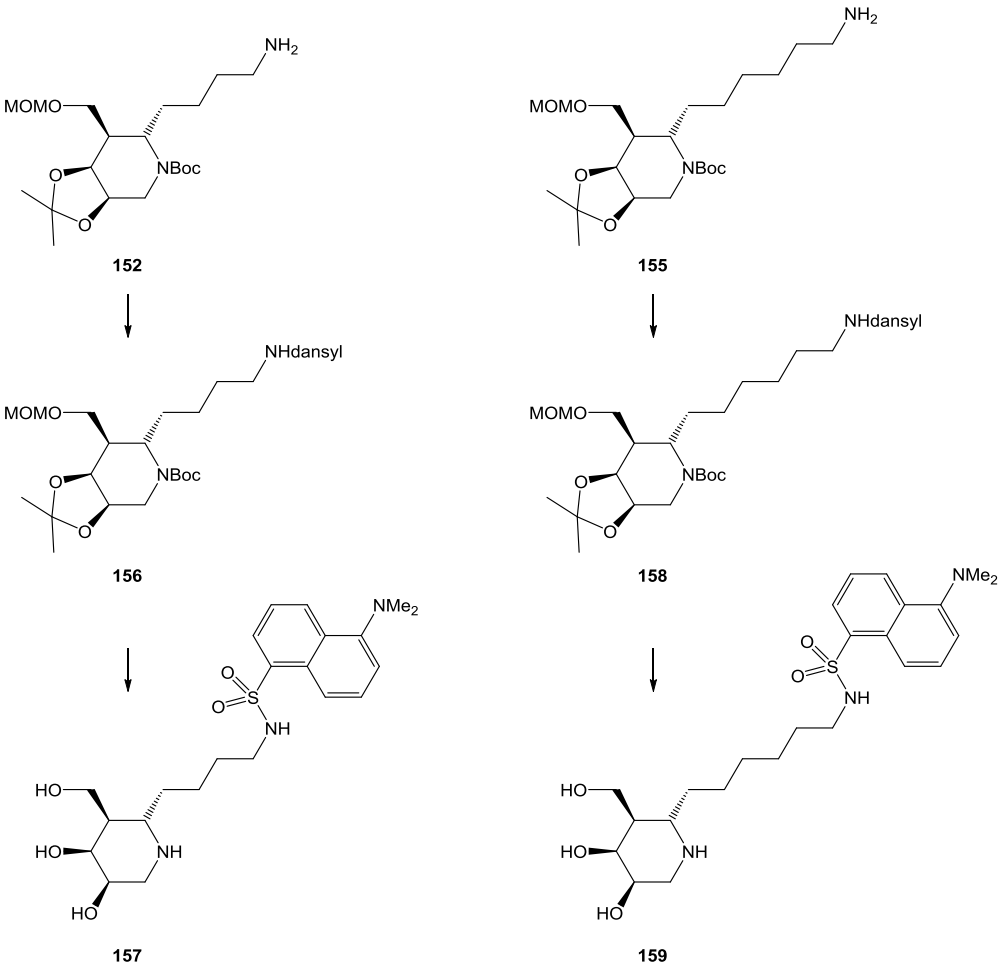


Scheme 24: Synthesis of *C*-5a-elongated 4-*epi*-isofagomines providing a terminal amine.

The incorporation of diagnostic tags to biological active compounds produces powerful tools for the investigation of biological activity. Fluorescence is an important tool for the determination of the localization or concentrations of compounds within a cell.

A range of DGJ derivatives have been previously prepared employing the dansyl moiety. These powerful inhibitors, many of which also show significant chaperone activity, serve as comparable compounds.[67-71]

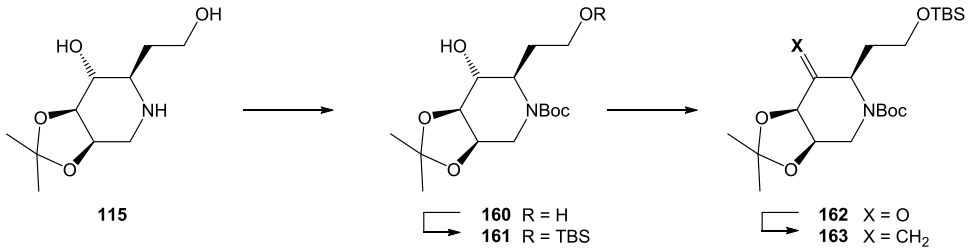
In this work, the 4-amino-butyl as well as the 6-amino-hexyl derivatives **152** and **155** were treated with dansyl chloride and Na_2CO_3 in MeOH, providing the *N*-dansyl analogues **156** and **158**. Acidic deprotection gave the fluorescent tagged inhibitors (5aR)-5a-*C*-(4-dansylamino)butyl-4-*epi*-isofagomine (**157**) and (5aR)-5a-*C*-(6-dansylamino)hexyl-4-*epi*-isofagomine (**159**). (Scheme 25)



Scheme 25: Synthesis of fluorescently tagged *C*-5a-elongated 4-*epi*-isofagomines **157** and **159**.

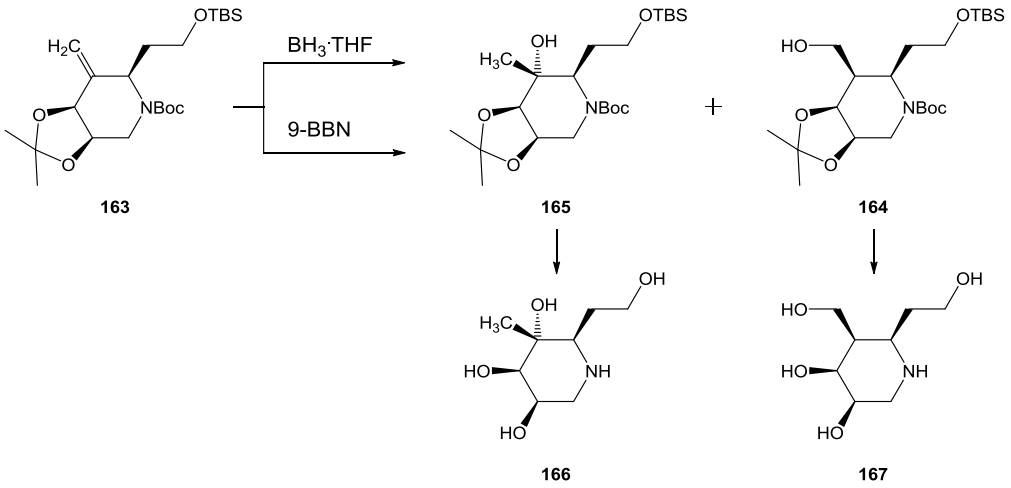
3.2.4.2 Synthesis of the (5a*R*)-Series

According to the presented synthesis of the (5a*S*)-series, this approach starts from the corresponding iminoalditol 1,5,6-trideoxy-1,5-imino-2,3-*O*-isopropylidene-D-*manno*-heptitol (**115**), prepared as shown in section 3.2.2.2. Treatment with Boc_2O in MeOH, using Et_3N as base gave **160**. Chemoselective protection of the primary alcohol employing TBSCl in DMF in the presence of imidazole provided compound **161**. Dess-Martin or Swern oxidation afforded ulose **162**, which could be easily converted into exocyclic alkene **163**. In contrast to the (5a*S*)-series, a conventional Wittig reaction with methylene triphenylphosphorane in dry THF yielded compound **163** in about 70% over 2 steps. (Scheme 26)



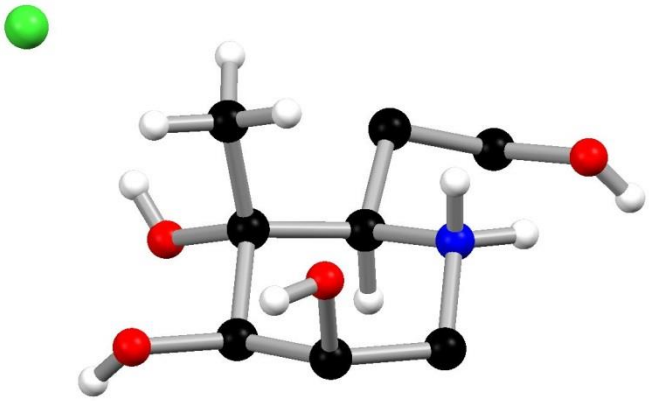
Scheme 26: Synthesis of compound **163**.

Hydrobaration of compound **163** employing BH_3·THF gave alcohol **164** in **70** % yield. Interestingly, the unexpected product of Markownikow addition **165** was also obtained in significant amounts (**5**-**10** %). Alternatively, treatment with the sterically demanding 9-BBN in THF afforded only the desired product **164** in satisfying yields. (Scheme 27)



Scheme 27: Synthesis of polyols **166** and **167**.

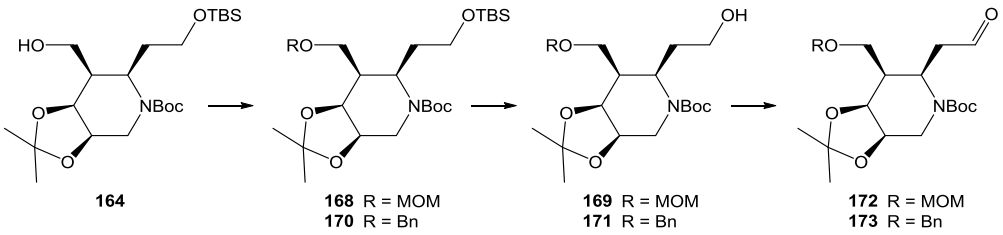
Acidic deprotecion of **165** gave polyol **166** of which a crystal structure could be obtained (Picture 6). This confirmed the attack of the borane from the less hindered *exo*-face, opposite to the isopropylidene group respectively the C-5a side-chain. Similarly, treatment of **164** with methanolic HCl provided (5a*R*)-5a-*C*-(2-hydroxyethyl)-4-*epi*-isofagomine (**167**). (Scheme 27)



Picture 6: Crystal structure of compound **166**·HCl.

For further modifications, standard O-protection (MOMCl) of **164** gave compound **168**. Subsequent cleavage of the silyl ether, employing TBAF·3H_2O in THF, provided alcohol **169** which served as starting material for further derivatizations of the side chain.

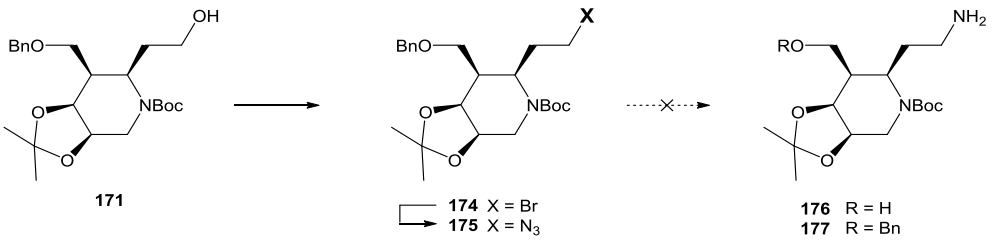
In a similar manner, compound **171** was prepared employing a conventional *O*-benzylation as intermediate step. Analogously to the (5a*S*)-series, Dess Martin oxidation furnishing alcohols **169** and **171** provided the corresponding aldehydes **172** and **173**.



Scheme 28: Synthesis of starting materials for subsequent *C*-5a side chain elongation.

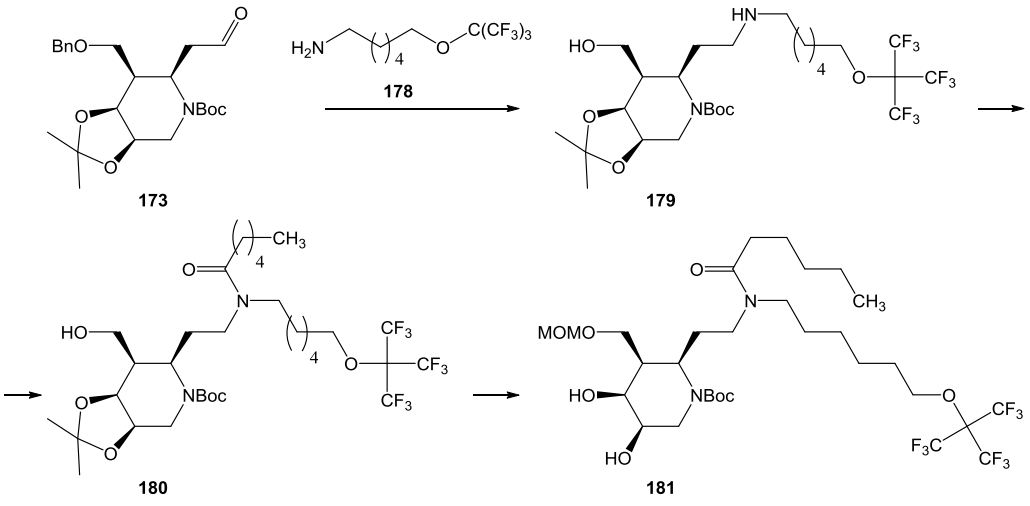
In preliminary studies, alcohol **171** was converted to bromodeoxy compound **174** using Ph_3PBr_2 in the presence of pyridine in CH_2Cl_2. Treatment with NaN_3 in DMF gave azide **175** which was supposed to be transformed into the corresponding amine. Unfortunately, this conversion was not successful, neither employing a hydrogenation reaction over Perlman´s catalyst leading to **176**, nor under Staudinger conditions forming **177**.

The reason, amongst other side reactions, might be an intramolecular nucleophilic attack of the liberated amine at the carbonyl moiety of the *N*-Boc group, resulting in the formation of a bicyclic carbamate. Due to the complex reaction mixture, this approach was not further investigated.



Scheme 29: Unsuccessful synthesis of amines **176** and **177**.

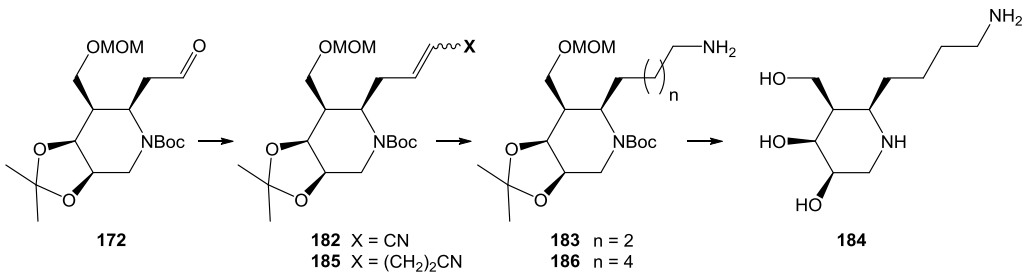
Alternatively, aldehydes **172** and **173** served as useful starting materials for chain elongation reactions. Two major strategies were investigated, starting with a conventional reductive amination furnishing **173** with fluorously tagged amine **178** under an atmosphere of hydrogen in MeOH using Pd/C (10%) as catalyst providing compound **179** in 60% yield. Interestingly, attack of the secondary amine at the *N*-Boc group was not observed. Nonetheless, amine **179** was immediately transformed into amide **180** employing hexanoic anhydride in pyridine. Due to problems during the purification procedure, the reaction mixture was directly treated with methanolic HCl providing free inhibitor **181** in reasonable yields (23% over 2 steps). (Scheme 30)



Scheme 30: Synthesis of compound **181**.

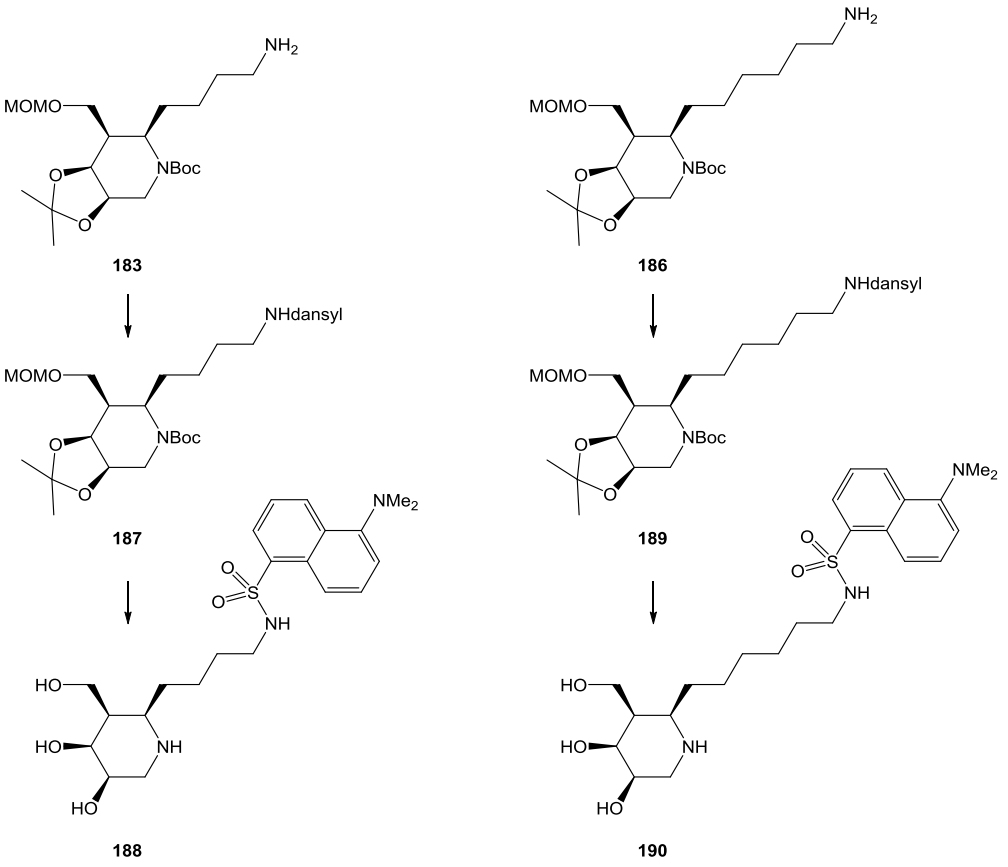
Furthermore, starting from **172**, two different amino-spacers were prepared employing the already mentioned HWE or Wittig reaction. Reaction with diethyl cyanomethyl phosphonate and *t*-BuOK in THF at 0°C provided unsaturated nitrile **182** as a mixture of *E*/*Z*-isomers.

Subsequent reduction (H_2, Raney-Ni) in MeOH liberated amine **183**, which after deprotection gave (5a*R*)-5a-*C*-(4-amino)butyl-4-*epi*-isofagomine (**184**). In a similar manner **172** was treated with triphenyl-(3-cyano)propyl phosphonium bromide and LDA in THF at -40°C providing nitrile **185**, which was converted into amine **186**. (Scheme 31)



Scheme 31: Synthesis of *C*-5a elongated 4-*epi*-isofagomines providing a terminal amine.

The respective amines serve as possible connection points for diverse functional tags. According to the mentioned strategy, compounds **183** and **186** were treated with dansyl chloride in MeOH employing Na_2CO_3 as base, providing the *N*-dansyl analogues **187** and **189**. Acidic deprotection gave (5a*R*)-5a-*C*-(4-dansylamino)butyl-4-*epi*-isofagomine (**188**) and (5a*R*)-5a-*C*-(6-dansylamino)-hexyl-4-*epi*-isofagomine (**190**), analogously to the (5a*S*)-series. (Scheme 32)



Scheme 32: Synthesis of fluorescently tagged *C*-5a-elongated 4-*epi*-IFGs **188** and **190**.

NOE-Spectroscopy of compounds **188** and **190** was performed. Both inhibitors were found to maintain $^{1,4}B$-boats in solution. Due to the large substituents in position C-5 and C-5a, this unnatural conformation is observed for the first time in in this type of compounds (Figure 30).

These results also match the XRDs of compound **116** and **166** which both show an equatorial positioned side chain next to the ring nitrogen.

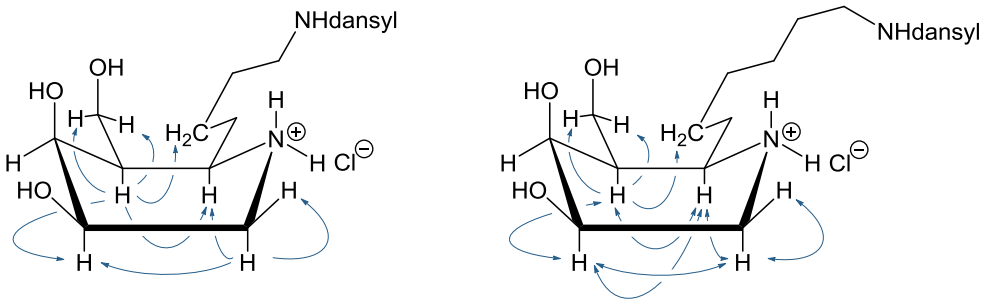
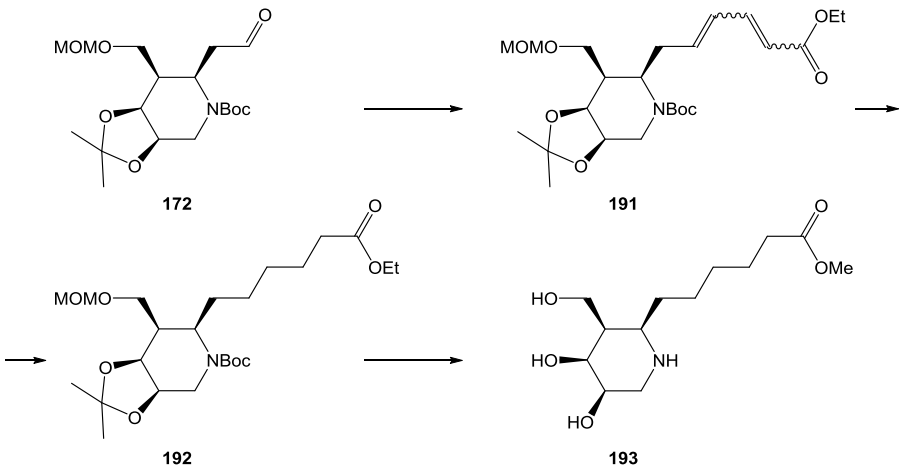


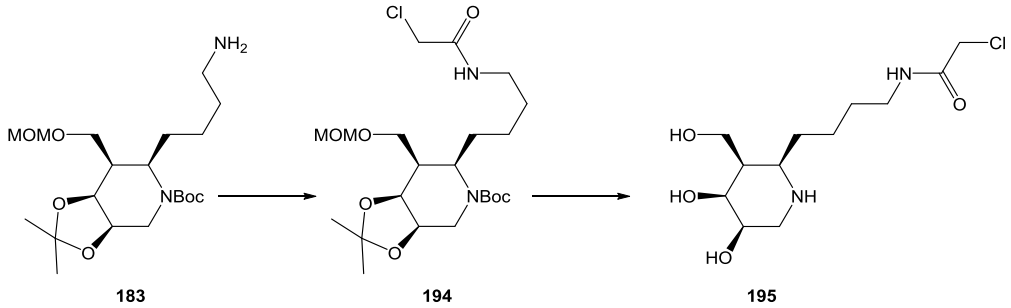
Figure 30: NOEs studies of compound **188**·HCl (left) and **190**·HCl (right).

In subsequent studies, spacer-arms containing a functional group which can be attacked by a nucleophile, for example an amino group of a respective glycosylhydrolase, were prepared. For example, aldehyde **172** was treated with triethyl 4-phosphonocrotonate in the presences of *t*-BuOK in THF, providing **191**, which was converted into saturated ester **192** employing hydrogen over Pd/C (10%) in MeOH. Removal of all protecting groups and concomitant transesterification in methanolic HCl gave methyl 6-[(5a*R*)-4-*epi*-isofagomin-5a-yl]-capronate (**193**).



Scheme 33: Synthesis of compound **193**.

Gratifyingly, ester **193** showed interesting biological activities which will be discussed in the next section. Following these results, related modifications of the side chain were investigated. To create a homologue in terms of reactivity, amine **183** was treated with chloroacetyl chloride to provide **194**. Deprotection in methanolic MeOH gave compound **195** which was unfortunately too unstable for biological evaluations. (Scheme 34)



Scheme 34: Synthesis of compound **195**.

3.2.5 Synthesis of structurally related derivatives of 4-*epi*-isofagomine

In order to demonstrate the versatility of this synthetic concept, the synthesis of additional compounds was investigated. As indicated in Figure 31, two compound classes related to the D-*galacto* series presented here are available following this approach. (Figure 31)

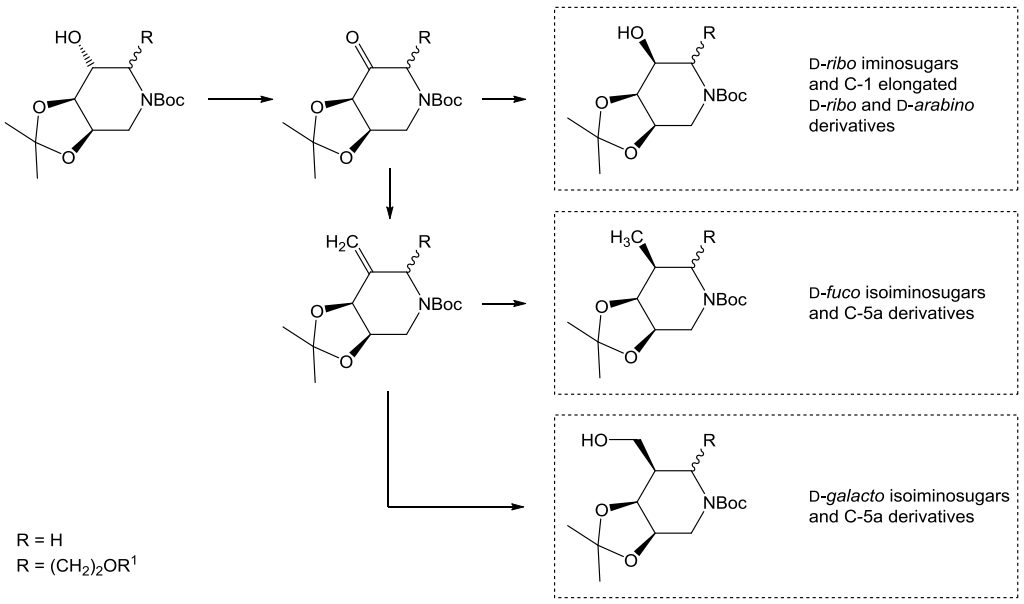
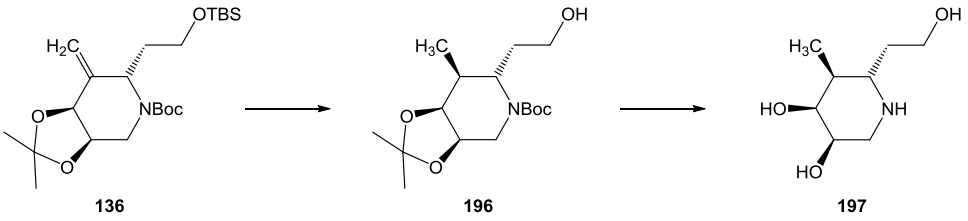


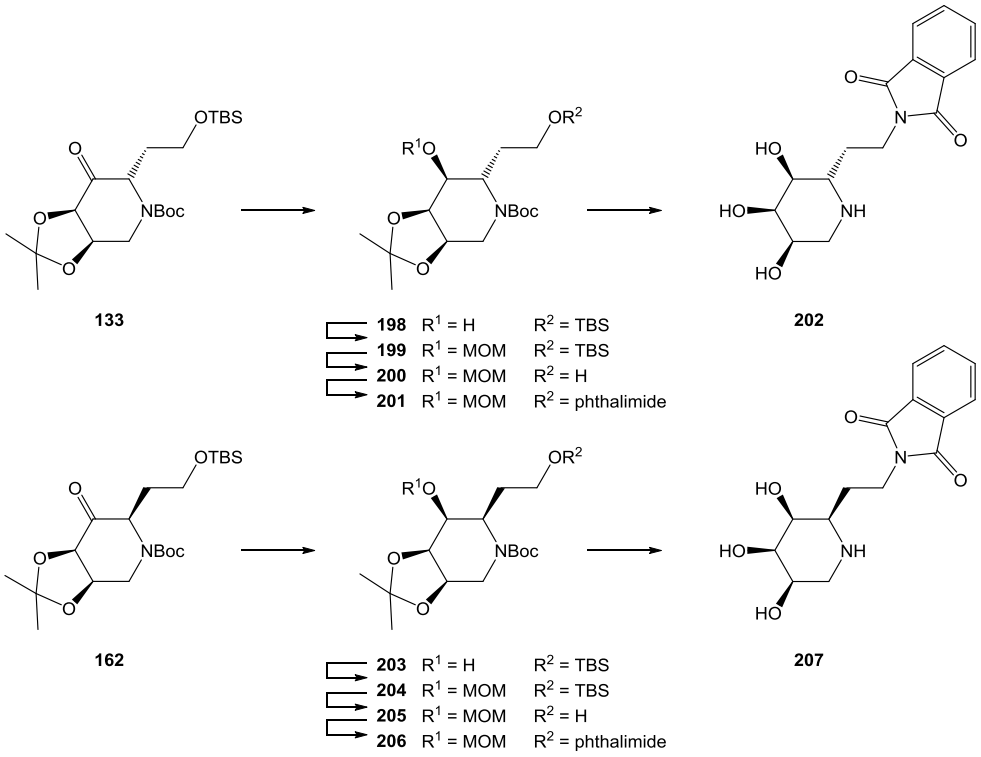
Figure 31: Possible modifications of the presented synthesis.

Following our interest in lysosomal storage diseases such as G_{M1}-gangliosidosis and Morquio B, entry into the D-*fuco* series was investigated. Catalytic hydrogenation (Perlman´s cat. in MeOH) of alkene **136** provided **196**. Particularly advantageous was the simultaneous cleavage of the silylether liberating alcohol which serves as potential precursor for further functionalization of the *C*-5a side chain. Conventional acidic deprotection in MeOH provided inhibitor **197**, the 6-deoxy-analogue to tetraol **147**. (Scheme 35)



Scheme 35: Synthesis of D-*fuco*-configurated analogue **197**.

Additionally, inversion of configuration at C-5, employing $NaBH_4$ in MeOH via uloses **133** and **162**, provided alcohols **198** and **203** respectively. Followed by an established protecting group strategy, compounds **200** as well as **205** were prepared. Conversion into phthalimido derivatives **201** and **206** was realised by a conventional Mitsunobu reaction, employing phthalimide, Ph_3P and DEAD in THF. Finally, acidic deprotection gave compounds **202** and **207**. (Scheme 36)

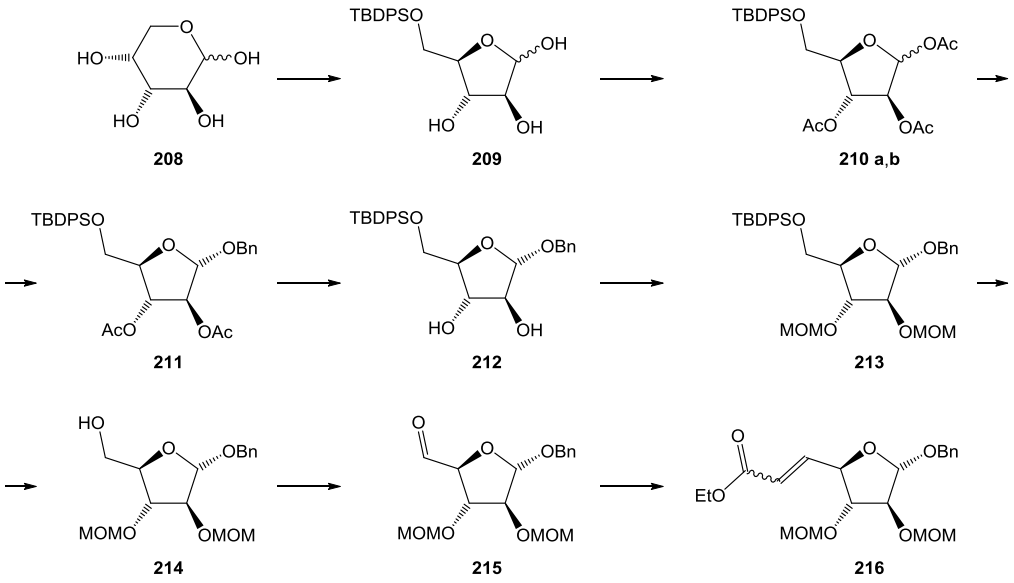


Scheme 36: Synthesis of compound **202** and **207**.

3.3 Synthesis of D-*gluco*-analogues

In order to prove the general applicability of the presented synthesis, the entry into the biological interesting D-*gluco*-series was investigated. Thus, compared to the D-*galacto*-series, the corresponding D-*gluco*-aldehyde **213** had to be prepared as starting material for the remaining synthetic sequence.

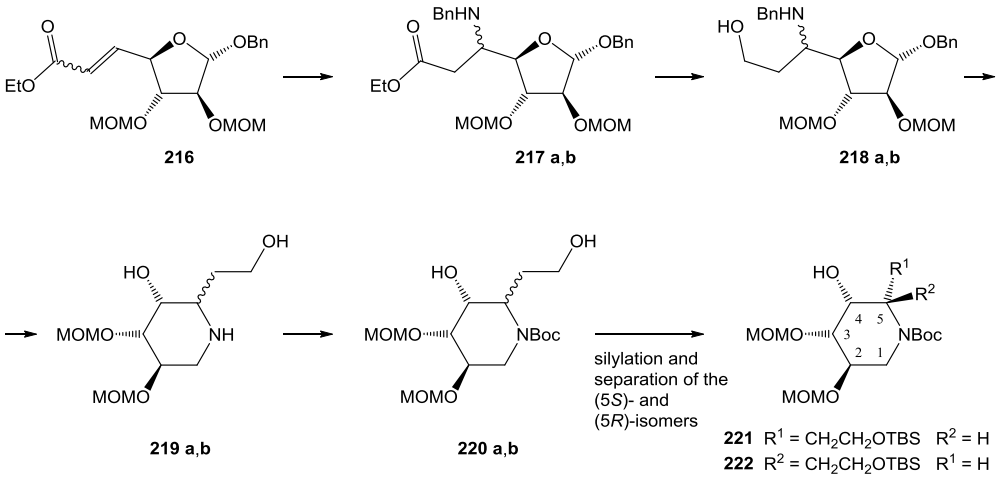
As indicated in Scheme 37, D-arabinose (**208**), treatment with TBDPSCl in DMF employing imidazole as base gave silyl ether **209**. Per-*O*-acetylation under standard conditions afforded **210**[171,172]as anomeric mixture. Transacetalisation in BnOH containing anhydrous HCl provided **211** in fair yield. Conventional ester saponification followed by O-protection employing MOMCl and DIPEA in CH_2Cl_2 gave compound **213**. Treatment of silyl ether **213** with TBAF $3H_2O$ in THF gave alcohol **214** which was immediately converted into aldehyde **215** employing Swern´s protocol. For the preparation of α,β-unsaturated ester **216** a conventional HWE reaction was investigated.



Scheme 37: Synthesis of α,β unsaturated ester **216**.

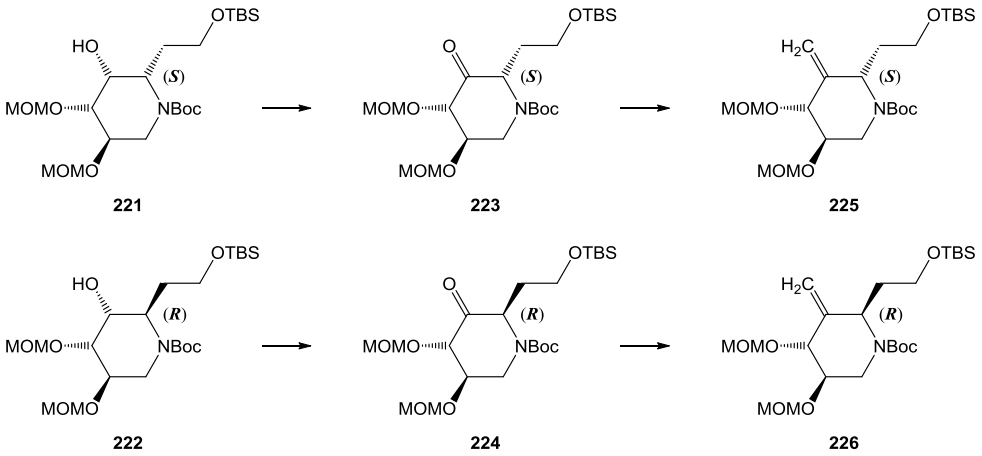
Analogously to the D-*galacto*-series, aza-Michael addition employing $BnNH_2$ in MeCN (1.1 v/v) provided amines **217 a** and **217 b** (2:1). Due to the impracticable separation of the prepared amines, investigations towards the following steps employed mixtures of the two isomers.

Reduction of the ester moieties gave the respective alcohols **218a** and **218b** which could be easily converted into iminoalditols **219a** and **219b** under hydrogenolytic conditions. Conventional N-protection (Boc_2O) and regioselective *O*-silylation provided alcohols **221** and **222** which could be separated on silica gel. Thus, each of the isomers served as starting material for either the (5*S*)- or the corresponding (5*R*)-series. (Scheme 38)



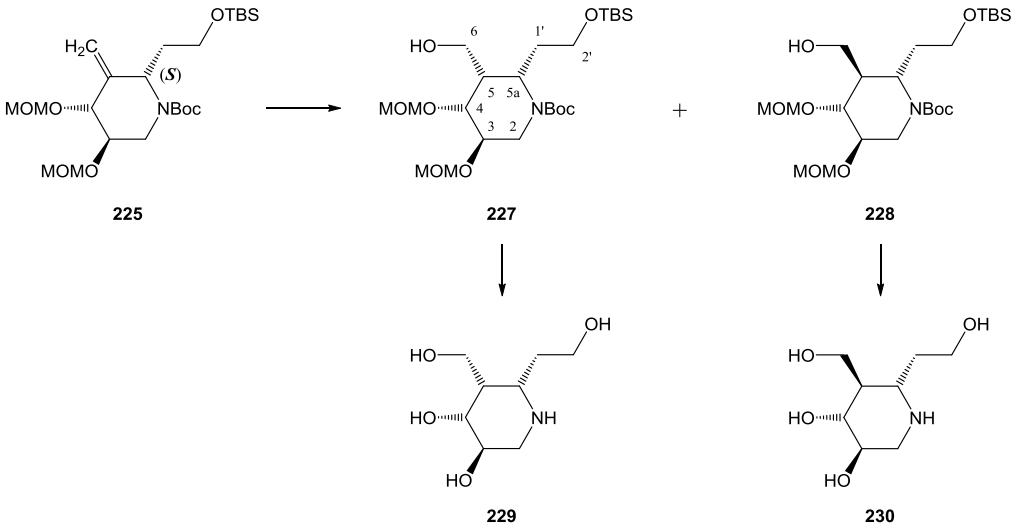
Scheme 38: Synthesis of iminoalditols **221** and **222**.

Conversion of compounds **221** and **222** employing Dess Martin periodinane in CH_2Cl_2 afforded the corresponding uloses **223** and **224**. For the introduction of the exocyclic methylene group, **223** and **224** were treated with Tebbe´s reagent in THF providing **225** and **226**. To circumvent problems comparable to the ones in the D-*galacto*-(5a*S*)-series, other conventional C-C branching reactions have not been investigated, as yet.



Scheme 39: Synthesis of compounds **225** and **226**.

Hydroboration of the alkene **225**, employing 9-BBN in THF at 0°C provided the corresponding alcohol **227** and isofagomine derivative **228** (ratio ca. 4:1). Due to a simplified purification, acidic deprotection of the resulting reaction mixture was employed, providing 5-*epi*-isofagomine derivative **229** and (5a*S*)-5a-*C*-(2-hydroxy)ethyl-isofagomine (**230**). (Scheme 40)



Scheme 40: Synthesis of compounds **229** and **230**.

The conformation of the respective starting material **225** ($^4C_1$-chair) as indicated in Figure 32 is expected due to the all equatorially positioned substituents. This serves as explanation for the favoured attack of the borane leading to the undesired L-*ido* series.

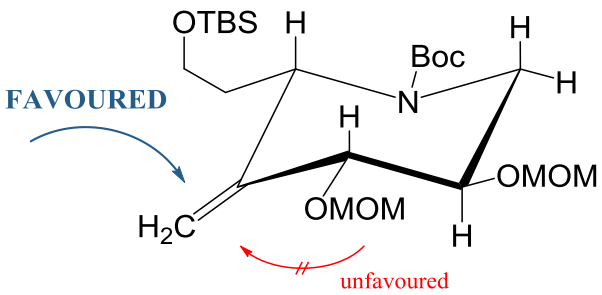


Figure 32: Possible attacks of borane at alkene **225**.

Chemical shifts in NMR-spectra of compound **230** fit to those of related compounds from literature (e.g. **21**, **22**, and **25**). This serves as rational for the indicated configuration of **230** at C-5 and C-5a. NOE spectroscopy of compound **229** confirmed the depicted configurations at the

newly introduced stereo centres (C-5 via hydroboration and C-5a via via aza.Michael addition). Furthermore, these studies suggested a $^{1,4}B$ conformation of **229**·HCl in solution. (Figure 33)

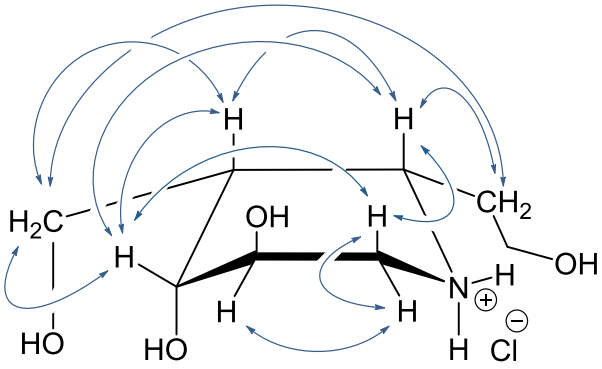
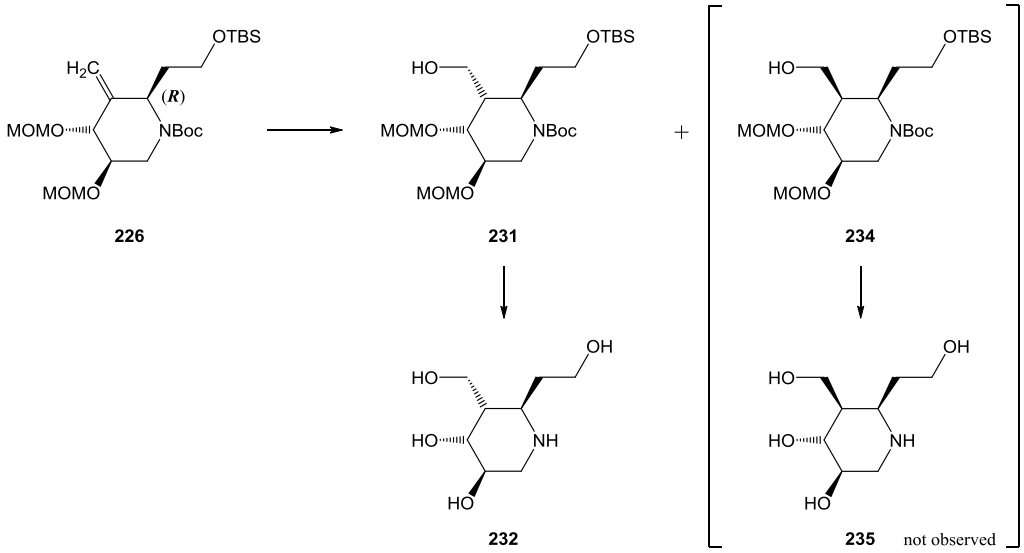


Figure 33: Relevant H-H couplings found in NOE studies of polyol **229**·HCl.

Hydroboration of (5a*R*)-isomer **226**, employing 9-BBN in THF at 0°C, followed by acidic deprotection liberated polyol **232**. Interestingly, the formation of the desired D-*gluco* derivative **235** was only observed in insignificant traces. (Scheme 41)



Scheme 41: Synthesis of compound **232**.

In conclusion, the investigated approach towards D-*galacto* configurated *C*5a-elongated isoiminosugars can be simultaneously applied in the synthesis of related D-*gluco* analogues.

4 Biological Evaluation of New Compounds

A selected set of the newly synthesised compounds presented in this thesis were evaluated concerning their activities against several glycosylhydrolases from various sources. Due to our interests in the studies and development of new active site chaperones, the most promising candidates were further investigated. Relevant tests were performed by Prof. Withers and co-workers at the UBC Vancouver and Prof. Windischhofer and co-workers at the Medical University of Graz.

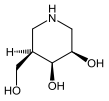
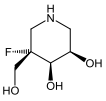
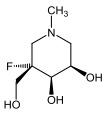
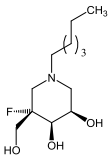
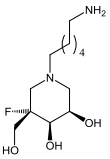
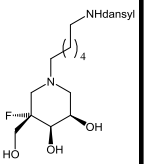
4.1 Kinetic studies with glycoside hydrolases

4.1.1 Inhibition profile of 5-fluoro derivatives of 4-*epi*-isofagomine

In Table 2, the inhibition constants of the new fluorous derivatives are collected and compared with parent compound 4-*epi*-IFG (**9**). The substances were tested for their inhibition of common glycosylhydrolases. In general, the screening showed a remarkable change in the potency and selectivity whereby the introduction of the tertiary fluorine atom improved the activity against α-galactosidases compared to non-fluorinated parent **9**. Simultaneously, a diminished inhibition of β-galactosidases was observed. This trend is clearly noticeable comparing **9** with fluoro derivative **121** and its *N*-Methyl analogue **123**. However, it is important to mention that for some reason further elaborations of the N-alkyl substituents (**125** and **127**) in some cases partially subvert the loss of potency towards β-galactosidases.

Nonetheless, the new inhibitors, due to the influence of the fluorine atom at C-5 on the chemical character of the system, provided at least a 10-fold increase in potency (from **9** to **123**) against clinically relevant Fabrazyme, which was further improved into the low micromolar range (**126** and **127**) upon elaboration of the N-alkyl substituent.

Table 2: K_i-Values of 5-Fluoro derivatives of 4-*epi*-IFG (**9**):

K_i- values [μM]		Compounds					
		4-*epi*-IFG	5-fluoro derivatives				
							
Enzyme		**9**	**121**	**123**	**124**	**126**	**127**
β-Gal	ABG	0.29	24	1700	N.I.	1825	332
β-Gal	*E.coli*	0.031	0.61	140	360	248	59.2
β-Gal	Bovine liver	10	100	3300	11.9	18.8	4.7
α-Gal	GCB	140	25	12	0.50	0.80	1.22
α-Gal	Fabrazyme (pH = 7.0)	2700	790	160	n.d.	n.d.	n.d.
α-Gal	Fabrazyme (pH = 5.5)	2800	240	90	116	96.6	31.1
β-Glc	GCase	n.d.	n.d.	n.d.	n.d.	n.d.	1228
α-Glc	*S.cerevisae*	n.d.	n.d.	n.d.	n.d.	n.d.	193

ABG = β-glucosidase/β-galactosidase from *Agrobacterium sp.*; *E. coli* = lac Z β-galactosidase from *E. coli*; GCB = α-galactosidase from green coffee beans; Fabrazyme = commercial recombinant lysosomal α-galactosidase; N.I. = no inhibition, with Ki > 2mM; n.d., not determined.

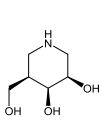
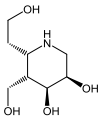
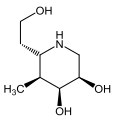
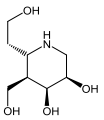
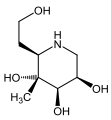
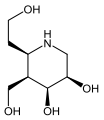
4.1.2 Inhibition profile of *C*-5a-(2-hydroxy)ethyl-4-*epi*-isofagomines

As expected, the prepared polyols did not serve as "outstanding" inhibitors of the tested enzymes. However, screening in an analogous manner to the clinically relevant D-*gluco*-configurated related iminosugar Miglitol (**11**) seemed to be essential. The measured data are summarized in Table 3.

Iminotetraols **146** and **166** were devoid of any mentionable activity. It is noteworthy that 6-deoxygenation (compound **197**) mainly reduces the activity against *E.coli* β-galactosidase whereas inhibitory power with the remaining enzymes probed was practically retained when compared with 4-*epi*-IFG (**9**). Further, both *C*-5a elongated 4-*epi*-IFGs (**147** and **167**) were found good inhibitors of β-galactosylhydrolases. In particular, an increased activity against bovine liver β-galactosidase can be observed. Simultaneously, inhibition properties against the other enzymes probed comply to parent **9**.

α-Galactosidases and glucosidases were not inhibited by any of these new compounds. Additionally, a significant improvement of the reported activities of (5a*R*)-epimer **167** compared to the corresponding (5a*S*) derivative **147** should be noted.

Table 3: K_i-Values of the prepared polyols:

K_i - values [µM]		Compounds					
		4-*epi*-IFG	(5a*S*)-series			(5a*R*)-series	
							
Enzyme		**9**	**146**	**197**	**147**	**166**	**167**
β-Gal	ABG	0.29	927	3.6	2.9	29.1	1.5
β-Gal	*E.coli*	0.031	147.5	305	0.106	22.0	0.049
β-Gal	Bovine liver	10	N.I.	6.57	4.89	20.0	4.0
α-Gal	GCB	140	n.d.	n.d.	n.d.	n.d.	n.d.
α-Gal	Fabrazyme	2800	N.I.	N.I.	N.I.	N.I.	N.I.
β-Glc	GCase	n.d.	N.I.	N.I.	N.I.	N.I.	229.5
α-Glc	*S.cerevisae*	n.d.	N.I.	N.I.	N.I.	N.I.	N.I.

ABG = β-glucosidase/β-galactosidase from *Agrobacterium sp.*; *E. coli* = lac Z β-galactosidase from *E. coli*; GCB = α-galactosidase from green coffee beans. Fabrazyme = commercial recombinant lysosomal α-galactosidase; N.I. = no inhibition, with Ki > 2mM; n.d., not determined.

4.1.3 Inhibition profile of *C*-5a derivatives of 4-*epi*-IFG

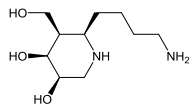
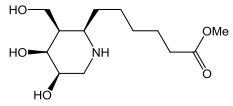
K_i-Values of (5a*R*)-configurated 4-*epi*-IFG derivatives **184** and **193** towards selected glycosidase hydrolases are listed in Table 4. Amine **184** showed no mentionable activity towards the enzymes probed. In contrast, ester **193** turned out as notable β-galactosidase inhibitor, whereas no affinity to clinically relevant Fabrazyme (α-Gal) was observed.

Despite its D-*galacto* configuration, compound **193** showed potent activity against GCase (β-glucosidase), but more interestingly, the extent of this inhibition was time-dependent, which means that the longer they incubated together, the smaller the residual activity was, indicating that there may be a bond-forming reaction going on between them. Further kinetic studies revealed compound **193** as an excellent GCase inactivator, similar to the best GCase mechanism-based inactivator reported so far. This behavior is expected due to the presence of the terminal ester moiety, which is probably susceptible of nucleophilic attack by one of the enzyme´s residues within the cleft leading to the active site.

This finding may be relevant for the development of GCase activators/inactivators that can be used as pharmacological chaperones for the treatment of Gaucher disease is an important topic in carbohydrate chemistry. Current investigations in this field indicate very promising results.

The performance of **193** indicates a great therapeutic potential. Alternatively to ester **193**, chloroacetamide derivate **195** was intended for further explorations. Unfortunately, **195** was not stable. Nevertheless, due to the reported results, other modifications in the *C*-5a side-chain in terms of length and reactive functional groups (epoxides, acrylates, etc.) leading to comparable analogues of **193** may pave the way for the discovery of new potential therapeutics.

Table 4: K_i-Values of compound **184** and **193**:

K_i - values [µM]		Enzyme					
		β-Gal	β-Gal	β-Gal	α-Gal	β-Glc	α-Glc
Compound		ABG	*E.coli*	Bovine liver	Fabrazyme	GCase	*S.cerevisae*
184		18.9	0.28	8.94	N.I.	178.9	N.I.
193		0.06	0.022	0.085	N.I.	1.91	N.I.

ABG = β-glucosidase/β-galactosidase from *Agrobacterium sp.*; *E. coli* = lac Z β-galactosidase from *E. coli*; Fabrazyme = commercial recombinant lysosomal α-galactosidase; N.I. = no inhibition, with Ki > 2mM; n.d., not determined.

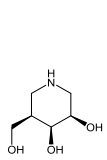
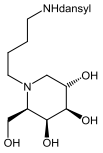
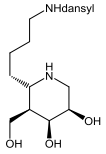
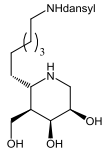
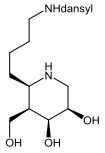
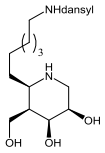
4.1.4 Inhibition profile of lipophilic, fluorescent *C*-5a derivatives of 4-*epi*-IFG

Table 5 shows the outstanding inhibition potential of the new compound class, the *C*-5a-elongated isofagomines. In order to prove their inhibition abilities relatively to the related iminosugars, compound **236** was prepared as standard following an in-house procedure[173]. In general, all of the newly prepared isoiminosugars showed a significantly increased activity against the tested β-galactosidases compared to parent compound **9** as well as iminosugar **236**. Furthermore, notable inhibition of β-glucosidases from GCase has to be mentioned. Gratifyingly, affinity to the employed α-galactosidases was not observed.

In detail, improvement of the inhibition potency depending on the length of the *C*-5a side-chain can be reported. Thus, dansylaminohexyl substituted derivate **159** and **190** showed enhanced activity to *N*-dansylaminobutyl analogues **157** and **188**. (up to 110-fold with β-galactosidase from bovine liver; **157** to **159**)

A maximization of the power of the (5a*R*)-series compared to the corresponding (5a*S*)-series has to be highlighted. On average, this modification causes a 7-fold better inhibition, whereby a 60-fold enhancement against ABG β-galactosidase (from compound **159** to **190**) was noted.

Table 5: K_i-Values of the prepared lipophilic *C*-5a derivatives of 4-*epi*-isofagomine (**9**):

K_i - values [μM]		Compounds					
		parent compounds		isoimino			
		isoimino	imino	(5a*S*)-series		(5a*R*)-series	
							
Enzyme		**9**	**236**	**157**	**159**	**188**	**190**
β-Gal	ABG	0.29	0.5	0.049	0.0175	0.020	0.0003
β-Gal	*E.coli*	0.031	2.1	0.0037	0.0021	0.0029	0.0004
β-Gal	Bovine liver	10	1.4	0.151	0.0014	0.0127	0.0005
β-Gal	C.jap.	n.d.	n.d.	n.d.	0.0025	n.d.	0.0004
α-Gal	GCB	140	n.d.	848	N.I.	N.I.	n.d.
α-Gal	Fabrazyme	2800	N.I.	N.I.	N.I.	N.I.	N.I.
β-Glc	GCase	n.d.	1.2	0.78	1.2	0.076	0.003
α-Glc	*S.cerevisae*	n.d.	N.I.	1110	N.I.	1677	N.I.

ABG = β-glucosidase/β-galactosidase from *Agrobacterium sp.*; *E. coli* = lac Z β-galactosidase from *E. coli*; C.jap. = β-galactosidase from *Cellvibrio japonicus*; GCB = α-galactosidase from green coffee beans; Fabrazyme = commercial recombinant lysosomal α-galactosidase; N.I. = no inhibition, with Ki > 2mM; n.d., not determined.

In terms of this, a 720-fold stronger inhibition of *E.coli* β-galactosidase employing isoiminosugar **188** relative to iminosugar analogue **236** is noteworthy. Furthermore, the investigated modifications of parent compound **9**, led to a 20 000-fold stronger Bovine Liver β-galactosidase inhibitor (**190**).

4.2 Evaluation with human patient´s fibroblasts

Selected substances prepared in this thesis were chosen for experiments in cell cultures with human patient fibroblasts, carrying galactosidase mutations leading to lysosomal storage diseases such as G_{M1}-gangliosidosis as well as Morquio B.

For comparison of relevant properties of the newly isoiminosugars, iminosugar **15** and **63** (the most potent chaperones introduced by our group) as well as benchmark molecule NOEV (**34**) served as standard. Measurements and appropriate evaluations were performed at the Medical University of Graz.

4.2.1 Inhibition profile with human lysosomal β-galactosidase

Prior to the chaperoning experiments, IC_{50}-values for human lysosomal β-galactosidase (wilde type) were determined in confluent fibroblasts from healthy patients. Accrued results are summarized in Figure 35. Properties of relevant compounds (**15** and **63**) as well as parent **9** and benchmark molecule **34** are listed in Figure 34.

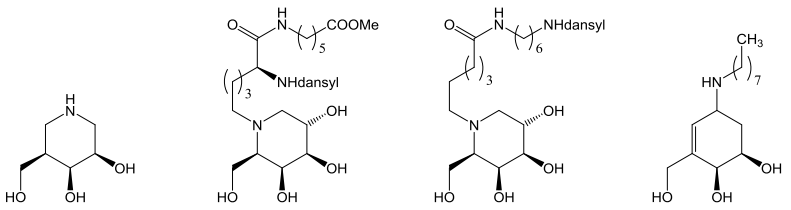


Figure 34: IC_{50}-values [μM] of comparative substances **9**[174], **15**[66], **63**[175] and **34**[82].

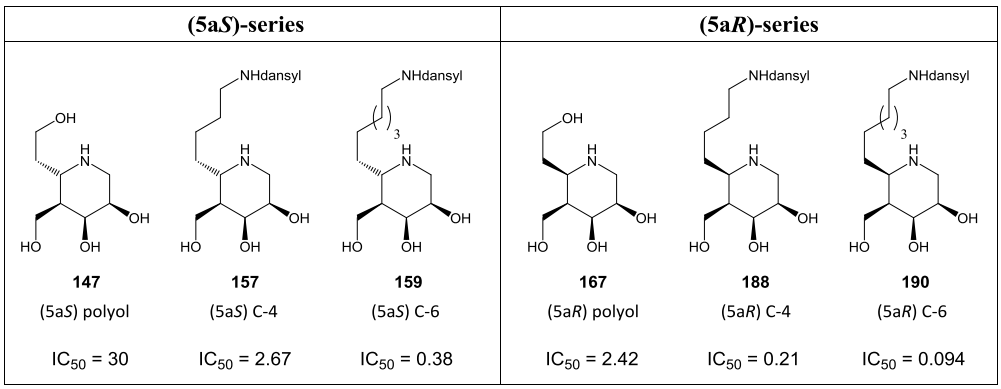


Figure 35: IC_{50}-values (μM ; human lys. β-galactosidase) of *C*-5a-elongated 4-*epi*-isofagomines.

In general, these tests proved an improvement of the activity against human β-galactosidase relative to parent 4-*epi*-IFG (**9**) (up to 1000-fold employing **190**). Even mediocre performances of aminodansylbutyl derivate **157** and polyol **167** resembled to comparative iminosugars **15** and **63**. In comparison with rivalling benchmark molecule NOEV (**34**), compounds **159**, **188** and **190** demonstrated inhibition activities in a similar order of magnitude, whereby (5a*R*)-*N*-aminodansylhexyl derivate **190** (IC_{50} = 0.094 μM) has to be highlighted as the most potent inhibitor.

In conclusion, confirming the above-mentioned trend, a significant improvement of the activities of the new compound class compared to standard compounds was clearly identifiable. Additionally, an increased potency of the (5a*R*)-series compared to the corresponding (5a*R*)-analogues and an enhanced inhibition power of longer spacer-arms was observed.

4.2.2 Chaperoning profiles towards mutant human lysosomal β-galactosidases

4.2.2.1 G_{M1}-gangliosidosis

In line with our interests in the studies of G_{M1}-gangliosidosis, the most promising candidates of above reported inhibitors were evaluated concerning their chaperoning affinities against the R201C mutant enzyme employing patients´ skin fibroblasts. The obtained profiles, compared to activities of benchmark representatives in this field, are shown in Figure 36.

Gratifyingly, all of the new inhibitors are powerful chaperones for the mutant β-galactosidase probed. All of the compounds provided a steady rise of β-galactosidase activity until the beneficial activity enhancement was gradually reduced by dose-dependent inhibition.

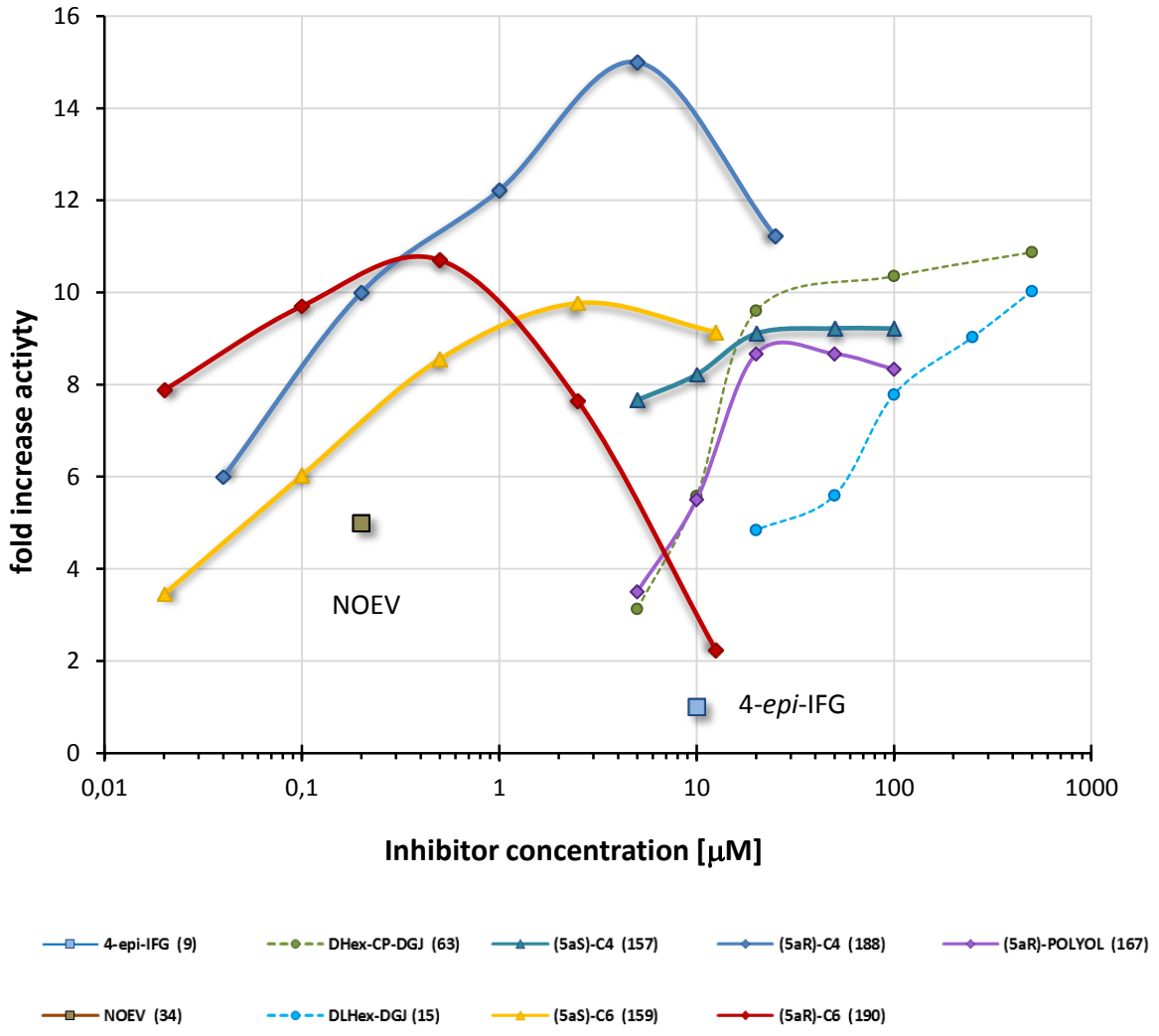


Figure 36: Chaperoning profile with human β-galactosidase (mutant R201C) of inhibitors **157**, **159**, **167**, **188**, and **190** in comparison with values of parent 4-*epi*-IFG (**9**), reference substance **15** and **63**, as well as NOEV (**34**) as activity enhancement at given concentrations.

As a striking example serves the unexpected high potency of (5a*R*)-polyol **167**, which showed practical identical effects with R201C as compound **63**. An enhancement of 9-fold β-galactosidase activity at a concentration of 20 μM was also achieved employing (5a*S*)-*N*-aminodansylbutyl derivative **157**, whereas a distinct superiority of the fluorescent compound was found at 5 μM (3.5-fold vs. 8-fold increased enzyme activity).

However, the actual potency reveal inhibitors **159**, **188**, and **190**. An obvious enhancement of β-galactosidase activity was identifiable, even at a low concentration of 0.02 μM, thus in a range of three orders of magnitude lower compared to parent **9** as well as isoiminosugars **157** and **167**. A maximum chaperone effect of 10-fold enhanced activity, similar to iminosugars **15** and **63** at 500 μM, was already achieved at concentrations of about 1μM employing **188** and **190**. Evaluation of (5aR)-*N*-aminodansylbutyl derivative **190** showed a therapeutic window spanning a concentration range of three orders of magnitude with 6-fold increase of β-galactosidase activity at a concertation as low as 0.04 μM and an impressive maximum chaperone effect of 15-fold activity enhancement at 5 μM.

4.2.2.2 Morquio B disease

Our interests in the development of chemical chaperones for the treatment of lysosomal storage disorders suggested further investigations towards other mutants of human β-galactosidase such as Morquio B-related cell line. For this purpose, (5a*S*)- and (5a*R*)-*N*-aminodansylhexl derivatives **159** and **190**, were chosen as most potent chaperones towards mutant W273L.

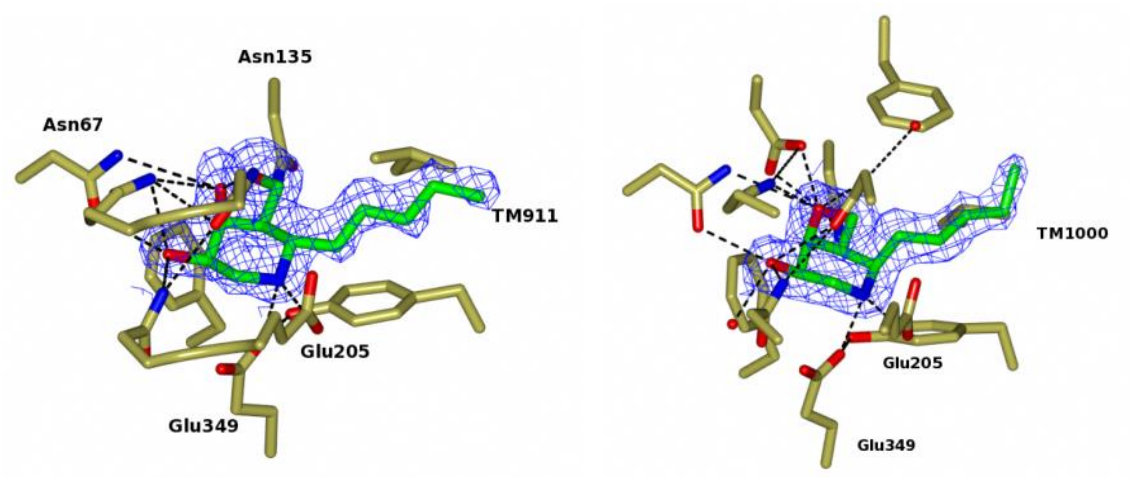
Unfortunately, none of the probed inhibitors showed a noteworthy activity against this defected human β-galactosidase. Furthermore, accumulation of a yet unidentified deposit was visually detected during the treatment of the probed fibroblasts with inhibitor **159**.

The reason behind this poor performance might be the mutation itself. According to other investigations employing W273L, the observed low activity is not surprising. Other mutant lines, for example R201H, are sensitive to the new inhibitors.

4.2.3 Extended investigations

Due to the unexpected superiority of the presented (5a*R*)-series compared to the corresponding (5a*S*)-series in terms of biological activity, further properties were investigated. In detail, the above discussed unusual $^{1,4}B$-conformation in protic solution was deemed interesting.

A co-crystal structure of the most potent, (5a*S*)-5a-*C*-(4-dansylamino)hexyl-4-*epi*-isofagomine (**159**) and (5a*R*)-5a-*C*-(4-dansylamino)hexyl-4-*epi*-isofagomine (**190**) with β-galactosidase from *Cellvibrio japonicus* was determined by Prof. Gideon Davis and his group. The resulting images are shown in Picture 7. (active site in detail)

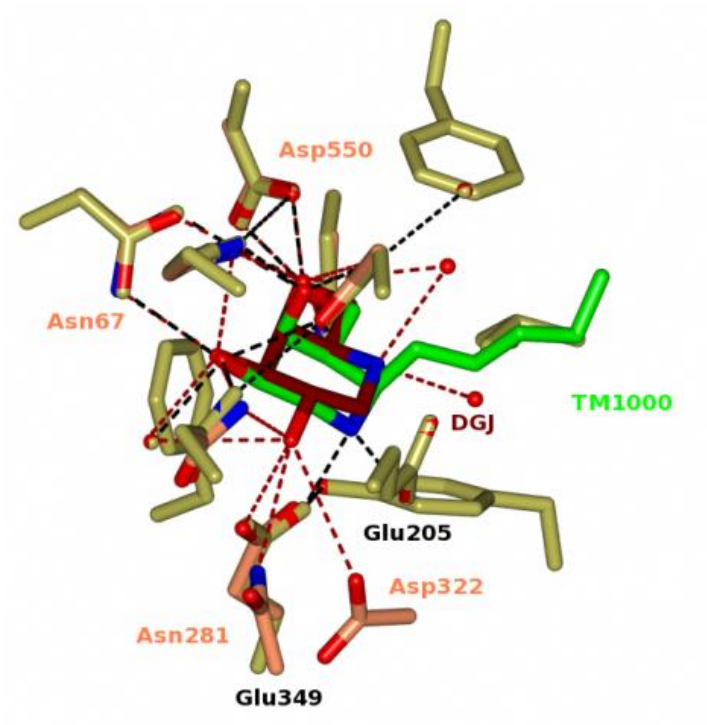


Picture 7: (5a*S*) configurated inhibitor **159** (left, TM 911) and (5a*R*) configurated inhibitor **190** (right, TM 1000) co-crystallized in β-galactosidase from *Cellvibrio japonicus*.

Inhibitor **159** was clearly found to maintain a relaxed $^4C_1$-chair as expected. Interestingly, compound **190** also exhibits this conformation. Additionally, it is noteworthy to mention that the observed interactions between the enzyme towards the spacer-arms became weaker in terms of distance to the sugar-ring. Due to this, resolution of the terminal CH_2-*N*-dansyl moiety was not possible.

These results may serve as explanation for the powerful inhibition effects of the newly prepared compound class compared to 1-deoxygalactonojirimycin (**7**). Picture 8 unambiguously showed a minimized clearance between Glu205 and Glu349 and the ring nitrogen of the newly isoiminosugar **190** compared to **7**.

According to the generally accepted enzymatic mechanism of the catalytic cleavage by hydrolases and the consequential inhibition mechanism, these reduced distances form stronger ionic bonds between the active moieties, resulting the presented outstanding performances of the *C*-5a-elongated 4-*epi*-isofagomines.



Picture 8: Stereo overlay of CjGH35A structures with DGJ (**7**) (pdb entry 4d1j) (ligand in tan, C atoms of neighbouring residues in coral, hydrogen bonds in tan) and (5a*R*) inhibitor **190** (TM1000, ligand in green, residue C atoms in gold, hydrogen bonds in black).

5 Conclusion and Outlook

The synthetic approaches developed in this thesis enable the syntheses of new families of isoiminosugars, namely *C*-5 fluorinated as well as *C*-5a elongated 4-*epi*-isofagomines, of which both were found to exhibit outstanding behaviors against glycosylhydrolases.

5.1 5-Fluoro-Series

Starting from iminoalditol **100**, introduction of an exocyclic methoxymethylene group provided enolether **118** which could be easily converted into fluoro-aldehyde **119** employing an electrophilic fluorination reaction as key-step of the presented synthetic approach. Reduction and subsequent removal of the protecting group liberated 5-fluoro-4-*epi*-isofagomine (**121**) as parent of a new family of isoiminosugars. Conventional derivatization of **121** provided a set of N-alkylated analogues.

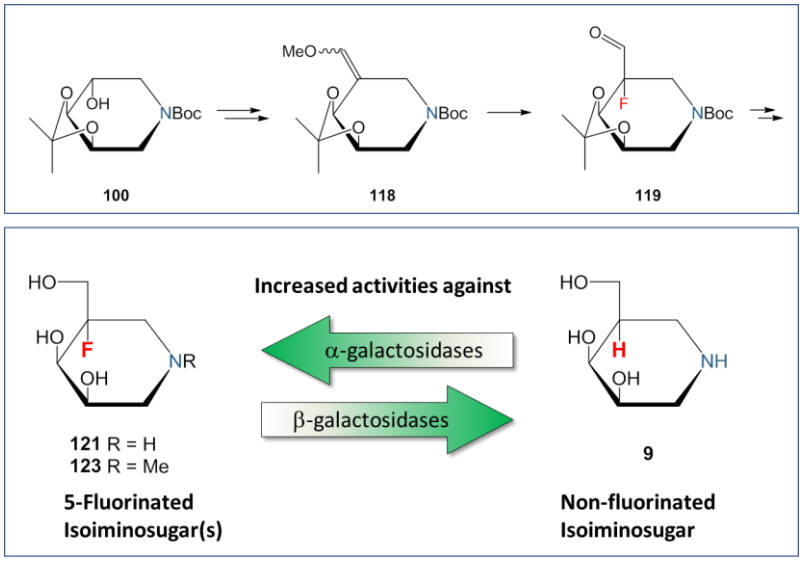


Figure 37: Summarized synthesis and biological relevant properties of 5-fluorinated 4-*epi*-IFGs.

Investigations of the new compounds for their inhibition of common glycosyl hydrolases showed a remarkable change in the potency and selectivity. In general, the introduction of the tertiary fluorine atom improved the activity against clinically relevant α-galactosidases (Fabrazyme) compared to non-fluorinated 4-*epi*-isofagomine **9**. Simultaneously a diminished inhibition of β-galactosidases was observed.

Possibly, these results help to create a new generation of more selective pharmaceutical chaperones for the treatment of lysosomal storage diseases.

5.2 *C*-5a-elongated isofagomines

For the syntheses of *C*-5a-elongated isofagomines, a novel synthetic approach had to be developed. In order to gain access to the new families, namely the (5a*S*)- and (5a*R*)- isomers of the D-*galacto*-series respectively the (5a*R*)- configurated D-*gluco*-series, as well as to the known (5a*S*)-D-*gluco* analogues, compounds **G_{gal}** and **G_{glu}** were intended as potent intermediates.

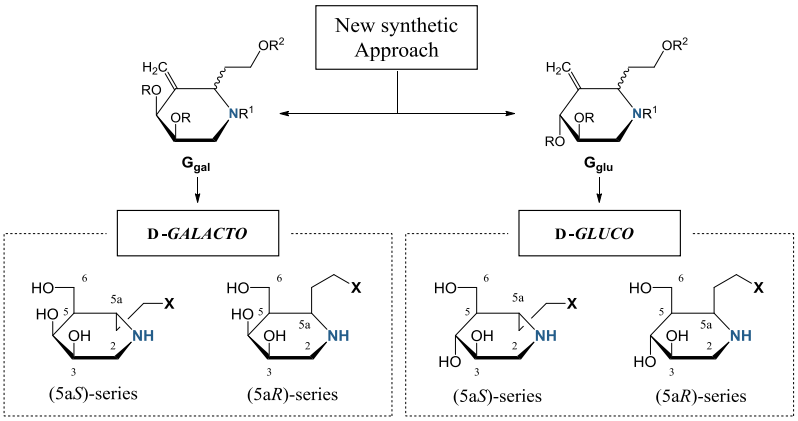
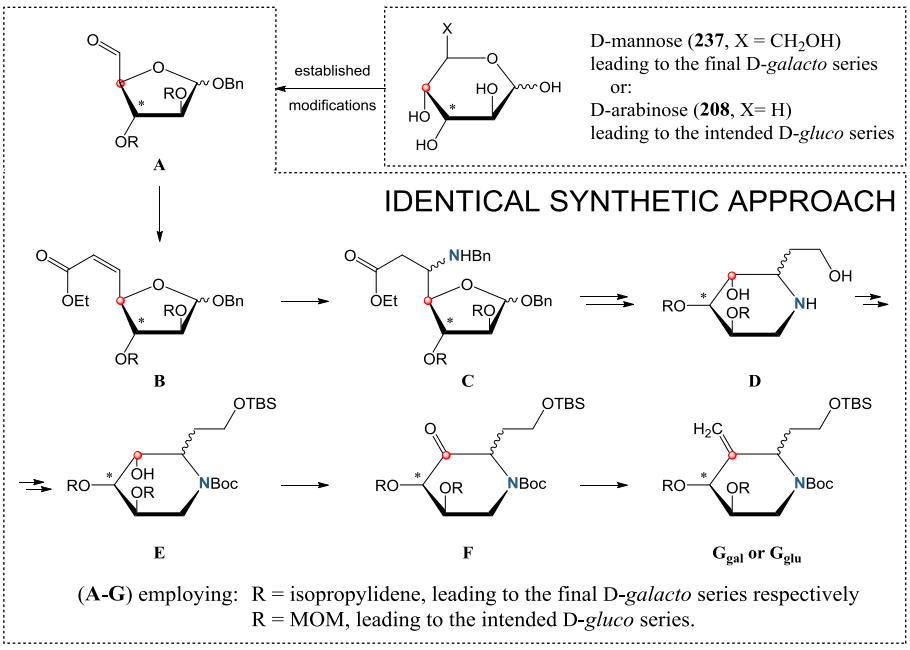


Figure 38: Fundamental structures of the families of *C*-5a-elongated isofagomines.

Starting from the respective aldehydes **A**, easy available from the simple and commercially available carbohydrates D-mannose (**237**) or D-arabinose (**208**), identical synthetic modifications provided the above-mentioned intermediates **G_{gal}** and **G_{glu}**.

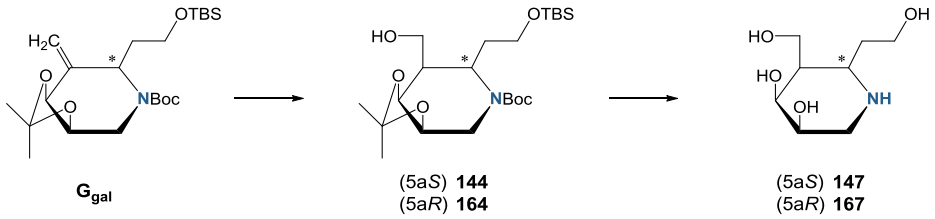


Scheme 42: : General synthetic approach towards versatile intermediates en route to *C*-5a-elongated isoiminosugars.

Elaboration of **A**, employing a Horner-Wadsworth-Emmons reaction, gave unsaturated esters **B** as powerful Michael acceptors. Treatment with $BnNH_2$ provided amines **C** which, after reduction of the ester moiety, could be easily converted into the respective iminoalditols **D** of each series. Further, an established protecting group strategy provided the associated alcohols **E** which were transformed into the corresponding uloses **F** as starting materials for C-C branching reactions. A conventional Wittig respectively Tebbe reaction gave both desired isomers of the respective iminoalditols $\mathbf{G_{gal}}$ and $\mathbf{G_{glu}}$.

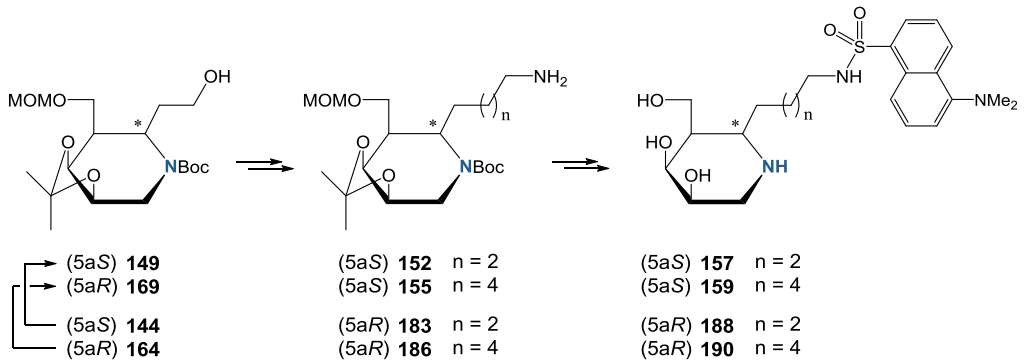
5.2.1 D-*galacto*-Series

Starting from the respective compounds $\mathbf{G_{gal}}$, conventional hydroboration gave the desired alcohols **144** and **164**. Removal of all protecting groups provided the corresponding polyols **147** and **167** as prototypes of the new family of *C*-5a-elongated 4-*epi*-isofagomines. Additionally, hydrogenolysis of **136** provided access to the related D-*fuco* series.



Scheme 43: Synthesis of compounds **147** and **167**.

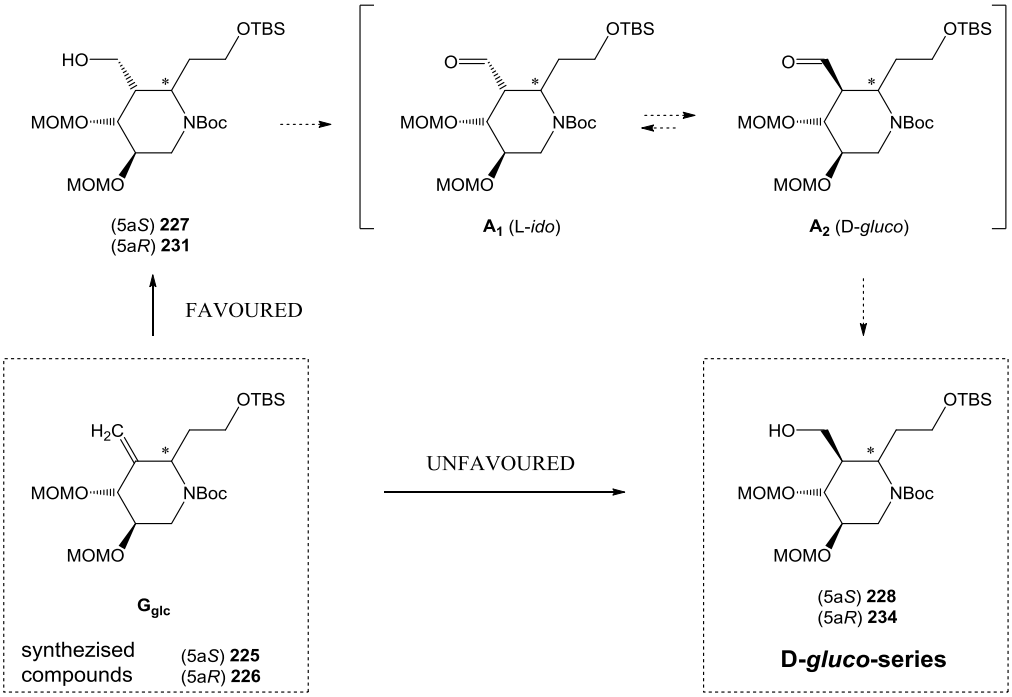
Furthermore, a simple protecting group manipulation of (5a*S*)-isomer **144** liberated alcohol **149** as potent starting material for advanced elaborations. For example, oxidation followed by established C-C branching reactions provided elongated spacer-arms bearing a terminal nitrile which could be easily converted into the corresponding amines **152** and **155**. Conventional *N*-dansylation and subsequent removal of all protecting groups furnished fluorescently tagged inhibitors **157** and **159**. Similarly, the related (5a*R*)-series was prepared.



Scheme 44: Synthesis of fluorescently tagged *C*-5a-elongated 4-*epi*-isofagomines.

5.2.2 D-*gluco*-Series

Investigations towards the D-*gluco*-series indicated the need of an additional step beyond the preparation of the hydroxymethyl group at C-5. Due to the stereo chemical outcome of the hydroboration of intermediates $\mathbf{G_{glc}}$ resulting the formation of undesired L-*ido*-series (**227** and **231**) as major products, epimerization at C-5, employing an oxidation and subsequent reduction step, is intended to give the correct D-*gluco*-series (**228** and **234**). This sequence, due to epimerisation at C-5 and in consequence new rearranged conformations of the ring-systems, enables the equatorially orientation of all substituents which is intended as driving force for these conversions. (Scheme 45 and Figure 39)



Scheme 45: Alternative synthetic modifications towards the D-*gluco*-series.

Current investigations towards the entry into the D-*gluco*-series are part of the Master thesis of Patrick Weber, BSc. The synthesis of *C*-5a-elongated derivatives of isofagomine (**9**) in the (5a*S*)- and (5a*R*)-series, similar to the presented D-*galacto*-analogues (e.g. compounds **159** and **190**), is an additional aim of the mentioned work.

Possibly, these developments help to create a new generation of inhibitors and pharmaceutical chaperones for the treatment of lysosomal storage diseases.

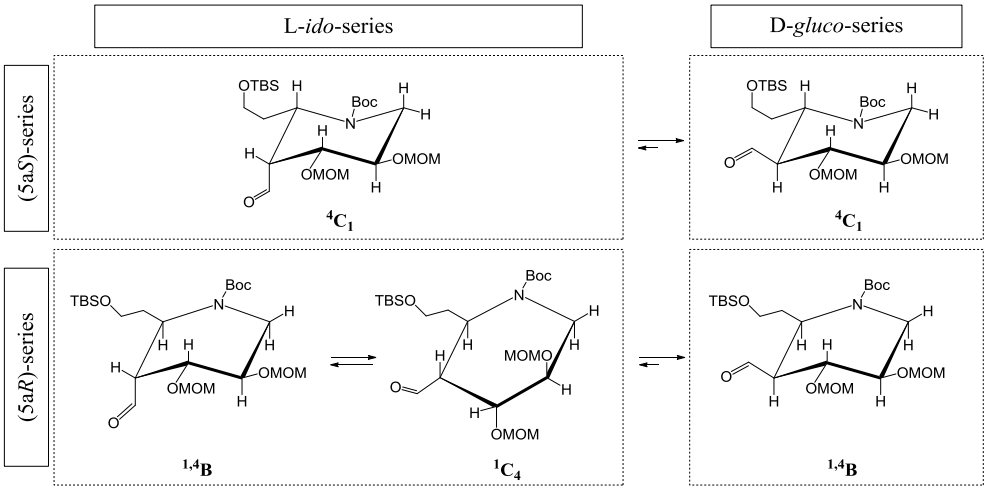


Figure 39: Intended epimerization at C-5.

5.2.3 Biological evaluation of the D-*galacto*-Series

Biological evaluations of the new families showed a notable potency as outstanding inhibitors of β-galactosidases, whereas affinity to α-galactosidases was not observed. Gratifyingly, the *C*-5a-elongated isoiminosugars were found to be superior to non-substituted parent **9**, competing iminosugars (e.g. **236**) as well as benchmark molecule NOEV (**34**).

In detail, an expected dependency of activities on the length of the *C*-5a side-chain can be reported. Thus, extended spacer-arms ensure a stronger inhibition of β-galactosidases. The validity of this trend was supported by the introduction of the respective dansylaminobutyl- and dansylaminohexyl-derivatives. Effects of longer spacer-arms remain to be investigated. Furthermore, the unexpected improved activities of the (5a*R*)-series compared to the corresponding (5a*S*)-series have to be mentioned.

Additionally, investigations of the new families indicated a remarkable potency as pharmacological chaperones for the treatment of G_{M1}-gangliosidosis (R201C). For example, compounds **159**, **188** and **190** provided a rise of the β-galactosidase activity (up to 15-fold) at low concentrations ranging between 0.02 – 10 μM.

In summary, the investigated modifications of parent **9** led to noteworthy biological activities. An optimized composition of the spacer-arm (compound **190**) provided the best inhibitor of β-galactosidases (K_i = 0.3 nM, ABG; K_i = 0.5 nM, bovine liv.; K_i = 0.4 nM, *E.coli*) I known thus far.

6 Experimental

6.1 General Methods

Analytical TLC was performed on precoated aluminum plates silica gel 60 F_{254} (E. Merck 5554). Respective compounds were detected with UV light (254 nm). For staining one of the below-mentioned solutions was employed followed by heating with a heat gun.

VAN: Vanillin/sulfuric acid: vanillin (9 g) in H_2O (950 mL), EtOH (750 mL) and H_2SO_4 (120 mL).

CAM: Ceric ammonium molybdate: ammonium heptamolybdate tetrahydrate (100g) in 10% H_2SO_4 (1000 mL) and ceric sulfate (8 g) in 10% H_2SO_4 (80 mL).

NIP: Ninhydrin/pyridine: ninhydrin (2.5 g) in pyridine (50 mL) and MeOH (950mL).

Flash chromatography was performed with the indicated solvent systems on silica gel 60 (230-400 mesh, E. Merck 9385) or silica gel 60 (Acros Organics, AC 24036).

Optical rotations were measured at 20° C on a Perkin Elmer 341 polarimeter at a wave length of 589 nm and a path length of 10 cm. MALDI-TOF Mass Spectrometry was performed on a Micromass TofSpec 2E Time-of-Flight Mass Spectrometer.

NMR spectra were recorded on a Varian INOVA 500 operating at 599.82 MHz (^{1}H), and at 125.894 MHz (^{13}C) or on a Bruker Ultrashield spectrometer at 300.36 and 75.53 MHz, respectively. $CDCl_3$ was employed for protected compounds and methanol-d_4 or D_2O for unprotected inhibitors. Chemical shifts are listed in delta employing residual, non-deuterated solvent as the internal standard. Structures of crucial intermediates have been unambiguously assigned by APT, COSY, HSQC and NOE spectroscopy.

NMR-Abbreviations:

s	singlet	dd	double doublet
d	doublet	ddd	double double doublet
t	triplet	m	multibelt
bm	broad multiblet	bs	broad singlet

6.2 General Procedures:

Reactions were performed at ambient temperature and ambient pressure. Otherwise, conditions are explicitly specified. Reaction monitoring was performed by TLC employing the indicated solvent systems. Due to two stable rotameric populations of the *N*-Boc group, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution of the ^{1}H-NMR and ^{13}C-NMR spectra. Signals of *N*-Boc protected compounds may be retarded or show up as pairs.

6.2.1 General Procedure A: (LAH Reduction)

To a 10% suspension of LAH (1.2-2.0 equiv) in dry THF, a 3% solution of the respective ester in dry THF was added dropwise at 0°C. After completed conversion of the starting material (5-15 min), the reaction was quenched with H_2O. By addition of NaOH (3 N) and stirring for further 60 min aluminium salts were precipitated which could be removed by filtration. The filtrate was dried over Na_2SO_4 and concentrated under reduced pressure.

6.2.2 General Procedure B: (Hydrogenolysis over Pearlman´s catalyst)

A 10% solution of the starting material in MeOH was stirred with $Pd(OH)_2/C$ under an atmosphere of H_2 at ambient pressure for 18h. After completed conversion of the starting material (8-12h), the catalyst was filtered off and the solvent was removed under reduced pressure.

6.2.3 General Procedure C: (*N*-Boc Protection)

To a 5% solution of the respective amine in MeOH, Et_3N (3-4 equiv) and Boc_2O (1.1-1.5 equiv) were added. After completed conversion of the starting material, the reaction mixture was concentrated and the remaining residue was diluted with CH_2Cl_2 and consecutively washed with HCl (6 %) and saturated aq $NaHCO_3$. The combined organic layers were dried (Na_2SO_4), filtered and evaporated to dryness.

6.2.4 General Procedure D: (*O*-TBS Protection)

To a 20% solution of the selected alcohol in dry DMF, imidazole (2.5-3.0 equiv) and TBSCl (1.1-1.2 equiv) were added. After completed conversion of the starting material, the reaction was quenched with MeOH and the solvents were removed under reduced pressure. The remaining residue was diluted with CH_2Cl_2 and consecutively washed with HCl (6 %) and saturated $NaHCO_3$. The organic layers were dried (Na_2SO_4), filtered off and concentrated.

6.2.5 General Procedure E: (Dess-Martin Oxidation)

To a 10% solution of the respective alcohol in CH_2Cl_2, Dess-Martin periodinane (1.1-1.3 equiv) was added. After completed conversion of the starting material, the reaction mixture was carefully quenched with saturated $NaHCO_3$. The organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure.

6.2.6 General Procedure F: (Swern Oxidation)

To a solution of DMSO (3.5 equiv, 15% in CH_2Cl_2), oxalyl chloride (3.3 equiv) was added dropwise at -78°C. After stirring for 30 min, the respective alcohol (dissolved in CH_2Cl_2) was added dropwise and the reaction mixture was stirred for 20 min. Addition of Et_3N (5.5-7.0 equiv) and stirring for additionally 20-30 min completed the conversion of the reactants. The reaction mixture was consecutively washed with HCl (6 %) and saturated $NaHCO_3$. Removal of the dried (Na_2SO_4) solvents under reduced pressure gave the crude product.

6.2.7 General Procedure G: (Tebbe Reaction)

To a 10% solution of the respective ulose in dry THF, Tebbe´s reagent (1.1-1.5 equiv) was added under an atmosphere of nitrogen at -25 °C. After 120 min at -20 °C, the reaction was stirred at ambient temp for another 12 h. The reaction mixture was diluted with Et_2O, and NaOH (1.5-1.9 equiv, 1.5 *N*) was added dropwise. After vigorous stirring for further 12 h, the mixture was filtered. The filtrate was washed with water and the organic layer was dried (Na_2SO_4). Removal of the solvents under reduced pressure provided the crude product.

6.2.8 General Procedure H: (Wittig Reaction)

A solution of freshly prepared LDA [by dropwise addition of 2.5 M *n*-BuLi (3.3 equiv) in hexane to diisopropyl amine (3.4 equiv, 20% solution in dry THF)] in dry THF was added dropwise at -78°C under an atmosphere of nitrogen to a suspension of respepective phosphomium halide (3.5 equiv). After stirring at -40°C for 60 min, a solution of the starting material (3% in dry THF) was added dropwise. After having been stirred for 12 h, the reaction mixture was diluted with CH_2Cl_2 and consecutively washed with HCl (6 %) and saturated $NaHCO_3$. After drying over Na_2SO_4, the filtrate was concentrated under reduced pressure to provide the crude product.

6.2.9 General Procedure I: (Hydrobaration)

Variant 1:

To a 10% solution of the respective alkene in dry THF, BH_3·THF (1 M in THF, 3-5 equiv) was added dropwise at 0°C under an atmosphere of nitrogen. After stirring for 12 h, the reaction was allowed to reach ambient temperature. To the ice cooled reaction mixture, H_2O, NaOH (3 N) and H_2O_2 (33 %,) were added. After additional stirring for 8h, the organic layer was separated and dried (Na_2SO_4), filtered and the solvent was removed under reduced pressure.

Variant 2:

Alternatively, the respective starting material was treated with 9-BBN (1M in THF) instead of BH_3·THF. The remaining procedure is identical with variant 1.

6.2.10 General Procedure J: (*O*-MOM Protection)

To a 10% solution of the respective alcohol and DIPEA (2.5-4.0 equiv) in CH_2Cl_2, MOMCl (1.5-2.0 equiv) was added dropwise. After completed conversion, the reaction mixture was consecutively washed with HCl (6 %) and saturated $NaHCO_3$. After drying over Na_2SO_4, the filtrate was concentrated under reduced pressure to provide the crude product.

6.2.11 General Procedure K: (*O*-TBS Deprotection)

The *O*-silylated material and TBAF·$3H_2O$ (0.3-1.3 equiv) were stirred in THF/H_2O (100:1 v/v). After completed cleavage of the protecting group, the solvents were removed under reduced pressure to give the free alcohol.

6.2.12 General Procedure L: (Horner-Wadsworth-Emmons Reaction)

To a suspension of *t*-BuOK (1.5 equiv) in dry THF, the respective phosphonate (1.8-2 equiv) was added dropwise at 0°C. The mixture was stirred for 10 min before allowing the reaction to warm up to ambient temperature. After additional 40 min, a 3-5% solution of the respective starting material in dry THF was added dropwise. The reaction mixture was stirred until completed conversion of the starting material was detected. The reaction was extracted with HCl (6 %) and consecutively washed with saturated $NaHCO_3$. After drying over Na_2SO_4, the filtrate was concentrated under reduced pressure providing the crude *E*/*Z*-isomers of the corresponding product.

6.2.13 General Procedure M: (Hydrogenolysis over Raney-Ni)

A 10% solution of the respective cyanide in MeOH was stirred with Raney-Ni (moist in H_2O) under an atmosphere of H_2 at ambient pressure for 30-60 min. After completed conversion of the starting material, the catalyst was filtered off. Removal of the solvent under reduced pressure afforded the crude amine.

6.2.14 General Procedure N: (N-Alkylation)

A 5% solution of the respective amine in dry DMF was stirred with Na_2CO_3 (2.5-3.0 equiv) and the selected halocarbon (1.1 equiv) at 20 - 50°C. After completed conversion of the starting material, the solvent was removed under reduced pressure to give the crude product.

6.2.15 General Procedure O: (*N*-Dansylation)

To a suspension of the respective amine and Na_2CO_3 (2.5-3.0 equiv) in MeOH, dansyl chloride (1.2 equiv) was added. After completed conversion of the starting material, the reaction mixture was evaporated to dryness.

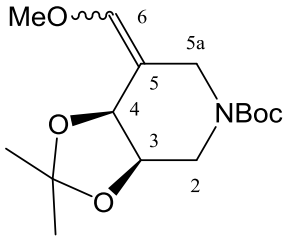
6.2.16 General Procedure P: (Removal of all Acid labile Protecting Groups)

The respective starting material was dissolved in MeOH and HCl (12 *N*) was added dropwise to adjust pH 1. After completed removal of all protecting groups, the solvents were removed under reduced pressure.

6.3 5-Fluoro derivatives of 4-*epi*-isofagomine

N-tert-Butyloxycarbonyl-1,5-dideoxy-1,5-imino-3,4-O-isopropylidene-2-C-(methoxy)methylene-D-erythro-pentitol (118)

A solution of 2.5 M *n*-BuLi (1.16 mL, 2.89 mmol) in hexane was added dropwise to a cooled suspension of (methoxymethyl)triphenylphosphonium chloride (1.05 g, 3.07 mmol) in dry THF (40 mL) under an atmosphere of nitrogen at -78°C. After stirring for 40 min at -20°C a solution of *N-tert*-butyloxycarbonyl-1,5-dideoxy-1,5-imino-3,4-*O*-isopropylidene-D-*erythro*-pent-2-ulose[50] (**117**, 238 mg, 0.88 mmol) in dry THF (5 mL) was added dropwise, and the mixture was stirred for 12 h and allowed to warm up to ambient temperature. The reaction was diluted with CH_2Cl_2 and successively washed with HCl (6 %) and saturated $NaHCO_3$. The combined organic layers where dried over Na_2SO_4, filtered off and evaporated to dryness. The remaining residue was quickly passed through silica gel (C/EA 8:1 v/v) to give highly unstable compound **118** (151 mg, 0.50 mmol, 57.5%, *E/Z* ca. 1:1) as a pale yellow syrup which had to be immediately employed for the next step without further purification.



$C_{15}H_{25}NO_5$

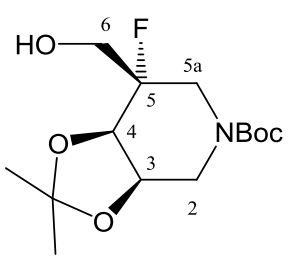
MW: 299.37 g/mol

TLC: UV, CAM

R_f: 0.6 and 0.7 (C/EA 1:1 v/v)

N-tert-Butyloxycarbonyl-5-fluoro-3,4-O-isopropylidene-4-epi-isofagomine (120)

To a solution of compound **118** (151 mg, 0.50 mmol) in MeCN/H_2O (6 mL, 5:1 v/v), Selectfluor® (190 mg, 0.56 mmol) was added. After completed conversion of the starting material the reaction was neutralized with $NaHCO_3$. The solvent was removed under reduced pressure and the remaining residue was diluted with THF (15 mL). The resulting suspension was sonicated and $NaBH_4$ (95 mg, 2.5 mmol) was added carefully. After 4 h, the reaction mixture was diluted with CH_2Cl_2 and successively washed with HCl (6 %) and saturated $NaHCO_3$. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified on silica gel (C/EA 6:1 v/v) to provide compound **120** (86 mg, 0.28 mmol, 55.8%) as colourless syrup.



$C_{14}H_{24}FNO_5$

MW: 305.35 g/mol

TLC: CAM

R_f: 0.5 (C/EA 1:1 v/v)

$[\alpha]_D^{20}$ = +14.2 (c 5.08, $CHCl_3$)

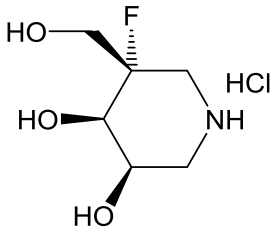
^{1}H NMR (300 MHz, $CDCl_3$) δ = 4.38 (m, 1H, H-3), 4.25 (m, 1H, H-4), 3.90-3.50 (m, 4H, H-2, H-5a, H-6, H-6´), 3.50-3.20 (m, 2H, H-2´, H-5a´), 2.63 (bs, 1H, OH), 1.39 (s, 9H, Boc), 1.37, 1.28 (2s, 3H each, isopropylidene). Due to two pronounced rotameric populations (14´, 14´´) of the *N*-Boc group, signal splitting in the respective NMR spectra has been observed.

^{13}C NMR (75.5 MHz, $CDCl_3$): **14´:** δ = 155.6 (Boc), 109.9 (isopropylidene), 94.3 (d, 173.04 Hz, C-5), 77.4 (Boc), 72.3 (C-3), 71.5 (d, 36.8 Hz, C-4), 64.3 (d, 21.7 Hz, C-6), 42.9 (d, 20.4 Hz, C-5a), 42.6 (C-2), 28.5 (Boc), 26.7, 24.5 (isopropylidene). **14´´:** δ = 155.6 (Boc), 109.9 (isopropylidene), 94.0 (d, 171.9 Hz, C-5), 80.2 (Boc), 72.2 (C-3), 71.7 (d, 36.9 Hz, C-4), 64.6 (d, 21.7 Hz, C-6), 43.8 (d, 19.3 Hz, C-5a), 41.5 (C-2), 28.5 (Boc), 26.9, 24.6 (isopropylidene).

MS: Calcd for [$C_{14}H_{24}FNO_5$]: *m/z* 305.1638 $[MI]^+$; Found $[MI]^+$ 305.1633.

5-Fluoro-4-epi-isofagomine (121)

Following general procedure P, compound **120** (103 mg, 0.34 mmol) was converted into free base **121** (50 mg, 0.30 mmol, 89.7%) as colourless syrup. Recrystallization with HCl in MeOH and Et_2O afforded the corresponding hydrochloride (**121**·HCl) as white crystals.



$C_6H_{13}ClFNO_3$

MW: 305.35 g/mol

TLC: CAM, NIP

R_f: 0.5 ($CHCl_3$/MeOH/NH_4OH 4:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

$[\alpha]_D^{20}$ = -11.9 (c 1.47, H_2O) (hydrochloride)

$[\alpha]_D^{20}$ = -10.4 (c 0.43, MeOH) (free base)

^{1}H NMR (300 MHz, D_2O): (hydrochloride) δ = 4.15 (dddd, 1H, $J_{2,3}$ 7.5 Hz, $J_{2',3}$ 11.8 Hz, $J_{3,4}$ <1 Hz, $J_{3,F}$ 7.5 Hz, H-3), 4.10 (bs, 1H, H-4), 3.86 (dd, 1H, $J_{6,6'}$ 13.3 Hz, $J_{6,F}$ 24.5 Hz, H-6), 3.80 (dd, 1H, $J_{6',F}$ 24.9 Hz, H-6´), 3.46 (dd, 1H, $J_{5a,5a'}$ 14.0 Hz, $J_{5a,F}$ 9.5 Hz, H-5a), 3.30 (m, 1H, $J_{2,2'}$ 11.9 Hz, H-2), 3.25 (dd, 1H, $J_{5a',F}$ 36.5 Hz, H-5a´), 3.08 (dd, 1H, H-2´).

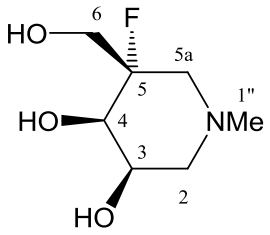
^{13}C NMR (300 MHz, D_2O): (hydrochloride) δ = 95.3 (d, 175.2 Hz, C-5), 65.6 (d, 28.9 Hz, C-4), 62.7 (C-3), 61.7 (d, 19.3 Hz, C-6), 42.2 (d, 20.9 Hz, C-5a), 41.6 (C-2); **15** (free base).

^{13}C NMR (75.5 MHz, MeOH-d_4): (free base) δ = 97.5 (d, 172.9 Hz, C-5), 69.6 (d, 28.8 Hz, C-4), 67.7 (C-3), 64.1 (d, 20.8 Hz, C-6), 46.3 (C-2), 45.7 (d, 21.8 Hz, C-5a).

MS: Calcd for $[C_6H_{12}FNO_3H]$: *m/z* 166.0880 $[M+H]^+$; Found $[M+H]^+$ 166.0870.

N-Methyl-5-fluoro-4-epi-isofagomine (123)

Following general procedure N, 5-fluoro-4-*epi*-isofagomine hydrochloride (**121**·HCl) (14 mg, 0.07 mmol) was treated with iodomethane (5 μL, 0.08 mmol) and Na_2CO_3 (22 mg, 0.21 mmol) to give compound **123** (9 mg, 0.05 mmol, 72.3%). Recrystallization with HCl in MeOH/Et_2O afforded the corresponding hydrochloride (**123**·HCl) as white crystals.



$C_7H_{14}FNO_3$

MW: 179.19 g/mol

TLC: CAM, NIP

R_f: 0.55 ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

$[\alpha]_D^{20}$ = -9.8 (c 0.335, MeOH) (free base)

^{1}H NMR (300 MHz, MeOH-d_4): (free base) δ = 4.06 (m, 1H, $J_{2,3}$ 4.5 Hz, $J_{2',3}$ 11.5 Hz, $J_{3,F}$ 7.4 Hz, H-3), 3.92 (bs, 1H, H-4), 3.80 (d, 2H, $J_{6,6'}$ <1 Hz, $J_{6,F} = J_{6',F}$ 25 Hz, H-6, H-6´), 3.37 (dd, 1H, $J_{5a,5a'}$ 13.0 Hz, $J_{5a,F}$ 9.1 Hz, H-5a), 3.13 (dd, 1H, $J_{2,2'}$ 11.2 Hz, H-2), 3.06 (dd, 1H, $J_{5a',F}$ 35.0 Hz, H-5a´), 2.95 (dd, 1H, H-2´), 2.80 (s, 3H, 3x H-1").

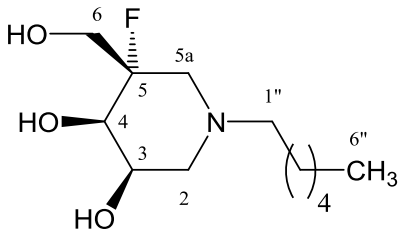
^{13}C NMR (75.5 MHz, MeOH-d_4): (free base) δ = 97.2 (d, 175.5 Hz, C-5), 67.4 (d, 28.0 Hz, C-4), 65.2 (C-3), 63.7 (d, 19.8 Hz, C-6), 54.3 (C-2), 54.0 (d, 20.4 Hz, C-5a), 44.7 (C-1").

^{13}C NMR (75.5 MHz, MeOH-d_4): (hydrochloride) δ = 97.1 (d, 175.8 Hz, C-5), 66.8 (d, 28.0 Hz, C-4), 64.5 (C-3), 63.5 (d, 19.53 Hz, C-6), 53.5 (d, 20.1 Hz, C-5a), 53.5 (C-2), 44.4 (C-1").

MS: Calcd for $[C_7H_{14}FNO_3]$: *m/z* 179.0958 $[M]^+$; Found $[M]^+$ 179.0957.

N-(n-Hexyl)-5-fluoro-4-epi-isofagomine (124)

Following general procedure N, 5-fluoro-4-*epi*-isofagomine hydrochloride (**121**·HCl) (30 mg, 0.15 mmol) was treated with 1-bromohexane (21 μL, 0.16 mmol) and Na_2CO_3 (39 mg, 0.37 mmol) to give compound **124** (33 mg, 0.13 mmol, 89.9%).



$C_{12}H_{24}FNO_3$

MW: 249.33 g/mol

TLC: CAM

R_f: 0.8 ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 3:1:0.01 v/v/v)

$[\alpha]_D^{20}$ = -9.9 (c 0.21, MeOH) (hydrochloride)

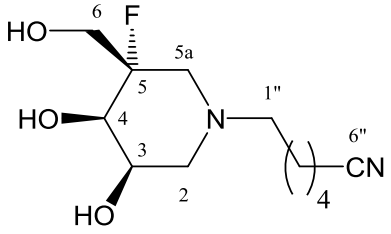
^{1}H NMR (300 MHz, MeOH-d_4): (free base) δ = 3.95 (ddd, 1H, $J_{2,3}$ 4.2 Hz, $J_{2',3}$ 10.5 Hz, $J_{3,4}$ 3.9 Hz, $J_{3,F}$ 7.6 Hz, H-3), 3.83 (m, 1H, H-4), 3.77 (dd, 1H, $J_{6,6'}$ 12.7 Hz, $J_{6,F}$ 22.9 Hz, H-6), 3.71 (dd, 1H, $J_{6',F}$ 24.6 Hz, H-6´), 2.86 (dd, 1H, $J_{5a,5a'}$ 12.0 Hz, $J_{5a,F}$ 12.0Hz, H-5a), 2.70 (dd, 1H, $J_{2,2'}$ 10.5 Hz, H-2), 2.35 (dd, 1H, $J_{5a',F}$ 32.8 Hz, H-5a´), 2.40 (m, 3H, H-2´, 2x H-1"), 1.54 (bs, 2H, 2x H-2"), 1.33 (bs, 6H, 2x H-3", 2x H-4", 2x H-5"), 0.9 (t, 3H, 3x H-6").

^{13}C NMR (75.5 MHz, MeOH-d_4): (free base) δ = 97.2 (d, 175.0 Hz, C-5), 69.4 (d, 28.9 Hz, C-4), 67.3 (C-3), 64.6 (d, 20.4 Hz, C-6), 59.4 (C-1"), 54.4 (C-2), 53.2 (d, 19.80 Hz, C-5a), 32.9, 28.3, 27.1, 23.6 (C-2", C-3", C-4", C-5"), 14.4 (C-6").

MS: Calcd for [$C_{12}H_{24}FNO_3H$]: *m/z* 250.1819 $[M+H]^+$; Found $[M+H]^+$ 250.1935.

N-(ω-C-Cyano)pentyl-5-fluoro-4-epi-isofagomine (125)

Following general procedure N, 5-fluoro-4-*epi*-isofagomine hydrochloride (**121**·HCl) (29 mg, 0.14 mmol) was treated with ω-bromohexanoic nitrile (19 μL, 0.16 mmol) and Na_2CO_3 (46 mg, 0.43 mmol) to give compound **125** (35 mg, 0.13 mmol, 93.5%).



$C_{12}H_{21}FN_2O_3$

MW: 260.31 g/mol

TLC: CAM

R_f: 0.8 ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 3:1:0.01 v/v/v)

$[\alpha]_D^{20}$ = -7.8 (c 1.35, MeOH)

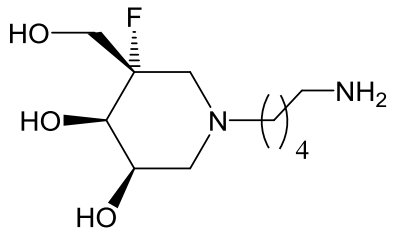
^{1}H NMR (300 MHz, MeOH-d_4): δ = 3.95 (m, 1H, $J_{2,3}$= $J_{3,4}$ 4.0 Hz, $J_{2',3}$ 10.0 Hz, $J_{3,F}$ 7.5 Hz, H-3), 3.84 (bs, 1H, H-4), 3.77 (dd, 1H, $J_{6,6'}$ 12.7 Hz, $J_{6,F}$ 22.7 Hz, H-6), 3.73 (dd, 1H, $J_{6',F}$ 24.5 Hz, H-6´), 2.91 (poorly resolved dd, 1H, $J_{5a,F}$ ca.12 Hz, H-5a), 2.75 (dd, 1H, $J_{2,2'}$ 10.5 Hz, H-2), 2.60-2.35 (m, 6H, H-2´, H-5a´, 2x H-1´´, 2x H-5´´), 1.75-1.40 (m, 6H, 2x H-2", 2x H-3", 2x H-4").

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 121.1 (C-6”), 97.2 (d, 175.0 Hz, C-5), 69.3 (d, 29.2 Hz, C-4), 67.1 (C-3), 64.5 (d, 20.1 Hz, C-6), 58.8 (C-1”), 54.2 (C-2), 53.1 (d, 20.1 Hz, C-5a), 27.5, 26.3, 26.2 (C-2”, C-3”, C-4”), 17.2 (C-5”).

MS: Calcd for [$C_{12}H_{21}FN_2O_3H$]: *m/z* 261.1614 $[M+H]^+$; Found $[M+H]^+$ 261.1630.

N-(6-Amino)hexyl-5-fluoro-4-epi-isofagomine (126)

Following general procedure M, compound **125** (34 mg, 0.13 mmol) was converted into amine **126** (28 mg, 0.11 mmol, 81.1%) as a yellow syrup.



$C_{12}H_{21}FN_2O_3$

MW: 260.31 g/mol

TLC: CAM, NIP

R_f: 0.45 ($CHCl_3$/MeOH/NH_4OH 4:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

$[\alpha]_D^{20}$ = -12.4 (c 0.205, MeOH)

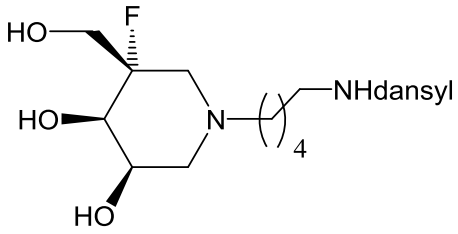
^{1}H NMR (300 MHz, MeOH-d_4): δ = 3.93 (m, 1H, $J_{2,3}$ 4.0 Hz, $J_{2',3}$ 10.0 Hz, $J_{3,F}$ 7.6 Hz, H-3), 3.82 (dd, 1H, $J_{3,4}$= $J_{4,F}$ 4.0 Hz, H-4), 3.75 (dd, 1H, $J_{6,6'}$ 12.7 Hz, $J_{6,F}$ 22.8 Hz, H-6), 3.72 (dd, 1H, $J_{6',F}$ 24.8 Hz, H-6´), 2.81 (poorly resolved dd, 1H, $J_{5a,F}$ ca. 12 Hz, H-5a), 2.74 (t, 2H, *J* 7.3 Hz, 2x H-6”), 2.65 (dd, 1H, $J_{2,2'}$ 10.5 Hz, H-2), 2.30 (dd, 1H, $J_{5a,5a'}$ 12.6 Hz, $J_{5a',F}$ 33.0 Hz, H-5a´), 2.50-2.28 (m, 3H, H-2´, 2x H-1”), 1.68-1.30 (2m, each 4H, 2x H-2”, 2x H-3”, 2x H-4”, 2x H-5”).

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 97.3 (d, 174.7 Hz, C-5), 69.6 (d, 28.9 Hz, C-4), 67.5 (C-3), 64.7 (d, 20.4 Hz, C-6), 59.2 (C-1”), 53.4 (d, 19.8 Hz, C-5a), 54.6 (C-2), 41.8 (C-6”), 31.7, 28.3, 27.6, 27.1 (C-2”, C-3”, C-4”, C-5”).

MS: Calcd for [$C_{12}H_{25}FN_2O_3H$]: *m/z* 265.1927 $[M+H]^+$; Found $[M+H]^+$ 265.1951.

N-(6-Dansylamino)hexyl-5-fluoro-4-epi-isofagomine (127)

Following general procedure O, compound **126** (45 mg, 0.17 mmol) was treated with dansyl chloride (51 mg, 0.19 mmol) and Na_2CO_3 (55 mg, 0.52 mmol). Chromatographic purification provided compound **127** (64 mg, 0.13 mmol, 74.8%) as fluorescent wax.



$C_{24}H_{36}FN_3O_5S$

MW: 497.63 g/mol

TLC: UV, CAM, NIP

R_f: 0.6 ($CHCl_3$/MeOH/NH_4OH 3:1:0.01 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 3:1:0.01 v/v/v)

$[\alpha]_D^{20}$ = -6.6 (c 1.25, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 8.59-7.28 (6H, dansyl), 3.95 (poorly resolved m, 1H, H-3), 3.84 (bs, 1H, H-4), 3.75 (dd, 1H, $J_{6,6'}$ 12.7 Hz, $J_{6,F}$ 22.6 Hz, H-6), 3.72 (dd, 1H, $J_{6',F}$ 24.6 Hz, H-6´), 2.80 (poorly resolved dd, 1H, $J_{5a,5a'}$ ca. 10 Hz, H-5a), 2.95-2.85 (m, 8H, 2x H-6", NMe_2 dansyl), 2.65 (dd, 1H, $J_{2,2'}$ 10.0 Hz, $J_{2,3}$ 3.8 Hz, H-2), 2.40-2.70 (m, 4H, H-2´, H-5a´, 2x H-1"), 1.40-0.98 (2x m, each 4H, 2x H-2", 2x H-3", 2x H-4", 2x H-5").

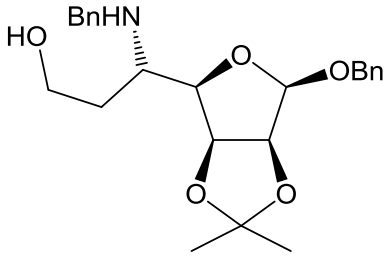
^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 153.2-116.4 (dansyl), 97.2 (d, 175.24 Hz, C-5), 69.5 (d, 30.0 Hz, C-4), 67.4 (C-3), 64.6 (d, 20.4 Hz, C-5a), 59.1 (C-1"), 54.4 (C-2), 53.2 (d, 19.5 Hz, C-6), 45.8 (NMe_2, dansyl), 43.7 (C-6"), 30.4, 27.9, 27.3, 26.8 (C-2", C-3", C-4", C-5").

MS: Calcd for [$C_{24}H_{36}FN_3O_5SNa$]: *m/z* 520.2258 $[M+Na]^+$; Found $[M+Na]^+$ 520.2473.

6.4 (5a*S*)-4-*epi*-isofagomines

Benzyl N-benzyl-5-amino-5,6-dideoxy-2,3-O-isopropylidene-α-L-gulo-heptofuranoside (112)

Following general procedure A, ester **108** (5.3 g, 12.00 mmol) was treated with $LiAlH_4$ (0.68 g, 18.0 mmol), H_2O (3 mL) and 10 mL NaOH (3 N). Purification of the remaining residue on silica gel provided alcohol **112** (4.5 g, 11.3 mmol, 93.8%).



$C_{24}H_{31}NO_5$

MW: 413.51 g/mol

TLC: UV, VAN

R_f: 0.6 (EA/MeOH 10:2 v/v)

SCG: (EA/MeOH 15:1 v/v)

$[\alpha]_D^{20}$ = -20.3 (c 2.02, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.72 (d, 1H, $J_{1,2}$ 4.2 Hz, H-1), 4.60 (dd, 1H, $J_{2,3}$ 6.0 Hz, $J_{3,4}$ 3.6 Hz, H-3), 4.49 (dd, 1H, H-2), 3.63 (m, 2H, 2x H-7), 3.53 (dd, 1H, $J_{4,5}$ 8.8 Hz, H-4), 3.11 (ddd, 1H, $J_{5,6a}$ 4 Hz, $J_{5,6b}$ 7.5 Hz, H-5), 1.80-1.53 (bm, 2H, H-6a, H-6b).

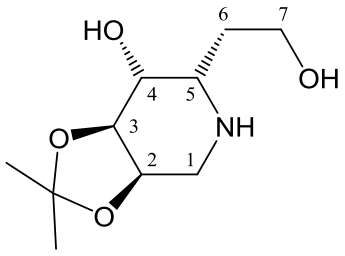
^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 140.6, 139,0 (*ipso* NHBn, *ipso* OBn), 129.7-128.3 (aromatic OBn, NHBn), 114.4 (isopropylidene), 103.4 (C-1), 81.1, 81.0, 80.1 (C-2, C-3, C-4), 73.0 (O*CH*$_2$Ph), 61.2 (C-7), 56.6 (C-5), 52.1 (NH*CH*$_2$Ph), 32.8 (C-6), 26.2, 25.6 (isopropylidene).

MS: Calcd for [$C_{20}H_{31}NO_5$H]: *m/z* 414.2281[M+H]$^+$; Found [M+H]$^+$ 414.2273.

MS: Calcd for [$C_{20}H_{31}NO_5$Na]: *m/z* 436.2100 [M+Na]$^+$; Found [M+Na]$^+$ 436.2107.

1,5,6-Trideoxy-1,5-imino-2,3-O-isopropylidene-L-gulo-heptitol (114)

Following general procedure B, compound **110** (4.5 g, 11.26 mmol) was converted into crude **114**. Purification on silica gel afforded **114** (2.1 g, 9.66 mmol, 85.8%) as colourless syrup.



$C_{10}H_{19}NO_4$

MW: 217.27 g/mol

TLC: CAM, NIP

R_f: 0.5 ($CHCl_3$/MeOH/NH_4OH 3:1:0.01 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)

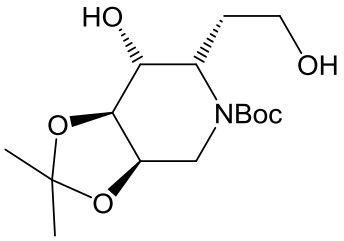
$[\alpha]_D^{20}$ = -28.4 (c 1.12, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.30 (ddd, 1H, $J_{1,2ax}$ 6.3 Hz, $J_{1,2eq}$ 4.8 Hz, $J_{2,3}$ 6 Hz, H-2), 4.18 (dd, 1H, $J_{3,4}$ 3Hz, H-3), 3.80 (dd, 1H, $J_{4,5}$ 3 Hz, H-4), 3.68 (m, 2H, 2x H-7), 3.25-3.13 (m, 2H, H-1eq, H-5), 2.88 (dd, 1H, $J_{1ax,1eq}$ 13.5 Hz, H-1ax), 1.77 (m, 2H, 2x H-6).

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 110.3 (isopropylidene), 76.9 (C-3), 71.4 (C-2), 68.5 (C-4), 59.98 (C-7), 52.6 (C-5), 45.4 (C-1), 33.8 (C-6), 27.9, 25.7 (isopropylidene).

N-tert-Butyloxycarbonyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-L-gulo-heptitol (128)

Following general procedure C, **114** (2.1 g, 9.66 mmol) was treated with Et_3N (4.0 mL, 28.99 mmol) and $(Boc)_2O$ (2.5 g, 11.59 mmol). Chromatographic purification on silica gel gave compound **128** (2.75 g, 8.66 mmol, 89.7%) as a pale yellow syrup.



$C_{15}H_{27}NO_6$

MW: 317.38 g/mol

TLC: CAM

R_f: 0.5 (C/EA 1:4 v/v/v)

SCG: (C/EA 1:1 v/v)

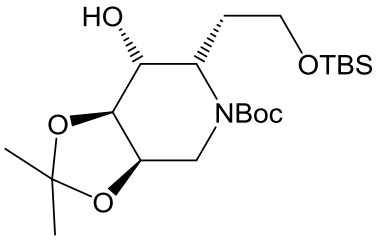
$[a]_D^{20}$ = +25.3 (c 4.75, $CHCl_3$)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.18 (m, 2H, H-2, H-3), 4.00 (dd, 1H, $J_{1ax/1eq}$ 14.Hz, $J_{1eq,2}$ 2.8 Hz, H-1eq), 3.95 (m, 2H, H-5), 3.88-3.83 (m, 1H, H-4), 3.58-3.40 (m, 2H, 2x H-7), 3.28-3.08 (m, 1H, H-1ax), 2.00-1.60 (m, 2H, 2x H-6).

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 158.0, (Boc), 110.3 (isopropylidene), 81.2, 81.1 (Boc), 76.6, 76.2, 77.5, 77.4 (C-2, C-3), 68.2 (C-4), 59.8 (C-7), 53.0, 51.9 (C-5), 52.6, 41.5 (C-1), 31.8, 30.7 (C-6), 28.7 (Boc), 27.3, 24.9 (isopropylidene).

N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-L-gulo-heptitol (130)

Following general procedure D, compound **128** (2.75 g, 8.66 mmol) was treated with imidazole (1.77 g, 25.99 mmol) and TBSCl (1.40g, 9.53 mmol). Purification on silica gel afforded **130** (3.1 g, 7.18 mmol, 82.9%) as a pale colourless syrup.



$C_{21}H_{41}NO_6Si$

MW: 431.64 g/mol

TLC: CAM

R_f: 0.5 (C/EA 2:1 v/v/v)

SCG: (C/EA 10:1 v/v)

$[a]_D^{20}$ = +53.5 (c 0.94, $CHCl_3$)

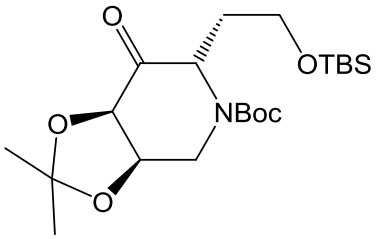
^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.48-4.35 (m, 2H, H-2, H-3), 4.35-3.89 (m, 2H, 2x H-7), 3.40-3.19 (m, 1H, H-1ax), 2.08-1.88 (m, 2H, 2x H-6).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.7 (Boc), 109.3 (isopropylidene), 79.5 (Boc), 73.8 (C-3), 73.1, 72.9 (C-4), 66.7, 66.6 (C-2), 60.6, 60.4 (C-7), 53.3, 52.8 (C-5), 41.9, 40.9 (C-1), 32.4, 31.4 (C-6), 26.5, 24.4 (isopropylidene), 25.94, 18.25, -5.6 (TBS).

MS: Calcd for [$C_{21}H_{41}NO_6SiNa$]: *m/z* 454.2601 $[M+Na]^+$; Found $[M+Na]^+$ 454.2617.

N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-L-ribo-hept-4-ulose (133)

Following general procedure E, alcohol **130** (1.39 g, 3.22 mmol) was treated with Dess-Martin periodinane (1.64 g, 3.86 mmol). Purification on silica gel afforded **133** (1.34g, 3.12 mmol, 96.9%) as a colourless syrup.



$C_{22}H_{41}NO_5Si$

MW: 427.66 g/mol

TLC: UV, CAM

R_f: 0.45 (C/EA 4:1 v/v/v)

SCG: (C/EA 7:1 v/v)

$[a]_D^{20}$ = +24.5 (c 2.17, $CHCl_3$)

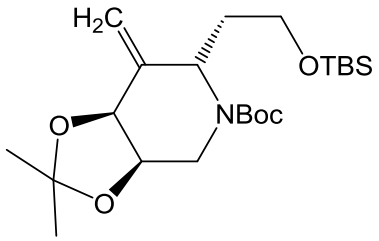
^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.80 (bs, 1H, H-5), 4.60-4.19 (m, 3H, H-1eq, H-2, H-3), 3.66 (m, 2H, 2x H-7), 3.08 (m, 1H, H-1ax), 1.99 (m, 2H, 2x H-6).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 204.8 (C-4), 155.2 (Boc), 111.7 (isopropylidene), 80.7 (Boc), 76.7, 76.6 (C-3, C-2), 59.7 (C-7), 59.1 (C-2), 42.2 (C-1), 32.8 (C-6), 28.5 (Boc), 26.6, 25.0 (isopropylidene), 26.1, 18.43, -5.2, -5.3 (TBS).

MS: Calcd for [$C_{21}H_{39}NO_6SiNa$]: *m/z* 452.2444 $[M+Na]^+$; Found $[M+Na]^+$ 452.2544.

N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-4-C-methylene-L-ribo-heptitol (136)

Following general procedure G, ulose **133** (0.96 g, 2.24 mmol) was treated with Tebbe´s reagent (0.5 M in toluene, 4.9 mL, 2.45 mmol), Et_2O (80 mL) and NaOH (1.5 N, 1.95 mL). Purification on silica gel provided compound **136** (897 mg, 2.09 mmol, 93.5%).



$C_{21}H_{39}NO_6Si$

MW: 429.63 g/mol

TLC: CAM

R_f: 0.55 (C/EA 4:1 v/v/v)

SCG: (C/EA 9:1 v/v)

$[\alpha]_D^{20}$ = +9.0 (c 4.33, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.30, 5.19 (2s, 2H, 2x exo-methylene), 4.72 (bs, 1H, H-5), 4.64 (d, 1H, $J_{2,3}$ 7.1 Hz, H-3), 4.25 (bs, 1H, H-2), 4.05 (m, 1H, H-1eq), 3.75-3.57 (2H, 2x H-7), 2.82 (m, 1H, H-1ax), 2.09-1.76 (m, 2H, 2x H-6).

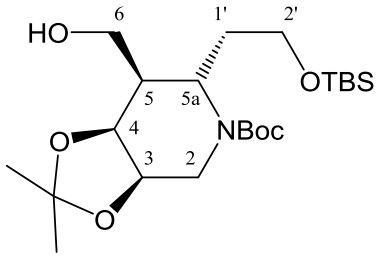
^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.6 (Boc), 143.0 (C-4), 116.0 (exo-methylene), 109.5 (isopropylidene), 79.9, 79.6 (Boc), 76.9, 75.3 (C-2, C-3), 60.7, 60.4 (C-7), 52.5, 51.7 (C-5), 43.4, 42.4 (C-1), 35.4, 35.3 (C-6), 28.5 (Boc), 26.1 (TBS), 26.7, 24.9 (isopropylidene), 18.4, -5.2 (TBS).

MS: Calcd for $[C_{22}H_{41}NO_5SiNa]$: *m/z* 450.2652 $[M+Na]^+$; Found $[M+Na]^+$ 450.2767.

(5aS)-N-tert-Butyloxycarbonyl-3,4-O-isopropylidene-5a-C-(tert-butyldimethylsilyloxy)ethyl-4-epi-isofagomine (144) *and*

(5aS)-N-tert-Butyloxycarbonyl-3,4-O-isopropylidene-5a-C-(tert-butyldimethylsilyloxy)ethyl-4,5-di-epi-isofagomine (145)

Following general procedure I (variant 1), compound **136** (453 mg, 1.06 mmol) was treated with BH_3·THF (1 M, 5.3 mL), H_2O (5 mL), NaOH (3 *N*, 8 mL) and H_2O_2 (33%, 8 mL). Purification of the on silica gel provided afforded alcohols **144** (241 mg, 0.54 mmol, 51.0%) and **145** (101 mg, 0.23 mmol, 21.4%).



Compound: **144**

$C_{22}H_{43}NO_6Si$

MW: 445.67 g/mol

TLC: CAM

R_f: 0.25 (C/EA 2:1 v/v/v)

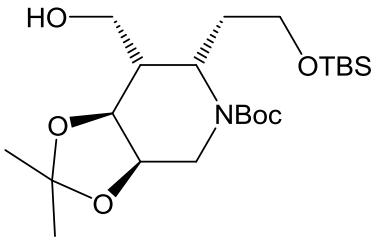
SCG: (C/EA 15:1 v/v) to (C:EA 10:1 v/v)

$[\alpha]_D^{20}$ = +52.6 (5.32, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.49 (m, 1H, H-4), 4.32-3.94 (m, 3H, H-2eq, H-3, H-5a), 3.82 (bs, 2H, 2x H-6), 3.69 (bs, 2H, 2x H-2´), 2.94-2.48 (m, 2H, H-2ax, 6-OH), 2.05-1.60 (m, 3H, H-5, 2x H1´).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.8 (Boc), 108.9 (isopropylidene), 79.8, 79.4 (Boc), 74.6, 74.5 (C-4), 74.0, 73.8 (C-3), 63.2, 63.0 (C-6), 60.7, 60.6 (C-2´), 47.5, 47.0 (C-5a), 42.9, 41.9 (C-2), 41.1, 40.8 (C-5), 36.0, 35.7 (C-1´), 28.6 (Boc), 26.2, 24.3 (isopropylidene), 26.0, 18.3, -5.3 (TBS).

MS: Calcd for [$C_{22}H_{43}NO_6SiNa$]: *m/z* 468.2757 $[M+Na]^+$; Found $[M+Na]^+$ 468.3375.



Compound: **145**

$C_{22}H_{43}NO_6Si$

MW: 445.67 g/mol

TLC: CAM

R_f: 0.4 (C/EA 2:1 v/v/v)

SCG: (C:EA 15:1 v/v) to (C:EA 10:1 v/v)

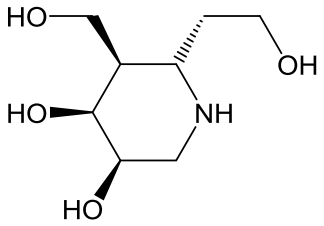
$[\alpha]_D^{20}$ = -24.1 (c 1.75, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.45-4.17 (bs, 2H, H-2eq, H-5a), 4.16-4.02 (m, 2H, H-3, H-4), 3.89-3.49 (m, 4H, 2x H-6, 2x H-2´), 3.24 (bs, 1H, H-2ax), 2.09 (m, 1H, H-5), 1.76-1.51 (m, 2H, 2x H-1´).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.5 (Boc), 108.9 (isopropylidene), 80.1 (Boc), 74.4, 72.0 (C-3, C-4), 63.3 (C-6), 61.0 (C-2´), 49.1 (C-5a), 43.9 (C-5), 40.4, 39.4 (C-2), 29.7 (C-1´), 28.4 (Boc), 28.3, 26.4 (isopropylidene), 26.0, 18.4, -5.3, -5.4 (TBS).

(5aS)-5a-C-(2-Hydroxy)ethyl-4-epi-isofagomine (147)

Following general procedure P, compound **144** (61 mg, 0.14 mmol) was converted into crude **147**. Purification on silica gel afforded **147** (24 mg, 0.13 mmol, 91.7%) as amorphous solid.



$C_8H_{17}NO_4$

MW: 191.23 g/mol

TLC: CAM, NIP

R_f: 0.45 ($CHCl_3$/MeOH/NH_4OH 4:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

$[a]_D^{20}$ = -18.1 (c 0.73, MeOH) (hydrochloride)

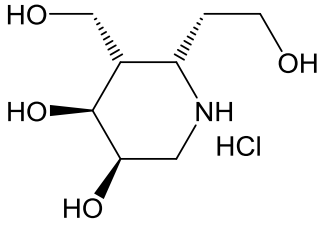
^{1}H NMR (300 MHz, D_2O) (hydrochloride): δ = 4.22 (dd, 1H, $J_{3,4}$ 2.7 Hz, $J_{4,5}$ 2.5 Hz, H-4), 3.94 (ddd, 1H, $J_{2ax,3}$ 11.7 Hz, $J_{2eq,3}$ 5 Hz, H-3), 3.87-3.73 (m, 3H, $J_{5,6}$ 5 Hz, 1x H-6, 2x H-2´), 3.70 (dd, 1H, $J_{5,6}$ 7.3 Hz, $J_{6,6}$ 11.5 Hz, 1x H-6), 3.45 (ddd, 1H, $J_{5,5a}$ 11.5 Hz, $J_{5,1´}$ 3.2 Hz, $J_{5a,\ 1´}$ 8.4 Hz, H-5a), 3.25 (dd, 1H, $J_{2ax,2eq}$ 12 Hz, H-2eq), 3.12 (dd, 1H, H-2ax), 2.15-2.05 (m, 1H, 1x H-1´), 2.03-1-92 (m, 1H, H-5), 1.86-1.71 (m, 1H, 1x H-1´).

^{13}C NMR (75.5 MHz, D_2O) (hydrochloride): δ = 66.9 (C-4), 65.6 (C-3), 59.6 (C-6), 58.2 (C-2´), 51.7 (C-5a), 42.5 (C-5), 42.2 (C-2), 31.0 (C-1´).

MS: Calcd for [$C_8H_{17}NO_4H$]: *m/z* 520.2258 $[M+H]^+$; Found $[M+H]^+$ 192.1240.

(5aS)-5a-C-(2-Hydroxy)ethyl-4,5-di-epi-isofagomine (146)

Following general procedure P, compound **145** (79 mg, 0.18 mmol) was converted into title compound **146** which was an amorphous solid. Recrystallization in MeOH and Et_2O in the presence of a few drops of HCl (12 *N*) afforded the corresponding hydrochloride **146**·HCl (36 mg, 0.16 mmol, 89.2%) as colourless crystals.



$C_6H_{18}ClNO_4$

MW: 227.69 g/mol

TLC: CAM, NIP

R_f: 0.4 ($CHCl_3$/MeOH/NH_4OH 4:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

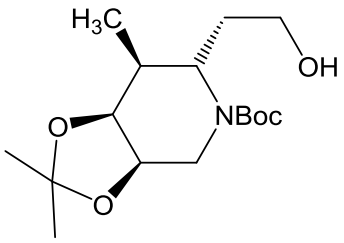
$[a]_D^{20}$ = -13.6 (c 0.91, H_2O)

^{1}H NMR (300 MHz, D_2O) (hydrochloride): δ = 4.22 (ddd, 1H, $J_{2ax,3}$ 7.9 Hz, $J_{2eq,3}$ 3.8 Hz, $J_{3,4}$ 6.8 Hz, H-3), 4.04 (dd, 1H, $J_{4,5}$ 3.1Hz, H-4), 3.96 (dd, 1H, $J_{5,6}$ 5.4 Hz, $J_{6,6}$ 11.7 Hz, 1x H-6), 3.88-3.68 (m, 4H, H-5a, 1x H-6, 2x H-2´), 3.35 (dd, 1H, $J_{2ax,2eq}$ 12.9 Hz, H-2eq), 3.23 (dd, 1H, H-2ax), 2.35 (m, 1H, H-5), 2.05-1.93 (m, 2H, 2x H-1´);

^{13}C NMR (75.5 MHz, D_2O) (hydrochloride): δ = 67.9 (C-4), 64.1 (C-3), 59.0 (C-2´), 58.9 (C-6), 52.5 (C-5a), 43.0 (C-2), 40.8 (C-5), 28.0 (C-1´).

(5aS)-N-tert-Butyloxycarbonyl-6-deoxy-3,4-O-isopropylidene-5a-C-(tert-butyldimethylsilyloxy)ethyl-4-epi-isofagomine (196)

Compound **136** (165 mg, 0.39 mmol) was converted into **196** employing general procedure B. Chromatographic purification on silica gel gave **196** (107 mg, 0.34 mmol, 87.9%) as pale yellow syrup.



$C_{16}H_{29}NO_5$

MW: 315.41 g/mol

TLC: CAM, NIP

R_f: 0.25 (C/EA 1:1 v/v)

SCG: (C/EA 3:1 v/v)

$[a]_D^{20}$ = n.d.

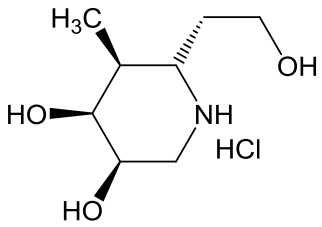
^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.39-4.09 (m, 2H, H-3, H-4), 3.94 (dd, 1H, $J_{2ax,2eq}$ 14.8 Hz, $J_{2eq,\ 3}$ < 1 Hz, H-2eq), 3.87 (m, 1H, H-5a), 3.60-3.38 (m, 2H, 2x H-2´), 2.71 (dd, 1H, $J_{2ax,3}$ < 1 Hz, H-2ax), 1.92 (m, 1H, 1x H-1´), 1.46 (m, 1H, H-5a), 1.22 (m, 1H, 1x H-1´), 1.16 (d, 3H, 3x H-6).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 157.4 (Boc), 108.4 (isopropylidene), 80.3 (Boc), 76.0, 73.9 (C-3, C-4), 58.2 (C-2´), 50.2 (C-5a), 42.0 (C-2), 36.4 (C-1´), 35.5 (C-5), 28.5 (Boc), 26.33, 24.2 (isopropylidene), 16.7 (C-6).

MS: Calcd for [$C_{16}H_{29}NO_5Na$]: *m/z* 338.1943 $[M+Na]^+$; Found $[M+Na]^+$ 338.1945.

(5aS)-6-Deoxy-5a-C-(2-hydroxy)ethyl-4-epi-isofagomine (197)

Following general procedure P, compound **196** (37 mg, 0.12 mmol) was converted into title compound **197** which was an amorphous solid. Recrystallization in MeOH and Et_2O in the presence of a few drops of HCl (12 *N*) afforded the corresponding hydrochloride **197**·HCl (21 mg, 0.10 mmol, 84.6%) as white crystals.



$C_8H_{18}ClNO_3$

MW: 211.69 g/mol

TLC: CAM, NIP

R_f: 0.55 ($CHCl_3$/MeOH/NH_4OH 4:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

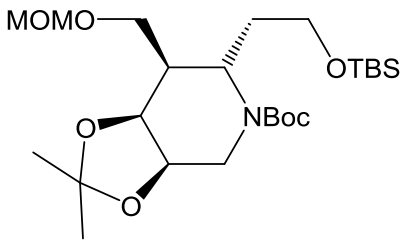
$[\alpha]_D^{20}$ = -10.7 (c 0.48, H_2O) (hydrochloride)

^{1}H NMR (300 MHz, D_2O) (hydrochloride): δ = 3.97 (m, 1H, H-3), 3.92 (m, 1H, H-4), 3.78 (m, 2H, 2x H-2´), 3.30-3.17 (m, 2H, H-2eq, H-5a), 3.08 (dd, 1H, $J_{2ax,2eq}$ = $J_{2ax,3}$ 11.5 Hz, H-2ax), 2.13-2.00 (m, 1H, 1x H-1´), 1.92 (ddq, 1H, $J_{4,5}$ 2Hz, J5,5a 13.7 Hz, $J_{5,6}$ 6.9 Hz, H-5), 1.82-1.67 (m, 1H, 1x H-1´), 1.06 (d, 3H, 3x H-6).

^{13}C NMR (75.5 MHz, D_2O) (hydrochloride): δ = 70.4 (C-4), 65.6 (C-3), 58.2 (C-2´), 54.2 (C-5a), 42.35 (C-2), 35.83 (C-5), 30.9 (C-1´), 13.9 (C-6).

(5aS)-N-tert-Butyloxycarbonyl-5a-C-(tert-butyldimethylsilyloxy)ethyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (148)

Following general procedure J, compound **144** (162 mg, 0.36 mmol), was treated with diisopropyl ethyl amine (172 µL, 1.02 mmol) and chloromethyl methyl ether (36 µL, 0.47 mmol). Chromatographic purification on silica gel provided **148** (155 mg, 0.32 mmol, 87.1 %) as a yellow syrup.



$C_{24}H_{47}NO_7Si$

MW: 489.72 g/mol

TLC: CAM

R_f: 0.8 (C/EA 1:2 v/v)

SCG: (C/EA 8:1 v/v)

$[\alpha]_D^{20}$ = +86.5 (c 1.10, $CDCl_3$)

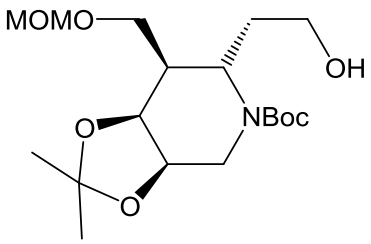
^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.48 (m, 1H, H-4), 4.20-3.87 (m, 2H, H-2eq, H-3), 3.82-3-54 (m, 5H, H-5a, 2x H-6, 2x H-2´), 2.88-2.67 (m, 1H, H-2ax), 1.94-1.54 (m, 3H, H-5, 2x H-1´).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.4 (Boc), 108.2 (isopropylidene), 96.5 (Boc), 79.6, 79.2 (Boc), 73.3, 73.2 (C-3), 71.3, 71.2 (C-4), 66.9 (C-6), 60.6, 60.4 (C-2´), 55.2 (MOM), 48.3, 47.5 (C-5a), 52.9, 41.9 (C-2), 39.9, 39.4 (C-5), 36.7, 36.3 (C-1´), 28.4 (Boc), 26.2, 24.2 (isopropylidene), 26.0, 18.3, -5.3 (TBS).

MS: Calcd for [$C_{24}H_{47}NO_7SiNa$]: *m/z* 512.3019 $[M+Na]^+$; Found $[M+Na]^+$ 512.3071.

(5aS)-N-tert-Butyloxycarbonyl-5a-C-(2-hydroxy)ethyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (149)

Silyl ether **148** (98 mg, 0.20 mmol) was converted into **149** employing general procedure K. Purification on silica gel gave alcohol **149** (69 mg, 0.18 mmol, 91.7%). Recrystallization in EA afforded colourless needles.



$C_{24}H_{47}NO_7Si$

MW: 489.72 g/mol

TLC: CAM

R_f: 0.3 (C/EA 1:2 v/v)

SCG: (C/EA 1:1 v/v)

$[\alpha]_D^{20}$ = +37.1 (c 1.93, $CHCl_3$)

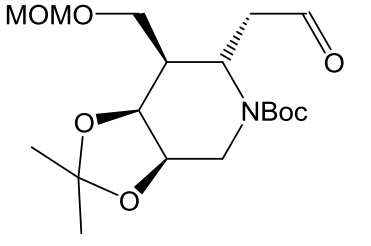
^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.52 (dd, 1H, $J_{3,4}$ 7.7 Hz, $J_{4,5}$ 2.4 Hz, H-4), 4.22-4.05 (m, 1H, H-3), 3.99-3.86 (m, 2H, H-2eq, H-5a), 3.75-3.60 (m, 2H, 2x H-6), 3.58-3.40 (m, 2H, 2x H-2´), 2.73 (dd, 1H, $J_{2ax,2eq}$ 14.7 Hz, $J_{2ax,3}$ 2 Hz, H-2ax), 1.99-1.87 (m, 1H, 1x H-1´), 1.73-1.62 (m, 1H, H-5), 1.40-1.29 (m, 1H, 1x H1´).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 157.2 (Boc), 108.5 (isopropylidene), 96.6 (MOM), 80.5 (Boc), 73.15 (C-3), 71.4 (C-4), 67.3 (C-6), 58.1 (C-2´), 55.4 (MOM), 46.5 (C-5a), 42.4 (C-2), 41.1 (C-5), 36.8 (C-1´), 28.4 (Boc), 26.3, 24.2 (isopropylidene).

MS: Calcd for [$C_{18}H_{33}NO_7Na$]: *m/z* 398.2155 $[M+Na]^+$; Found $[M+Na]^+$ 398.2517.

(5aS)-N-tert-Butyloxycarbonyl-5a-C-(1-oxo)ethyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (150)

Following general procedure E, alcohol **149** (110 mg, 0.29 mmol) was treated with Dess-Martin periodinane (149 mg, 0.35 mmol). Purification on silica gel afforded **149** (104 mg, 0.27 mmol, 94.8%) as a colourless syrup.



$C_{18}H_{31}NO_7$

MW: 372.45 g/mol

TLC: UV, CAM

R_f: 0.5 (C/EA 1:1 v/v)

SCG: (C/EA 1:1 v/v)

$[\alpha]_D^{20}$ = +56.2 (c 3.43, $CHCl_3$)

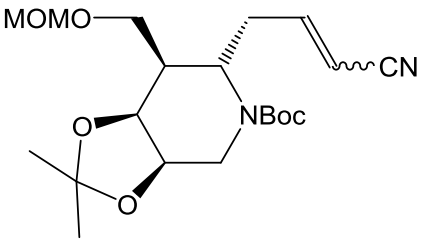
^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.96 (s, 1H, H-2´), 4.43 (m, 1H, H-4), 4.28-3.87 (m, 3H, H-2eq, H-3, H-5a), 3.74-3.48 (m, 2H, 2x H-6), 2.99-2.78 (m, 1H, H-2ax), 2.77-2.37 (m, 2H, 2x H-1´), 1.99-1.81 (m, 1H, H-5).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 199.9, 199.4 (C-2´), 154.5, 153.9 (Boc), 107.7 (isopropylidene), 95.6 (MOM), 79.7, 79.0 (Boc), 72.7 (C-4), 70.9 (C-3), 66.7 (C-6), 54.43 (MOM), 47.0, 46.3 (C-5a), 46.6, 46.3 (C-1´), 41.7, 40.7 (C-2), 39.3, 38.3 (C-5), 27.4 (Boc), 25.2, 23.3 (isopropylidene).

MS: (**XX hydrate**) Calcd for [$C_{18}H_{33}NO_8Na$]: *m/z* 414.2104 $[M+Na]^+$; Found $[M+Na]^+$ 414.0195.

4-[(5aS)-(N-tert-Butyloxycarbonyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomin-5a-yl)-but-2-enoic nitrile (151)

Following general procedure L, aldehyde **150** (83 mg, 0.22 mmol) was treated with diethyl cyanomethyl phosphonate (48 µL, 0.45 mmol) [deprotonated with *t*-BuOK (45 mg, 0.40 mmol]. Silica gel chromatography provided **151** (mixture of the *E*/*Z*-isomers, 64 mg, 0.16 mmol, 72.6%).



$C_{20}H_{32}N_2O_6$

MW: 396.48 g/mol

TLC: UV, CAM, NIP

R_f: 0.6 (C/EA 1:2 v/v)

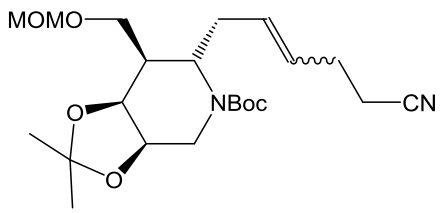
SCG: (C/EA 5:1 v/v)

^{1}H NMR (300 MHz, $CDCl_3$) (*E*/*Z*): δ = 6.83-6.45 (m, 1H, H-2´), 5.34 (m, 1H, H-3´), 4.49 (m, 1H, H-4), 4.30-3.78 (m, 3H, H-2eq, H-3, H-5a), 3.77-3.53 (m, 2H, 2x H-6), 2.91-2.53 (m, 3H, H-2ax, 2x H-1´), 1.76 (bs, 1H, H-5).

^{13}C NMR (75.5 MHz, $CDCl_3$) (*E*/*Z*): δ = 155.4, 155.1 (Boc), 152.2, 151.78 (C-2´), 117.2, 116.0 (C-4´), 108.6, 108.5 (isopropylidene), 102.0, 100.9 (C-3´), 96.6 (MOM), 80.1, 79.8 (Boc), 73.1-71.5 (C-3, C-4), 67.4, 67.3 (C-6), 55.4 (MOM), 50.6, 50.3, 49.6, 49.5 (C-5a), 43.2, 42.7, 42.1, 41.9 (C-2), 39.9, 39.8, 39.5, 38.8 (C-5), 37.9, 37.0, 36.6, 36.1 (C-1´), 28.4, 28.3 (Boc), 26.2, 24.2 (isopropylidene).

6-[(5aS)-(N-tert-Butyloxycarbonyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomin-5a-yl)-hex-4-enoic nitrile (154)

Following general procedure H, aldehyde **150** (110 mg, 0.29 mmol) was converted into **154**. Therefore, diisopropyl amine (0.14 mL, 0.99 mmol), 2.5 M *n*-BuLi (0.38 mL) and triphenyl-(3-cyano)propyl phosphonium bromide (421mg, 1.03 mmol) were used. Chromatographic purification afforded **154** (75 mg, 0.18 mmol, 60.1%) as a mixture of the *E*/*Z*-isomers.



$C_{22}H_{36}N_2O_6$

MW: 424.54 g/mol

TLC: UV, CAM, NIP

R_f: 0.62 (C/EA 1:1 v/v)

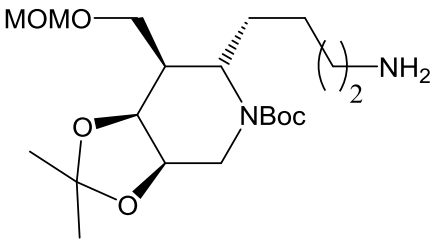
SCG: (C/EA 20:1 v/v) to (C/EA 10:1 v/v)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 5.71-5.39 (m, 2H, H-2´, H-3´), 4.51 (m, 1H, H-4), 4.26-3.94 (m, 2H, H-2eq, H-3), 3.93-3.69 (m, 1H, H-5a), 3.63 (bs, 2H, 2x H-6), 2.86-2.66 (m, 1H, H-2ax), 2.65-2.08 (m, 6H, 2x H-1´, 2x H-4´, 2x H-5´), 1.86 (bs, 1H, H-5).

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 155.4, 155.2 (Boc), 128.4, 128.3, 128.2 (C-2´, C-3´), 119.4 (C-6´), 108.5, 108.4 (isopropylidene), 96.5, 96.4 (MOM), 79.8, 79.5 (Boc), 73.3, 73.1, 71.7, 71.5 (C-3, C-4), 67.1, 66.7 (C-6), 55.3, 55.2 (MOM), 50.4, 49.6 (C-5a), 43.7, 42.8 (C-2), 38.5, 37.9 (C-5), 30.5, 29.4 (C-1´), 28.4 (Boc), 26.2, 24.2 (isopropylidene), 23.3 (C-4´), 17.4 (C-5´).

(5aS)-N-tert-Butyloxycarbonyl-5a-C-(4-amino)butyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (152)

Nitrile **151** (64 mg, 0.16 mmol) was converted into crude **152** employing general procedure M. Purification on silica gel provided amine **152** (49 mg, 0.12 mmol, 75.4%) as a yellow syrup.



$C_{20}H_{38}N_2O_6$

MW: 402.53 g/mol

TLC: CAM, NIP

R_f: 0.6 ($CHCl_3$/MeOH/NH_4OH 3:1:0.01 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)

$[a]_D^{20}$ = +33.8 (c 2.36, MeOH)

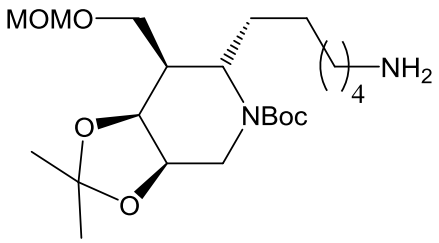
^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.56 (dd, 1H, $J_{3,4}$ 8 Hz, $J_{4,5}$ 2 Hz, H-4), 4.24 (m, 1H, H-3), 3.99 (m, 1H, H-2eq), 3.79 (ddd, 1H, $J_{5,5a}$ 10.6 Hz, $J_{5a,\,1´}$ 7.3 Hz, $J_{5a,1´}$ 3.0 Hz, H-5a), 3.72-3.55 (m, 2H, 2x H-6), 2.98-2.78 (m, 3H, H-2ax, 2x H-4´), 1.89 (m, 1H, H-5), 1.79-1.30 (m, 6H, 2x H-1´, 2x H-2´, 2x H-3´).

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 157.7, 157.4 (Boc), 109.4 (isopropylidene), 97.5 (Boc), 81.2, 80.9 (Boc), 81.2, 80.9 (C-3), 74.8, 74.6 (C-4), 68.4 (C-6), 55.6 (MOM), 52.0, 50.7 (C-5a), 43.8, 43.2 (C-2), 41.1 (C-4´), 40.4, 40.2 (C-5), 34.3, 34.1 (C-1´), 30.2, 29.4 (C-3´), 28.8 (Boc), 26.8, 24.4, 24.2 (isopropylidene), 23.5, 23.3 (C-2´).

MS: Calcd for [$C_{20}H_{38}N_2O_6$Na]: *m/z* 425.2628 $[M+Na]^+$; Found $[M+Na]^+$ 425.2699.

(5aS)-N-tert-Butyloxycarbonyl-5a-C-(6-amino)hexyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (38)

Nitrile **154** (75 mg, 0.17 mmol) was converted into crude amine **155** by general procedure M. Purification on silica gel provided **155** (64 mg, 0.15 mmol, 84.1%) as a pale yellow syrup.



$C_{22}H_{42}N_2O_6$

MW: 430.59 g/mol

TLC: CAM, NIP

R_f: 0.3 ($CHCl_3$/MeOH/NH_4OH 3:4:0.01 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)

$[a]_D^{20}$ = +27.4 (c 1.05, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.57 (dd, 1H, $J_{3,4}$ 7.9 Hz, $J_{4,5}$ 2.4 Hz, H-4), 4.25 (m, 1H, H-3), 3.99 (m, 1H, H-2eq), 3.84-3.55 (m, 3H, H-5a, 2x H-6), 2.96-2.77 (m, 3H, H-2ax, 2x H-6´), 1.88 (m, 1H, H-5), 1.76-1.32 (m, 10H, 2x H-1´, 2x H-2´, 2x H-3´, 2x H-4´, 2x H-5´).

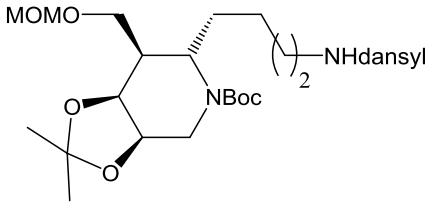
^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 157.7, 157.5 (Boc), 109.5 (isopropylidene), 97.6, 97.5 (MOM), 80.8 (Boc), 74.7, 74.5 (C-4), 73.4, 73.2 (C-3), 68.4 (C-6), 55.6 (MOM), 52.1, 50.8 (C-5a), 43.8, 43.1, 40.8, 40.7, 34.8, 34.6 (C-2, C-5, C-6´), 30.5, 30.4 (C-1´), 28.8 (Boc), 28.6, 26.8 (isopropylidene), 28.7-26.04 (C-2´, C-3´, C-4´, C-5´).

MS: Calcd for [$C_{22}H_{42}N_2O_6H$]: *m/z* 431.3121 $[M+H]^+$; Found $[M+H]^+$ 431.3120.

MS: Calcd for [$C_{22}H_{42}N_2O_6Na$]: *m/z* 453.2941 $[M+Na]^+$; Found $[M+Na]^+$ 453.2926.

(5aS)-N-tert-Butyloxycarbonyl-5a-C-(4-dansylamino)butyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (156)

Following general procedure O, compound **152** (28 mg, 0.07 mmol) was treated with Na_2CO_3 (19 mg, 0.18 mmol) and dansyl chloride (23 mg, 0.08 mmol) to give crude compound **156** which was immediately taken to the next step.



$C_{32}H_{49}N_3O_8S$

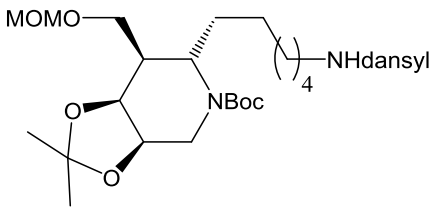
MW: 635.82 g/mol

TLC: UV, CAM, NIP

R_f: 0.5 (C/EA 1:1 v/v)

(5aS)-N-tert-Butyloxycarbonyl-5a-C-(6-dansylamino)hexyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (158)

Following general procedure O, compound **155** (64 mg, 0.15 mmol) was treated with Na_2CO_3 (44 mg, 0.42 mmol) and dansyl chloride (52 mg, 0.19 mmol) to give crude compound **158** which was immediately taken to the next step.



$C_{34}H_{53}N_3O_8S$

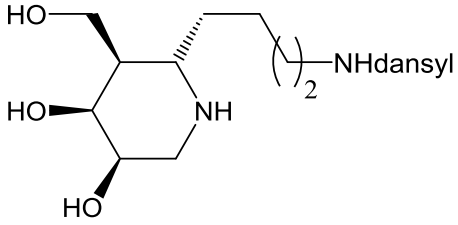
MW: 663.83 g/mol

TLC: UV, CAM, NIP

R_f: 0.3 (C/EA 2:1 v/v)

(5aS)-5a-C-(4-Dansylamino)butyl-4-epi-isofagomine (157)

Following general procedure P, crude compound **156** was converted into crude **157**. Purification on silica gel afforded **157**. Rrecrystallization in MeOH in the presence of HCl afforded **157**·HCl (21 mg, 0.41 mmol, 59.1% over 2 steps) as faintly green fluorescent solid.



$C_{22}H_{33}N_3O_5S$

MW: 451.58 g/mol

TLC: UV, CAM, NIP

R_f: 0.3 ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)

$[a]_D^{20}$ = -15.2 (c 1.1, MeOH) (free base)

^{1}H NMR (300 MHz, MeOH-d_4) (free base): δ = 4.10 (dd, $J_{3,4}$ = $J_{4,5}$ 2.5 Hz, Hz), 3.75 (ddd, 1H, $J_{2ax,3}$ 10.5 Hz, $J_{2eq,3}$ 6.1 Hz, H-3), 3.65 (bd, 2H, 2x H-6), 3.18 (ddd, 1H, $J_{5,5a}$ 10.9 Hz, $J_{5a,1'}$ 6.9 Hz, $J5_{a,1'}$ 3.1 Hz, H-5a), 3.14-3.00 (m, 2H, H-2eq, H-2ax) 2.85 (m, 2H, 2x H-4´), 1.69-1.155 (m, 8H, H-5, 2x H-1´, 2x H-2´, 2x H-3´).

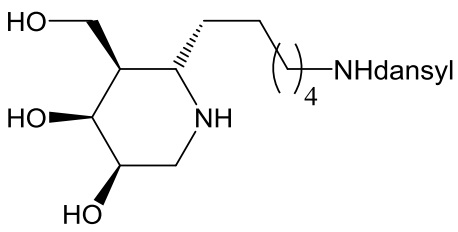
^{13}C NMR (75.5 MHz, MeOH-d_4) (free base): δ = 153.3-116.5 (dansyl), 69.1 (C-4), 67.6 (C-3), 61.6 (C-6), 53.6 (C-5a), 45.8 (2xNMe_2), 45.1 (C-5), 44.4 (C-2), 43.4 (C-4´), 30.8, 30.5 (C-1´, C-3´), 22.5 (C-2´).

MS: Calcd for [$C_{22}H_{33}N_3O_5SH$]: *m/z* 452.2219 $[M+H]^+$; Found $[M+H]^+$ 452.2336.

MS: Calcd for [$C_{22}H_{33}N_3O_5SNa$]: *m/z* 474.2039 $[M+Na]^+$; Found $[M+Na]^+$ 474.2052.

(5aS)-5a-C-(6-Dansylamino)hexyl-4-epi-isofagomine (159)

Following general procedure P, crude compound **158** was converted into crude **159**. Purification on silica gel provided **159** (35 mg, 0.07 mmol, 49.1% over 2 steps) as fluorescent wax.



$C_{24}H_{37}N_3O_5S$

MW: 479.64 g/mol

TLC: CAM, NIP

R_f: 0.25 ($CHCl_3$/MeOH/NH_4OH 3:1:0.01 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)

$[\alpha]_D^{20}$ =-10.3 (c 2.17, MeOH) (free base)

^{1}H NMR (300 MHz, MeOH-d_4) (free base): δ = 4.11 (dd, 1H, $J_{3,4}$ = $J_{4,5}$ 2.5 Hz, H-4), 3.66 (bd, 2H, 2x H-6), 3.62 (ddd, $J_{2ax,3}$ 10.58 Hz, $J_{2eq,3}$ 6.12 Hz, H-3), 2.95-2.78 (m, 5H, H-2ax, H-2eq, H-5a, 2x H-6´), 1.64-1.03 (m, 11H, H-5, 2x H1´, 2x H-2´, 2x H-3´, 2x H-4´, 2x H-5´).

^{13}C NMR (75.5 MHz, MeOH-d_4) (free base): δ = 153.2-116.4 (dansyl), 70.0 (C-4), 69.6 (C-3), 61.9 (C-6), 52.6 (C-5a), 46.8 (C-5), 45.9 (C-2), 45.8 (2xNMe_2), 43.7 (C-6´)32.5 (C-1´), 30.4, 30.1, 27.2, 25.7 (C-2´, C-3´, C-4´, C-5´).

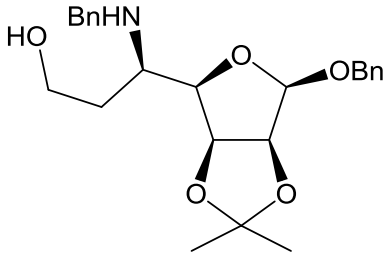
MS: Calcd for [$C_{24}H_{37}N_3O_5SH$]: *m/z* 480.2532 $[M+H]^+$; Found $[M+H]^+$ 480.2480.

MS: Calcd for [$C_{24}H_{37}N_3O_5SNa$]: *m/z* 502.2352 $[M+Na]^+$; Found $[M+Na]^+$ 502.2273.

6.5 (5a*R*)-4-*epi*-isofagomines

Benzyl N-benzyl-5-amino-5,6-dideoxy-2,3-O-isopropylidene-β-D-manno-heptofuranoside (113)

Following general procedure A, ester **109** (1.83 g, 4.0 mmol) was treated with $LiAlH_4$ (0.18g, 4.8 mmol), H_2O (3 mL) and 10 mL NaOH (3 N). Purification on silica gel provided alcohol **113** (1.59 g, 3.8 mmol, 95.7%).



$C_{24}H_{31}NO_5$

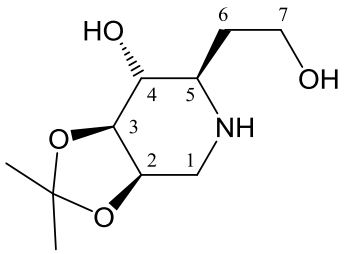
MW:	413.51 g/mol
TLC:	UV, VAN
R_f:	0.2 (C/EA 1:2 v/v)
SCG:	(C/EA 1:2 v/v)
$[a]_D^{20}$ =	-49.3 (c 0.58, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.66 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.60 (m, 1H, H-3), 4.51 (dd, 1H, $J_{2,3}$ 5.9 Hz, H-2), 3.86-3.58 (m, 3H, H-7a/b, 7-OH), 3.57 (dd, 1H, *J* 3.8 Hz, *J* 7.5 Hz, H-4) 3.30 (m, 1H, H-5), 2.51 (m, 2H, H-6a/b).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 139.9, 137.3 (*ipso* NHBn, *ipso* OBn), 128.5-127.2 (aromatic OBn, NHBn), 113.8 (isopropylidene), 101.0 (C-1), 80.0, 79.6, 78.0 (C-2, C-3, C-4), 62.5 (O*CH*$_2$Ph), 57.5 (C-7), 57.5 (C-5), 51.29 (NH*CH*$_2$Ph), 31.2 (C-6), 25.8, 25.2 (isopropylidene).

1,5,6-Trideoxy-1,5-imino-2,3-O-isopropylidene-D-manno-heptitol (115)

Following general procedure B, compound **113** (3.8 g, 9.2 mmol) was converted into crude **115**. Purification on silica gel afforded **XX** (1.9 g, 8.7 mmol, 95.2%) as colourless syrup.



$C_{10}H_{19}NO_4$

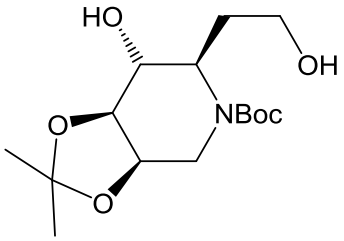
MW:	217.27 g/mol
TLC:	CAM, NIP
R_f:	0.55 ($CHCl_3$/MeOH/NH_4OH 3:1:0.01 v/v/v)
SCG:	($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)
$[a]_D^{20}$ =	-52.3 (c 1.84, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.20 (m, 1H, H-2), 3.89 (dd, 1H, $J_{2,3}$ 5.5 Hz, $J_{3,4}$ 7.1 Hz, H-3), 3.75 (t, 2H, J 6.3 Hz, 2x H-7), 3.38-3.25 (m, 2H, H-1eq, H-4), 2.99 (dd, 1H, $J_{1ax,2}$ 2.8 Hz, $J_{1eq,1ax}$ 14.8 Hz, H-1ax), 2.44 (ddd, 1H, $J_{4,5}$ 11.8 Hz, $J_{5,6}$ 3.3 Hz, $J_{5,6}$ 6.9 Hz, H-5), 2.12-1.98 (m, 2H, 2x H-6).

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 110.1 (isopropylidene), 81.5 (C-3), 75.3 (C-4), 75.1 (C-2), 61.2 (C-7), 59.1 (C-5), 46.4 (C-1), 34.9 (C-6), 28.5, 26.7 (isopropylidene).

N-tert-Butyloxycarbonyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-D-manno-heptitol (160)

Following general procedure C, **115** (1.9 g, 8.7 mmol) was treated with Et_3N (3.1 mL, 21.9 mmol) and $(Boc)_2O$ (2.3 g, 10.1 mmol). Chromatographic purification on silica gel gave compound **160** (2.6 g, 8.1 mmol, 92.5%) as a pale yellow syrup.



$C_{15}H_{27}NO_6$

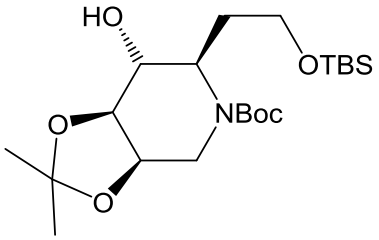
MW:	317.38 g/mol
TLC:	CAM
R_f:	0.5 (C/EA 1:4 v/v/v)
SCG:	(C:EA 1:2 v/v)
$[a]_D^{20}$ =	-34.8 (c 1.08, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.35 (m, 1H, H-2), 4.07 (bs, 1H, H-1eq), 4.01 (dd, 1H, $J_{2,3}$ = $J_{3,4}$ 6.5 Hz, H-3), 3.82 (m, 1H, H-5), 3.74 (m, 1H, H-4), 3.61 (m, 2H, 2x H-7), 2.88 (bs, 1H, H-1ax), 1.97 (bs, 2H, 2x H-6).

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 157.1 (Boc), 110.9 (isopropylidene), 81.7 (Boc), 79.0 (C-3), 72.6, 72.5 (C-4), 72.0, 71.9 (C-2), 60.2 (C-7), 56.1, 55.2 (C-5), 42.8, 41.3 (C-1), 34.9 (C-6), 28.6 (Boc), 27.8, 25.4 (isopropylidene).

N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-D-manno-heptitol (161)

Following general procedure D, compound **160** (640 mg, 2.02 mmol) was treated with imidazole (343 mg, 5.04 mmol) and TBSCl (330 mg, 2.22 mmol). Purification on silica gel afforded **161** (783 mg, 1.81 mmol, 89.9%) as a pale colourless syrup.



$C_{21}H_{41}NO_6Si$

MW: 431.64 g/mol

TLC: CAM

R_f: 0.45 (C/EA 2:1 v/v/v)

SCG: (C:EA 8:1 v/v)

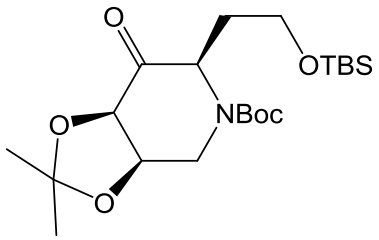
$[a]_D^{20}$ = +1.6 (c 3.46, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.38-4.12 (bm, 2H, H-1eq, H-3), 3.93 (m, 1H, H-2), 3.78-3.45 (m, 4H, H-4, H-5, 2x H-7), 2.67 (m, 1H, H-1ax), 1.97 -1.70 (m, 2H, 2x H-6).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 154.7 (Boc), 110.1 (isopropylidene), 80.4 (Boc), 78.2 (C-2), 73.5 (C-3), 71.3 (C-4), 61.4 (C-7), 56.7, 56.5 (C-5), 42.1, 40.9 (C-1), 36.6, 36.3 (C-6), 28.5 (Boc), 27.4, 24.9 (isopropylidene), 26.0, 18.4, -5.3, -5.4 (TBS).

N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-D-lyxo-hept-4-ulose (162)

Following general procedure F, alcohol **161** (0.98 g, 2.3 mmol, dissolved in 5 mL CH_2Cl_2) was added dropwise to a previously prepared reaction mixture of DMSO (484 µL, 6.8 mmol) and oxaly chloride (487 µL, 5.7 mmol). Addition of Et_3N (1.75 mL, 12.5 mmol) and purification on silica gel provided **162** (0.95 g, 2.2 mmol, 98.0%).



$C_{21}H_{39}NO_6Si$

MW: 429.63 g/mol

TLC: CAM

R_f: 0.6 (C/EA 2:1 v/v)

SCG: (C:EA 6:1 v/v)

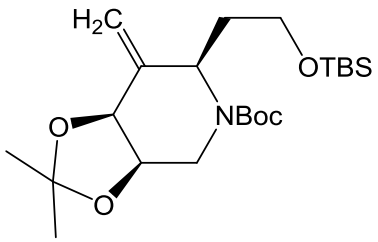
$[a]_D^{20}$ = n.d.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.80 (1H, bs, H-3), 4.46-4.23 (m, 3H, H-1eq, H-2, H-5), 3.67 (bs, 2H, 2x H-7), 2.68 (bs, 1H, H-1ax), 2.17 (bs, 1H, H-6), 1.82 (m, 1H, H-6).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 205.8 (C-4), 154.5 (Boc), 112.2 (isopropylidene), 81.2 (Boc), 79.2 (C-2), 74.3 (C-3), 59.9 (C-7), 59.8 (C-5), 45.2, 43.7 (C-1), 32.1, 31.9 (C-6), 28.4 (Boc), 27.1, 25.3 (isopropylidene), 26.0, 18.4, -5.2, -5.3 (TBS).

N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-O-isopropylidene-4-C-methylene-D-lyxo-heptitol (163)

A solution of 2.5 M *n*-BuLi (7.5 mL, 2.5 N) in hexane was added dropwise to a cooled suspension of methyltriphenylphosphonium bromide (2.8 g, 7.9 mmol) in dry THF (20 mL) under an atmosphere of nitrogen at -78°C. After stirring for 40 min at -20°C a solution of ulose **162** (0.95 g, 2.2 mmol) in dry THF (5 mL) was added dropwise, and the mixture was stirred for 12 h allowing to warm up to ambient temperature. The reaction was diluted with CH_2Cl_2 and successively washed with HCl (6 %) and saturated $NaHCO_3$. The combined organic layers where dried over Na_2SO_4, filtered off and evaporated to dryness. Chromatographic purification provided **163** (0.67 mg, 1.56 mmol, 83.8%) as a pale yellow syrup.



$C_{22}H_{41}NO_5Si$

MW:	427.66 g/mol
TLC:	UV, CAM
R_f:	0.7 (C/EA 3:1 v/v/v)
SCG:	(C:EA 100:1 v/v) to (C:EA 10:1 v/v)
$[\alpha]_D^{20}$ =	-23.3 (c 2.49, $CHCl_3$)

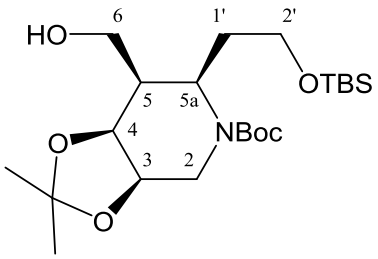
^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.30, 5.23 (2s, 2H, *exo*-methylene), 4.72 (bs, 1H, H-5), 4.51 (d, 1H, $J_{2,3}$ 5.4 Hz, H-3), 4.19 (bs, 1H, H-2), 4.04 (bs, 1H, H-1eq), 3.62 (m, 2H, 2x H-7), 2.84 (bs, 1H, H-1ax), 1.97 (bm, 2H, 2x H-6).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 154.8 (Boc), 140.3 (C-4), 118.7 (*exo*-methylene), 110.0 (isopropylidene), 80.1 (Boc), 77.1 (C-3), 72.9, 72.8 (C-2), 60.3, 60.1 (C-7), 53.8, 52.9 (C-5), 40.9, 39.7 (C-1), 35.3 (C-6), 28.5 (Boc), 28.4, 26.2 (isopropylidene), 26.1, 18.4, -5.2, (TBS).

(5aR)-N-tert-Butyloxycarbonyl-3,4-O-isopropylidene-5a-C-(tert-butyldimethylsilyloxy)ethyl-4-epi-isofagomine (164) *and* *(5aR)-N-tert-Butyloxycarbonyl-6-deoxy-5-C-hydroxy-3,4-O-isopropylidene-5a-C-(tert-butyldimethylsilyloxy)ethyl-4-epi-isofagomine (165)*

Following general procedure I (variant 1), compound **163** (897 mg, 2.09 mmol) was treated with BH_3·THF (1 M, 0.3 mL), H_2O (13 mL), NaOH (3 N, 18 mL) and H_2O_2 (33%, 18 mL). Chromatographic purification on silica gel provided alcohols **164** (668 mg, 1,5 mmol, 71.5%) and **165** (28 mg, 0.06 mmol, 3.0%).

Following general procedure I (variant 2), compound **163** (82 mg, 0,19 mmol) was treated with 9-BBN (1 M in THF, 1.5 mL), H_2O (4 mL), NaOH (3 N, 5 mL) and H_2O_2 (33%, 5 mL). Purification on silica gel provided alcohol **164** (68 mg, 1.5 mmol, 79.6%). The formation of **165** was not observed.



Compound: **164**

$C_{22}H_{43}NO_6Si$

MW: 445.67 g/mol

TLC: CAM

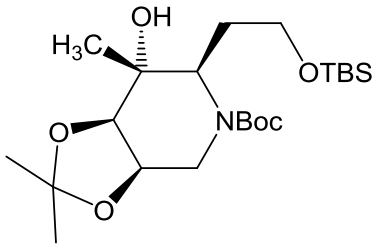
R_f: 0.6 (C/EA 1:1 v/v/v)

SCG: (C:EA 15:1 v/v) to (C:EA 10:1 v/v)

$[a]_D^{20}$ = +10.5 (c 2.03, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.35 (bm, 1H, H-5a), 4.32-3.87 (m, 3H, H-2eq, H-3, H-4), 3.86-3.70 (m, 2H, 2x H-6), 3.65-3.49 (m, 2H, 2x H-2'), 2.90-2.64 (bm, 1H, H-2ax), 2.36 (bd, 1H, 6-OH), 2.17 (bs, 1H, H-5), 2.10-1.71 (m, 2H, 2x H-1').

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 154.8 (Boc), 109.5 (isopropylidene), 80.2, 79.9 (Boc), 73.3, 73.2 (C-4), 71.3, 71.0 (C-3), 61.7 (C-6), 61.4 (C-2'), 48.5, 47.6 (C-5a), 42.2, 41.7 (C-5), 40.5, 39.2 (C-2), 28.7, 28.5 (C-1'), 28.7, 26.2 (isopropylidene), 28.5 (Boc), 26.1, 18.5, -5.2 (TBS).



Compound: **165**

$C_{22}H_{43}NO_6Si$

MW: 445.67 g/mol

TLC: CAM

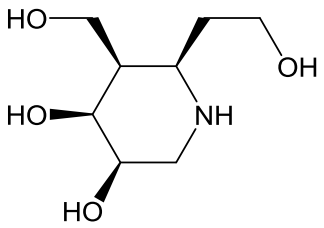
R_f: 0.8 (C/EA 1:1 v/v/v)

SCG: (C:EA 15:1 v/v) to (C:EA 10:1 v/v)

$[a]_D^{20}$ = n.d.

(5aR)-5a-C-(2-Hydroxy)ethyl-4-epi-isofagomine (167)

Following general procedure P, compound **164** (92 mg, 0.21 mmol) was converted into crude **167**. Purification on silica gel afforded **167** (37 mg, 0.19 mmol, 92.5 %).



$C_8H_{17}NO_4$

MW: 191.23 g/mol

TLC: CAM, NIP

R_f: 0.45 ($CHCl_3$/MeOH/NH_4OH 4:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

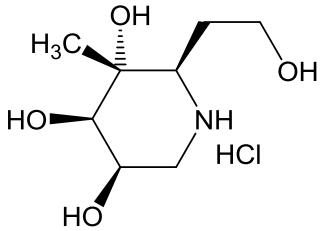
$[a]_D^{20}$ = -5.1 (c 1.83, MeOH (free base)

^{1}H NMR (300 MHz, D_2O) (hydrochloride): δ = 4.13 (dd, 1H, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 4.7 Hz, H-4), 4.09 (ddd, 1H, $J_{2eq,3}$ 5.6 Hz, $J_{2ax,3}$ 3.2 Hz, H-3), 3.91 (d, 2H, 2x H-6), 3.88-3.72 (m, 2H, 2x H-2'), 3.67 (m, 1H, $J_{5,5a}$ 4.8 Hz, H-5a), 3.39 (dd, 1H, $J_{2eq,2ax}$ 13.3 Hz, H-2eq) 3.27 (dd, 1H, H-2ax), 2.26 (m, 1H, H-5), 2.22-1.97 (m, 2H, 2x H-1').

^{13}C NMR (75.5 MHz, D_2O) (hydrochloride): δ = 67.4 (C-4), 65.0 (C-3), 59.0 (C-2'), 56.6 (C-6), 55.3 (C-5a), 45.1 (C-2), 41.0 (C-5), 30.0 (C-1')

(5aR)-6-deoxy-5a-C-(2-Hydroxy)ethyl-5-C-hydroxy-4-epi-isofagomine (166)

Following general procedure P, compound **165** (28 mg, 0.06 mmol) was converted into crude **166** which was an amorphous solid. Recrystallization in MeOH and Et_2O in the presence of a few drops of aq HCl afforded the corresponding hydrochloride **166**·HCl (12 mg, 0.05 mmol, 83.9%) as colourless crystals.



$C_6H_{18}ClNO_4$

MW: 227.69 g/mol

TLC: CAM, NIP

R_f: 0.55 ($CHCl_3$/MeOH/NH_4OH 4:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

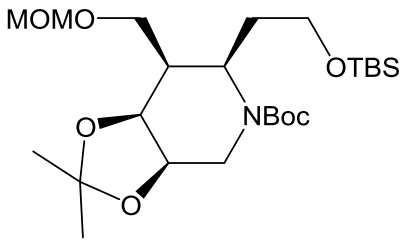
$[a]_D^{20}$ = n.d.

^{1}H NMR (300 MHz, D_2O) (hydrochloride): δ = 4.25 (ddd, 1H, $J_{2,3}$ 8.2 Hz, H-3), 3.85-3.71 (m, 2H, 2x H-2´), 3.70 (d, 1H, $J_{3,4}$ 3.5 Hz, H-4), 3.3 (m, 3H, H-2ax, H-2eq, H-5a), 2.16-1.85 (m, 2H, 2x H-1´), 1.33 (s, 3H, H-6).

^{13}C NMR (75.5 MHz, D_2O) (hydrochloride): δ = 73.7 (C-4), 72.6 (C-5), 64.9 (C-3), 61.9 (C-5a), 59.7 (C-2´), 44.6 (C-2), 27.6 (C-1´), 17.6 (C-6).

(5aR)-N-tert-Butyloxycarbonyl-5a-C-(tert-butyldimethylsilyloxy)ethyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (168)

Following general procedure J, compound **164** (672 mg, 1.5 mmol), was treated with diisopropyl ethyl amine (590 µL, 3.5 mmol) and chloromethyl methyl ether (130 µL, 1.7 mmol). Chromatographic purification on silica gel afforded **168** (648 mg, 1.3 mmol, 87.4 %) as a yellow syrup.



$C_{24}H_{47}NO_7Si$

MW: 489.72 g/mol

TLC: CAM

R_f: 0.6 (C/EA 2:1 v/v)

SCG: (C/EA 6:1 v/v)

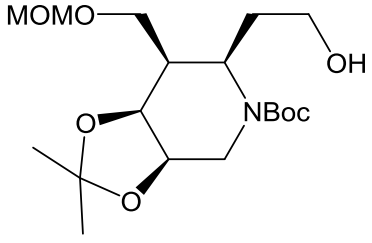
$[\alpha]_D^{20}$ = -3.2 (c 3.54, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.44-4.22 (m, 2H, H-3, H-5a), 4.21-3.88 (m, 2H, H-2eq, H-4), 3.74-3.56 (m, 2H, 2x H-6), 3.57-3.42 (m, 2H, 2x H-2'), 2.85-2.59 (m, 1H, $J_{2ax,3}$ 9.4 Hz, $J_{2ax,2eq}$ 12.3 Hz, H-2ax), 2.25 (m, 1H, H-5), 2.14 (m, 1H, H-2'), 1.60 (m, 1H, H-2').

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 154.7, 154.5 (Boc), 109.1 (isopropylidene), 96.5, 96.4 (MOM), 79.9, 79.6 (Boc), 72.9, 72.8 (C-4), 71.0, 70.7 (C-3), 66.2, 65.9 (C-6), 60.7 (C-2'), 55.2 (MOM), 48.2, 47.2 (C-5a), 40.1, 39.2 (C-5), 39.7, 38.8 (C-2), 30.2, 29.9 (C-1'), 28.5, 26.0 (isopropylidene), 28.5 (Boc), 25.9, 18.21, -5.4, -5.5 (TBS).

(5aR)-N-tert-Butyloxycarbonyl-5a-C-(2-hydroxy)ethyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (169)

Silyl ether **168** (648 mg, 1.3 mmol) was converted into crude **169** employing general procedure K. Purification on silica gel gave alcohol **169** (471 mg, 1.2 mmol, 94.8%).



$C_{24}H_{47}NO_7Si$

MW: 489.72 g/mol

TLC: CAM

R_f: 0.55 (C/EA 2:1 v/v)

SCG: (C/EA 8:1 v/v)

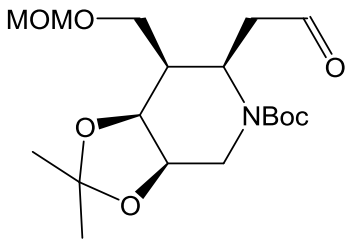
$[\alpha]_D^{20}$ = -3.3 (c 0.73, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.46 (m, 1H, H-5a), 4.31 (m, 1H, H-4), 4.10 (m, 1H, H-3), 4.00 (m, 1H, $J_{2eq,3}$ 7Hz, H-2eq), 3.76-3.61 (m, 2H, 2x H-6), 3.64-3.53 (m, 1H, H-2'), 3.35-3.26 (m, 1H, H-2'), 2.67 (dd, 1H, $J_{2ax,3}$ 9.8 Hz, $J_{2ax,2eq}$ 13.1 Hz, H-2ax), 2.37 (m, 1H, H-5), 2.05-1.60 (m, 2H, 2x H-1').

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 156.3 (Boc), 109.5 (isopropylidene), 96.7 (MOM), 81.0 (Boc), 72.8 (C-4), 71.1 (C-3), 66.0 (C-6), 58.5 (C-2'), 55.5 (MOM), 46.0 (C-5a), 40.5 (C-2), 39.3 (C-5), 29.1 (C-1'), 28.4 (Boc), 28.7, 26.12 (isopropylidene).

(5aR)-N-tert-Butyloxycarbonyl-5a-C-(1-oxo)ethyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (172)

Following general procedure E, alcohol **169** (223 mg, 0.59 mmol) was treated with Dess-Martin periodinane (310 mg, 0.72 mmol). Purification on silica gel afforded **172** (214 mg, 0.57 mmol, 96.5%) as a colourless syrup.



$C_{18}H_{31}NO_7$

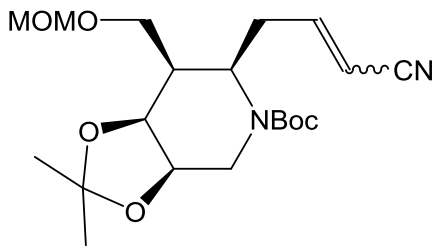
MW:	372.45 g/mol
TLC:	UV, CAM
R_f:	0.6 (C/EA 1:1 v/v)
SCG:	(C/EA 5:1 v/v)
$[\alpha]_D^{20}$ =	-0.68 (c 0.53, $CDCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.66 (s, 1H, H-2'), 5.05-4.69 (m, 1H, H-5a), 4.26 (dd, 1H, H-4), 4.22-4.01 (m, 1H, H-3), 3.92 (m, 1H, H-2eq), 3.73-3.54 (m, 2H, 2x H-6), 2.88 (m, 2H, H-2ax, H-1'), 2.68 (m. 1H, H-1'), 2.37 (bs, 1H, H-5).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 201.5 (C-1'), 154.74 (Boc), 109.6 (isopropylidene), 96.8, 96.6 (MOM), 81.0, 80.7 (Boc), 72.7, 72.6 (C-4), 71.0, 70.9 (C-3), 66.2, 66.0 (C-6), 55.6 (MOM), 45.2 (C-5a), 42.9, 42.8 (C-1'), 60.6, 39.6 (C-2), 39.0 (C-5), 28.4, 25.9 (Boc, isopropylidene).

4-[(5aR)-(N-tert-Butyloxycarbonyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomin-5a-yl)-but-2-enoic nitrile (182)

Following general procedure L, aldehyde **172** (169 mg, 0.45 mmol) was treated with diethyl cyanomethyl phosphonate (96 µL, 0.90 mmol) [deprotonated with *t*-BuOK (91 mg, 0.81 mmol]. Silica gel chromatography provided **182** (mixture of the *E*/*Z*-isomers, 148 mg, 0.37 mmol, 82.5%).



$C_{20}H_{32}N_2O_6$

MW: 396.48 g/mol

TLC: UV, CAM, NIP

R_f: 0.7 (C/EA 1:1 v/v)

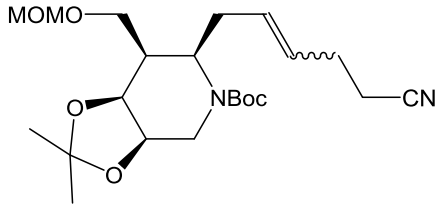
SCG: (C/EA 5:1 v/v)

^{1}H NMR (300 MHz, $CDCl_3$) (*E*/*Z*): δ = 6.69-6.28 (m, 1H, H-2'), 5.39-5.24 (m, 1H, H-3'), 4.67-4.51 (m, 1H, H-5a), 4.44-3.88 (m, 3H, H-2eq, H-3, H-4), 3.82-3.52 (m, 2H, 2x H-6), 3.22 (m, 1H, H-1'), 2.86 (m, 1H, H-1'), 2.78-2.52 (m, 1H, H-2ax), 2.53-2.23 (m, 1H, H-5)

^{13}C NMR (75.5 MHz, $CDCl_3$) (*E*/*Z*): δ = 154.8, 154.7 (Boc), 152.8, 152.7, 152.5, 151.6 (C-2'), 117.1, 117.0, 116.1, 116.1 (C-4'), 109.7, 109.4 (isopropylidene), 101.6, 101.4, 101.3, 100.8 (C-3'), 96.7, 96.5 (MOM), 80.8, 80.6, 80.5, 80.3 (Boc), 72.8, 72.7, 70.7, 70.6 (C-3, C-4), 66.4, 66.2, 65.9, 65.8 (C-6), 55.8, 55.4 (MOM), 48.4, 48.0 (C-5a), 40.2, 40.1, 39.7, 39.6, 39.1, 38.9 (C-2, C-5), 31.4, 31.0, 30.2, 30.0 (C-1'), 28.6, 28.4, 28.2, 26.1, 25.9 (Boc, isopropylidene)

6-[(5aR)-(N-tert-Butyloxycarbonyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomin-5a-yl)-hex-4-enoic nitrile (185)

Following general procedure H, aldehyde **172** (145 mg, 0.39 mmol) was converted into crude **185**. Therefore, diisopropyl amine (0.18 mL, 1.28 mmol), 2.5 M *n*-BuLi (2.0 mL) and triphenyl-(3-cyano)propylphosphonium bromide (557 mg, 1.36mmol) were used. Chromatographic purification afforded **185** (108 mg, 0.25 mmol, 65.5%) as a mixture of the *E*/*Z*-isomers.



$C_{22}H_{36}N_2O_6$

MW: 424.54 g/mol

TLC: UV, CAM, NIP

R_f: 0.62 (C/EA 1:1 v/v)

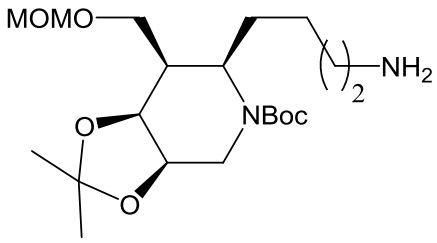
SCG: (C/EA 20:1 v/v) to (C/EA 10:1 v/v)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 5.4 (bs, 2H, H-2´, H-3´), 4.47-3.80 (m, 4H, H-2eq, H-3, H-4, H-5a), 3.60 (m, 2H, 2x H-6), 2.62 (m, 2H, H-2ax, 1x H-1´), 2.45-2.01 (H-5, 1x H-1´, 2x H-4´, 2x H-5´).

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 154.7, 154.4 (Boc), 129.7-126.7 (C-2´, C-3´), 119.1, 119.0 (C-6´), 109.2, 109. 2 (isopropylidene), 96.6, 96.5 (MOM), 80.6, 79.9 (Boc), 73.0, 72.9, 71.0, 70.4 (C-3, C-4), 66.3, 65.9 (C-6), 55.4, 55.3 (MOM), 50.5, 49.3 (C-5a), 40.2, 39.8, 39.3, 38.9 (C-2, C-5, C-4'), 29.7, 29.8, 28.7, 28.3, 25.9 (Boc, isopropylidene), 23.6, 23.3, 17.4 (C-1´, C-4', C-5').

(5aR)-N-tert-Butyloxycarbonyl-5a-C-(4-amino)butyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (183)

Nitrile **182** (148 mg, 0.37 mmol) was converted into **183** employing general procedure M. Purification on silica gel provided amine **183** (145 mg, 0.36 mmol, 96.5%) as a yellow syrup.



$C_{20}H_{38}N_2O_6$

MW: 402.53 g/mol

TLC: CAM, NIP

R_f: 0.4 ($CHCl_3$/MeOH/NH_4OH 3:4:0.01 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)

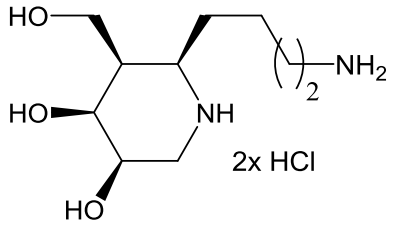
$[a]_D^{20}$ = -3.2 (c 1.05, $CHCl_3$)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.35 (m, 2H, H-4, H-5a), 4.14-3.92 (m, 2H, H-2eq, H-3), 3.69 (m, 2H, 2x H-6), 2.82-2.61 (m, 3H, H-2ax, 2x H-4'), 2.33 (m, 1H, H-5), 2.05 (m, 1H, H-1'), 1.72-1.13 (m, 5H, H-1', 2x H-2', 2x H-3').

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 156.7, 156.5 (Boc), 110.5 (isopropylidene), 97.8, 97.7 (MOM), 81.6, 81.4 (Boc), 74.5, 74.4, 72.2, 72.0 (C-3, C-4), 67.5, 67.3 (C-6), 55.7 (MOM), 52.3, 50.8 (C-5a), 42.1, 42.0, 41.2, 40.7, 40.0 (C-2, C-5, C-4'), 32.4, 31.8 (C-1'), 29.0, 28.7, 28.6, 26.2 (Boc, isopropylidene), 27.4, 27.0, 24.7, 24.3, (C-2', C-3').

(5aR)-5a-C-(4-Amino)butyl-4-epi-isofagomine (184)

Following general procedure P, compound **183** (22 mg, 0.055 mmol) was converted into **184**. Chromatographic purification and subsequent recrystallization of methanolic HCl provided title compound **184**·2HCl (14.5 mg, 0.050 mmol, 91.1%).



$C_{10}H_{24}Cl_2N_2O_4$

MW: 291.21 g/mol

TLC: CAM, NIP

R_f: 0.15 ($CHCl_3$/MeOH/NH_4OH 1:2:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 1:2:1 v/v/v)

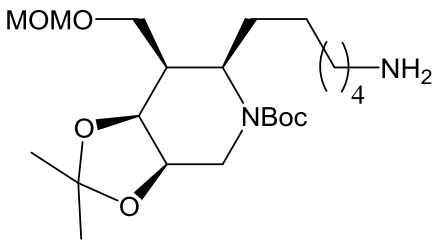
$[a]_D^{20}$ = -9.0 (c 0.36, MeOH) (hydrochloride)

^{1}H NMR (300 MHz, D_2O) (hydrochloride): δ = 4.17 (m, 2H, H-3, H-4), 3.98 (m, 2H, 2x H-6), 3.53 (m, 1H, H-5a), 3.44 (m, 1H, H-2eq), 3.30 (m, 1H, H-2ax), 3.08 (t, 2H, 2x H-4'), 2.35 (m, 1H, H-5), 1.97 (m, 2H, 2x H-1'), 1.80 (m, 2H, 2x H-3'), 1.58 (m, 2H, 2x H-2').

^{13}C NMR (75.5 MHz, D_2O) (hydrochloride): δ = 67.7 (C-3), 64.9 (C-4), 57.0 (C-5a), 55.9 (C-6), 46.4 (C-2), 40.3 (C-5), 39.1 (C-4'), 28.2 (C-1'), 26.4 (C-3'), 22.1 (C-2').

(5aR)-N-tert-Butyloxycarbonyl-5a-C-(4-amino)hexyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (186)

Nitrile **185** (108 mg, 0.25 mmol) was converted into **186** employing general procedure M. Purification on silica gel provided amine **186** (92 mg, 0.21 mmol, 83.9%) as a yellow syrup.



$C_{22}H_{42}N_2O_6$

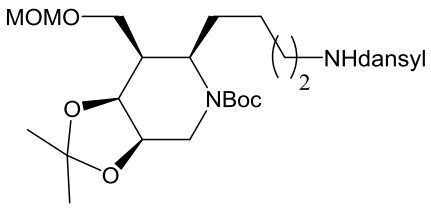
MW:	430.59 g/mol
TLC:	CAM, NIP
R_f:	0.3 ($CHCl_3$/MeOH/NH_4OH 3:4:0.01 v/v/v)
SCG:	($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)
$[\alpha]_D^{20}$ =	-1.2 (c 2.61, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 4.32 (m, 2H, H-4, H-5a), 4.13-3.91 (m, 2H, H-2eq, H-4), 3.68 (m, 2H, 2x H-6), 2.82 (t, 2H, 2x H-6´), 2.81 (m, 1H, H-2ax), 2.32 (m, 1H, H-5), 2.06 (m, 1H, 1x H-1´), 1.69-1.16 (m, 9H, 1x H-1´, 2x H-2´, 2x H-3´, 2x H-4´, 2x H-5´).

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 156.7, 156.5 (Boc), 110.5 (isopropylidene), 97.8, 97.6 (MOM), 81.5, 81.3 (Boc), 74.5, 72.2, 72.0 (C-3, C-4), 67.5, 67.3 (C-6), 55.7 (MOM), 52.2, 50.8 (C-5a), 41.9, 41.4, 41.1, 40.7, 39.9 (C-2, C-5, C-6'), 32.2-26.2 (C-1´, C-2´, C-3´, C-4´, C-5´, Boc, isopropylidene).

(5aR)-N-tert-Butyloxycarbonyl-5a-C-(6-dansylamino)butyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (187)

Following general procedure O, compound **183** (16 mg, 0.04 mmol) was treated with Na_2CO_3 (11 mg, 0.099 mmol) and dansyl chloride (13 mg, 0.048 mmol) to give **187**. Silica gel chromatography provided compound **187** (22 mg, 0.035 mmol, 87.1 %).



$C_{32}H_{49}N_3O_8S$

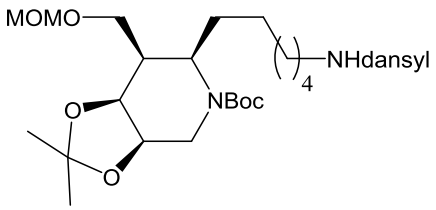
MW:	635.82 g/mol
TLC:	UV, CAM, NIP
R_f:	0.5 (C/EA 1:1 v/v)
SCG:	(C/EA 15:1 v/v) to (C/EA 5:1 v/v)
$[\alpha]_D^{20}$ =	-2.9 (c 0.79, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.24-4.10 (m, 2H, H-3, H-5a), 4.10-3.79 (m, 2H, H-2eq, H-4), 3.51 (m, 2H, 2x H-6), 2.78 (m, 2H, 2x H-4'), 2.47 (m, 1H, H-2ax), 2.17 (m, 1H, H-5), 1.77 (m, 1H, H-1'), 1.46-0.96 (m, 5H, H-1', 2x H-2', 2x H-3').

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.0 (Boc), 145.1-115.3 (dansyl), 109.3 (isopropylidene), 96.6 (MOM), 80.1 (Boc), 73.1, 73.0, 71.1, 70.8 (C-3, C-4), 66.4, 66.0 (C-6), 55.4 (MOM), 50.6, 49.2 (C-5a), 45.6 (NMe_2, dansyl), 43.4 (C-4'), 40.1, 38.7 (C-2), 40.0, 39.5 (C-5), 29.6, 29.3 (C-1'), 28.8, 26.2 (isopropylidene), 28.5 (Boc), 25.9, 23.3, 22.9 (C-2', C-3').

(5aR)-N-tert-Butyloxycarbonyl-5a-C-(6-dansylamino)hexyl-3,4-O-isopropylidene-6-O-methoxymethylene-4-epi-isofagomine (189)

Following general procedure O, compound **186** (92 mg, 0.21 mmol) was treated with Na_2CO_3 (68 mg, 0.64 mmol) and dansyl chloride (69 mg, 0.26 mmol). Purification on silica gel provided **189** (86 mg, 0.13 mmol, 60.6%).



$C_{34}H_{53}N_3O_8S$

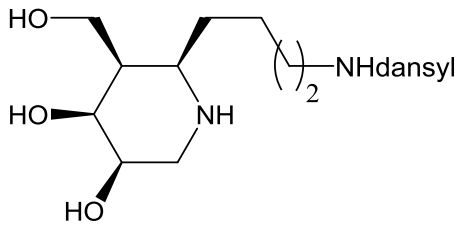
MW:	663.83 g/mol
TLC:	UV, CAM, NIP
R_f:	0.3 (C/EA 2:1 v/v)
SCG:	(C/EA 15:1 v/v)
$[\alpha]_D^{20}$ =	+0.7 (c 0.99, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.26-3.81 (m, 4H, H-2eq, H-3, H-4, H-5a), 3.55 (m, 2H, 2x H-6), 2.78 (m, 2H, 2x H-6´), 2.52 (m, 1H, H-2ax), 2.18 (m, 1H, H-5), 1.78 (m, 1H, 1x H-1´), 1.28-0.94 (m, 9H, 1x H-1´, 2x H-2´, 2x H-3´, 2x H-4´, 2x H-5´).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.0, 154.8 (Boc), 145.1-115.4 (dansyl), 109.3 (isopropylidene), 96.7, 96.6 (MOM), 80.0, 79.9 (Boc), 73.2, 73.1, 71.1, 70.9 (C-3, C-4), 66.5, 66.1 (C-6), 55.5, 55.4 (MOM), 50.8, 49.5 (C-5a), 45.6 (NMe_2, dansyl), 43.5, 43.4 (C-6'), 40.1, 39.5, 38.8 (C-2, C-5), 29.8, 29.6 (C-1'), 28.8, 26.5 (isopropylidene), 28.7, 28.6 (Boc), 27.1-26.2 (C-2', C-3', C-4', C-5').

(5aR)-5a-C-(4-Dansylamino)butyl-4-epi-isofagomine (188)

Following general procedure P, compound **187** (22 mg, 0.035 mmol) was converted into title compound **188**. Purification on silica gel provided **188** (14.7 mg, 0.033 mmol, 94.1%) as fluorescent wax.



$C_{22}H_{33}N_3O_4S$

MW: 451.58 g/mol

TLC: UV, CAM, NIP

R_f: 0.3 ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 3:1:0.01 v/v/v)

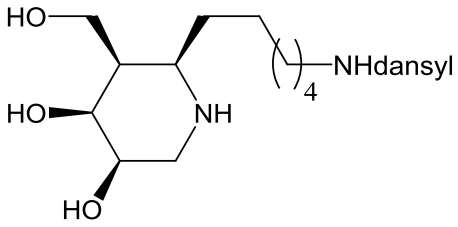
$[\alpha]_D^{20}$ = -9.1 (c 0.40, MeOH) (frEA base)

^{1}H NMR (300 MHz, MeOH-d_4) (free base): δ = 3.84 (dd, 1H, $J_{5,6}$ 3.8 Hz, $J_{6,6}$ 11.7 Hz, H-6), 3.81 (dd, 1H, $J_{3,4}$ 3.2 Hz, $J_{4,5}$ 5.5 Hz, H-4), 3.73 (ddd, 1H, $J_{3,4}$ 3.2 Hz, H-3), 3.70 (dd, 1H, $J_{5,6}$ 4.5 Hz, H-6), 2.85 (m, 2H, 2x H-4´), 2.62 (ddd, 1H, $J_{5,5a}$ 3.7 Hz, $J_{5a,1'}$ 7 Hz, H-5a), 3.02 (dd, 1H, $J_{2eq,3}$ 3.8 Hz, $J_{2ax,2eq}$ 13.6 Hz, H-2eq), 2.76 (dd, 1H, $J_{2ax,3}$ 2.6 Hz, H-2ax), 1.76 (m, 1H, H-5); 1.49 (m, 2H, 2x H-1´), 1.37 (m, 2H, 2x H-3´), 1.24 (m, 2H, 2x H-2´).

^{13}C NMR (75.5 MHz, MeOH-d_4) (free base): δ = 153.2-116.4 (dansyl), 71.2 (C-4), 68.3 (C-3), 58.4 (C-5a), 57.9 (C-6), 43.4 (2xNMe_2), 43.4, 42.3 (C-5, C-4'), 31.6 (C-1'), 30.5 (C-3'), 24.3 (C-2').

(5aR)-5a-C-(4-Dansylamino)hexyl-4-epi-isofagomine (190)

Following general procedure P, crude compound **189** (86 mg, 0.13 mmol) was converted into title compound **190**. Purification on silica gel provided **190** (46 mg, 0.09 mmol, 74.0%) as fluorescent wax.



$C_{24}H_{37}N_3O_4S$

MW: 479.64 g/mol

TLC: CAM, NIP

R_f: 0.3 ($CHCl_3$/MeOH/NH_4OH 3:1:0.01 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)

$[\alpha]_D^{20}$ = -19.1 (c 0.58, MeOH) (free base)

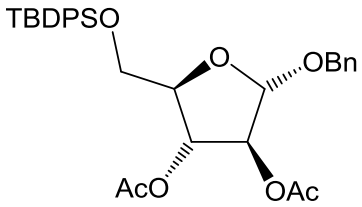
^{1}H NMR (300 MHz, MeOH-d_4) (free base): δ = 3.85 (m, 3H, H-3, H-4, H-6a), 3.70 (dd, 1H, $J_{6a,6b}$ 11.8 Hz, $J_{5,6b}$ 4.5 Hz, H-6b), 3.21 (m, 1H, H-5a), 3.12 (dd, 1H, $J_{2ax,2eq}$ 12.3 Hz, $J_{2eq,3}$ 2.9 Hz, H-2eq), 3.07 (dd, 1H, $J_{2ax,3}$ 2.3 Hz, H-2ax), 3.07 (dd, 1H, 2x H-6´), 2.01 (m, 1H, H-5), 1.66 (m, 2H, 2x H-1´), 1.34-0.96 (m, 8H, 2x H-2´, 2x H-3´, 2x H-4´, 2x H-5´).

^{13}C NMR (75.5 MHz, MeOH-d_4) (free base): δ = 153.2-116.4 (dansyl), 69.3 (C-4), 66.7 (C-3), 58.6 (C-5a), 57.4 (C-6), 45.8 (2xNMe_2), 43.7 (C-6'), 42.3 (C-5), 30.4, 30.1, 29.6, 27.1, 26.5 (C-1´, C-2´, C-3´, C-4´, C-5´).

6.6 *C*-5a-modified isofagomines

Benzyl 2,3-di-O-acetyl-5-O-tert-butyldiphenylsily-α-D-arabinofuranosid (221)

Compound **210 a**,**b** (28.5 g, 55.4 mmol, dissolved in 250 mL CH_2Cl_2) was treated with BnOH (6.9 mL, 66.5 mmol) and BF_3 Et_2O (3.9 mL, 38.8 mmol). After completed conversion of the starting material, the reaction mixture was carefully quenched with saturated $NaHCO_3$. The separated organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. Purification of the remaining residue by column chromatography on silica gel provided compound **211** (22.7 g, 40.4 mmol, 72.8%).



$C_{32}H_{38}O_7Si$

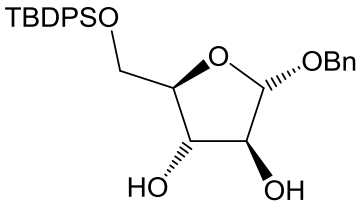
MW:	562.73 g/mol
TLC:	UV, VAN
R_f:	0.5 (C/EA 3:1 v/v)
SCG:	(C/EA 15:1 v/v)
$[\alpha]_D^{20}$ =	+76.2 (c 1.38, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.29 (dd, 1H, $J_{3,4}$ 5.7 Hz, H-3), 5.19 (s, 1H, H-2), 5.12 (s, 1H, H-1), 4.20 (ddd, $J_{4,5a}$ 4.3 Hz, $J_{4,5b}$ 9.3 Hz, H-4), 3.92 (m, 2H, H-5a, H-5b).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 170.1, 169.9 (2x Ac), 137.4-127.7 (2x *ipso* TBTPS, aromatic TBDPS, *ipso* OBn, aromatic OBn), 104.7 (C-1), 83.0 (C-4), 82.2 (C-2), 77.2 (C-3), 68.6 (O*CH$_2$*Ph), 63.3 (C-5), 28.1, 19.4 (TBDPS), 20.9, 20.8 (2x Ac).

Benzyl 5-O-tert-butyldiphenylsily-α-D-arabinofuranosid (212)

To a 10% solution of compound **211** (22.7 g, 40.4 mmol) in MeOH, 5 mL MeONa (1 M) was added dropwise. After completed conversion of the starting material, Amberlite IR120 was added in portions to adjust pH 7. The resin was filtered off and the solvents were evaporated. The remaining residue was quickly passed through silica gel to provide compound **212** (17.9 g, 37.4 mmol, 92.6%)



$C_{28}H_{34}O_5Si$

MW: 478.66 g/mol

TLC: UV, VAN

R_f: 0.3 (C/EA 3:1 v/v)

SCG: (C/EA 5:1 v/v)

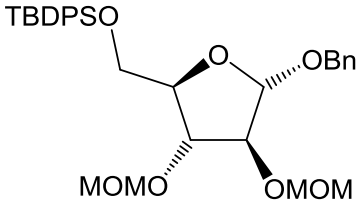
$[a]_D^{20}$ = +57.9 (c 1.00, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.18 (s, 1H, H-1), 4.07 (bs, 2H, H-2, H-4), 4.00 (s, 1H, H-3), 3.77 (dd, 1H, $J_{4,5a}$ 11.4 Hz, $J_{5a,5b}$ 11.4 Hz, H-5a), 3.67 (dd, 1H, $J_{4,5b}$ < 1 Hz).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 137.2-128.1 (2x *ipso* TBTPS, aromatic TBDPS, *ipso* OBn, aromatic OBn), 107.5 (C-1), 87.8 (C-4), 78.6 (C-3), 78.2 (C-2), 69.2 (O*CH*$_2$Ph), 64.2 (C-5), 26.8, 19.2 (TBDPS).

Benzyl 5-O-tert-butyldiphenylsily-2,3-di-O-methoxymethylene-α-D-arabinofuranosid (213):

Following general procedure J, compound **212** (17.4 g, 36.4 mmol), was treated with diisopropyl ethyl amine (55.6 mL, 327.2 mmol) and chloromethyl methyl ether (11.0 mL, 145.4 mmol). Modified to procedure F, the reaction mixture was refluxed for 24h. After addition of MeOH (40 mL), the solvents were removed under reduced pressure. Subsequent purification on silica gel provided **213** (15.4 g, 27.2 mmol, 74.7 %).



$C_{32}H_{42}O_7Si$

MW: 566.77 g/mol

TLC: UV, VAN

R_f: 0.6 (C/EA 3:1 v/v)

SCG: (C/EA 10:1 v/v)

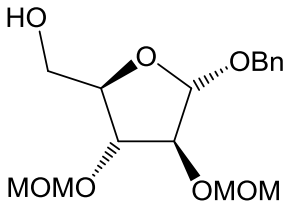
$[a]_D^{20}$ = +57.0 (c 0.97, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.98 (s, 1H, H-1), 4.15-4.02 (m, 3H, H-2, H-3, H-4), 3.79 (dd, 1H, $J_{4,5a}$ = $J_{4,5b}$ 4.6 Hz, $J_{5a,5b}$ 11.1 Hz, H-5a), 3.74 (dd, 1H, H-5b).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 137.9-127.8 (2x *ipso* TBTPS, aromatic TBDPS, *ipso* OBn, aromatic OBn), 105.5 (C-1), 96.2, 95.2 (2x MOM), 86.0 (C-4), 83.3, 81.9 (C-2, C-3), 68.7 (O*CH*$_2$Ph), 64.0 (C-5), 55.7, 55.6 (2x MOM), 26.9, 19.5 (TBDPS).

Benzyl 2,3-di-O-methoxymethylene-α-D-arabinofuranosid (214)

Silyl ether **213** (15.4 g, 27.2 mmol) was converted into crude **214** employing general procedure K. Purification on silica gel gave alcohol **214** (7.6 g, 23.2 mmol, 85.7 %).



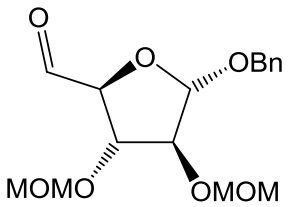
$C_{16}H_{24}O_7$

MW:	328.36 g/mol
TLC:	UV, VAN
R_f:	0.4 (C/EA 1:1 v/v)
SCG:	(C/EA 5:1 v/v)
$[\alpha]_D^{20}$ =	+127.1 (c 0.95, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.99 (s, 1H, H-1), 4.13-4.08 (m, 2H, H-2, H-4), 4.01 (dd, 1H, $J_{2,3}$ 2.2 Hz, $J_{3,4}$ 5.9 Hz, H-3), 3.82 (dd, 1H, $J_{4,5a}$ 3,1 Hz, $J_{5a,5b}$ 12.0 Hz, H-5a), 3.67 (dd, 1H, $J_{4,5b}$ 4.1 Hz, H-5b).
^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 136.6 (*ipso* OBn), 127.5-126.8 (aromatic OBn), 104.5 (C-1), 95.6, 95.0 (2x MOM), 84.9 (C-2), 81.9 (C-4), 80.9 (C-3), 67.8 (O*CH*$_2$Ph), 61.3 (C-5), 54.7, 54.6 (2x MOM).

Benzyl 2,3-di-O-methoxymethylene-5-ulo-α-D-arabinofuranoside (215)

Following general procedure F, alcohol **214** (11.0 g, 33.4 mmol, dissolved in 10 mL CH_2Cl_2) was added dropwise to a previously prepared reaction mixture of DMSO (7.1 mL, 100.5 mmol) and oxaly chloride (7.2 mL, 83.7 mmol). Addition of Et_3N (27.8 mL, 201.0 mmol) afforded crude **215** which was immediately used in the next step.

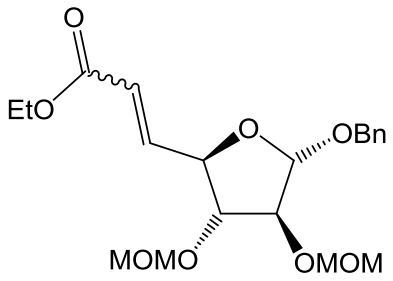


$C_{16}H_{22}O_7$

MW:	326.34 g/mol
TLC:	UV, VAN
R_f:	0.5-0.6 (C/EA 1:1 v/v)

Benzyl 5,6-dideoxy-2,3-di-O-methoxymethylene-hept-5-eno-α-D-arabinofuranoside ethyl uronate (216)

Following general procedure L, crude aldehyde **215** was treated with triethyl phosphonoacetate (10.3 mL, 50.1 mmol) [deprotonated with *t*-BuOK (4.9 g, 43.4 mmol)]. Silica gel chromatography provided **216** (ration *E*/*Z* ca. 95:5, 10.9 g, 27.5 mmol, 81.7% starting from **214**).



$C_{20}H_{28}O_8$

MW: 396.44 g/mol

TLC: UV, VAN

R_f: 0.8 (C/EA 1:1 v/v)

SCG: (C/EA 5:1 v/v)

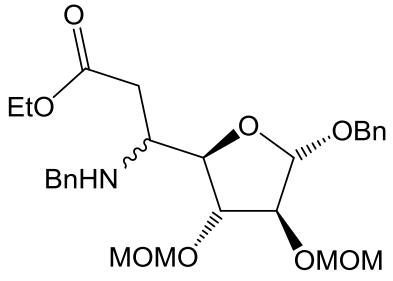
$[\alpha]_D^{20}$ = +79.2 (c 1.075, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.96 (dd, 1H, $J_{4,5}$ 5.4 Hz, $J_{5,6}$ 15.7 Hz, H-5), 6.08 (d, 1H, H-6), 5.03 (s, 1H, H-1), 4.53 (m, 1H, H-4), 4.11 (m, 3H, H-2, O*CH*$_2$CH$_3$), 3.85 (dd, 1H, *J* 6.2 Hz, J 2.0 Hz, H-3), 1.21 (t, 3H, *J* 7.1 Hz, OCH$_2$*CH*$_3$).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 166.1 (C-7), 144.2 (C-5), 137.5 (*ipso* OBn), 128.4-127.9 (aromatic OBn), 122.1 (C-6), 102.5 (C-1), 96.4, 96.1 (2x MOM), 86.1 (C-2), 85.7 (C-3), 80.7 (C-4), 68.9 (O*CH*$_2$Ph), 60.5 (O*CH*$_2$CH$_3$), 55.7 (2x MOM), 14.3 (OCH$_2$*CH*$_3$).

Benzyl 5-benzylamino-5,6-dideoxy-2,3-di-O-methoxymethylene-α-D-altrofuranoside ethyl uronate and Benzyl 5-benzylamino-5,6-dideoxy-2,3-di-O-methoxymethylene-β-L-galactofuranoside ethyl uronate (217 a and 217 b)

Ester **216** (10.9 g, 27.5 mmol) was stirred for 48h in a mixture of MeCN and $BnNH_2$ (10:1 v/v). Removal of the solvents and subsequent purification on silica gel provided a mixture of amines **217a** and **217b** (10.2 g, 20.3 mmol, 73.6 %, ratio ca. 1:1). A complete preparative separation of the achieved isomers was not investigated.



$C_{27}H_{37}NO_8$

MW: 503.59 g/mol

TLC: UV, VAN

SCG: (C/EA 5:1 v/v)

Isomer 217 a: R_f: 0.55 (C/EA 2:1 v/v); $[\alpha]_D^{20}$ = +81.1 (c 84, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.94 (s, 1H, H-1), 4.16-3.96 (m, 5H, H-2, H-3, H-4, O*CH*$_2$CH$_3$), 3.24 (m, 1H, H-5), 2.55 (dd, 1H, $J_{6a,6b}$ 13.7 Hz, $J_{5,6a}$ 4.5 Hz, H-6a), 2.48 (dd, 1H, $J_{5,6b}$ 5.1 Hz, H-6b), 1.15 (t, 3H, *J* 7.2 Hz, OCH$_2$*CH*$_3$).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 172.3 (C-7), 140.6 (*ipso* NHBn), 137.7 (*ispo* OBn), 128.4-127.0 (aromatic OBn, NHBn), 105.3 (C-1), 96.3, 95.8 (2x MOM), 85.9, 83.9, 82.1 (C-2, C-3, C-4), 68.9 (O*CH*$_2$Ph), 60.5 (O*CH*$_2$CH$_3$), 55.7 (2x MOM),54.6 (C-5), 51.4 (NH*CH*$_2$Ph), 36.8 (C-6), 14.3 (OCH$_2$*CH*$_3$).

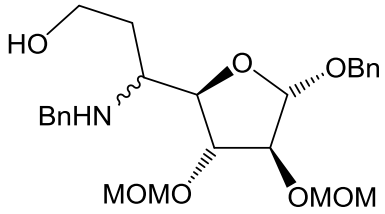
Isomer 217 b: R_f: 0.5 (C/EA 1:1 v/v); $[\alpha]_D^{20}$ = +60.1 (c 0.985, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.95 (s, 1H, H-1), 4.15-3.95 (m, 5H, H-2, H-3, H-4, O*C*$H_2$$CH_3$), 3.24 (m, 1H, H-5), 3.22 (dd, 1H, $J_{6a,6b}$ 16.1 Hz, $J_{5,6a}$ 5.3 Hz, H-6a), 2.55 (dd, 1H, $J_{5,6b}$ 7.2 Hz, H-6b), 1.15 (t, 3H, *J* 7.1 Hz, OCH_2*C*H_3).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 172.3 (C-7), 140.5 (*ipso* NHBn), 137.7 (*ispo* OBn), 128.4-126.9 (aromatic OBn, NHBn), 105.3 (C-1), 96.2, 95.6 (2x MOM), 85.1, 84.3, 82.7 (C-2, C-3, C-4), 68.5 (O*C*H_2Ph), 60.4 (O*C*$H_2$$CH_3$), 55.9, 55.7, 55.6 (C-5, 2x MOM), 51.4 (NH*C*H_2Ph), 35.7 (C-6), 14.3 (OCH_2*C*H_3).

Benzyl N-benzyl-5-amino-5,6-dideoxy-2,3-di-O-methoxymethylene-β-L-galacto-heptofuranoside and Benzyl N-benzyl-5-amino-5,6-dideoxy-2,3-di-O-methoxymethylene-α-D-altro-heptofuranoside (218 a and 218 b)

Following general procedure A, the isomeric mixture of compounds **217 a** and **217 b** (1.23 g, 2.44 mmol) were treated with $LiAlH_4$ (0.14 g, 3.66 mmol), H_2O (5 mL) and 14 mL NaOH (3 N). Purification on silica gel provided **218 a** and **218 b** (0.97 g, 2.10 mmol, 86.0 %) The completed preparative separation of the achieved isomers was not investigated.



$C_{25}H_{35}NO_7$

MW:	461.55 g/mol
TLC:	UV, VAN
SCG:	(C/EA 2:1 v/v)

Isomer 218a: $[\alpha]_D^{20}$ = +56.9 (c 0.91, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.96 (s, 1H, H-1), 4.11 (m, 2H, H-2, H-4), 3.92-3.66 (m, 3H, H-3, H-7a, H-7b), 2.99 (m, 1H, H-5), 1.81 (m, 1H, H-6a), 1.62 (m, 1H, H-6b).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 139.7 (*ipso* NHBn), 137.7 (*ispo* OBn), 128.6-127.5 (aromatic OBn, NHBn), 105.4 (C-1), 96.3, 95.9 (2x MOM), 85.5, 83.5, 83.0 (C-2, C-3, C-4), 68.9 (O*C*H_2Ph), 61.9 (C-7), 59.1 (C-5), 55.9, 55.7 (2x MOM), 55.1 (NH*C*H_2Ph), 30.9 (C-6).

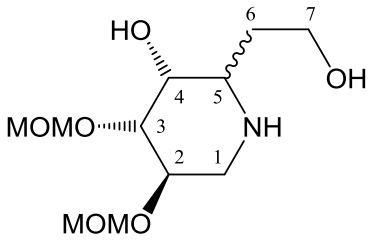
Isomer 218b: $[\alpha]_D^{20}$ = +83.4 (c 1.07, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.98 (s, 1H, H-1), 4.22 (m, 1H, H-4), 4.10 (bs, 1H, H-2), 3.86 (m, 1H, H-3), 3.82-3.64 (m, 2H, H-7a, H-7b), 2.97 (m, 1H, H-5), 1.86 (m, 2H, H-6a, H-6b).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 139.6 (*ipso* NHBn), 137.7 (*ispo* OBn), 128.5-127.2 (aromatic OBn, NHBn), 105.3 (C-1), 96.3, 95.9 (2x MOM), 85.6, 83.1, 82.3 (C-2, C-3, C-4), 68 .8 (O*CH*$_2$Ph), 62.3 (C-7), 59.0 (C-5), 55.7 (2x MOM), 55.5 (NH*CH*$_2$Ph), 30.4 (C-6).

1,5,6-Trideoxy-1,5-imino-2,3-O-methoxymethylene-L-galato-heptitol and 1,5,6-Trideoxy-1,5-imino-2,3-O-methoxymethylene-D-altro-heptitol (219 a and 219 b)

Following general procedure B, the isomeric mixture of compounds **218 a** and **218 b** (2.71 g, 5.85 mmol) was converted into iminoalditols **219 a** and **219 b** (1.35 g, 5.08 mmol, 86.9%), which were immediately employed for the next step.



$C_{11}H_{23}NO_6$

MW: 265.31 g/mol

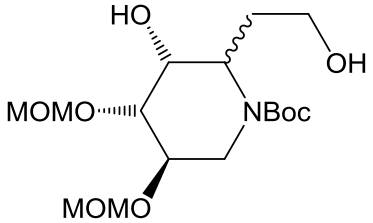
TLC: CAM

R_f: 0.35 ($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)

and 0.40 ($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)

N-tert-Butyloxycarbonyl-1,5,6-Trideoxy-1,5-imino-2,3-di-O-methoxymethylene-L-galato-heptitol and N-tert-Butyloxycarbonyl-1,5,6-Trideoxy-1,5-imino-2,3-di-O-methoxymethylene-D-altro-heptitol (220 a and 220 b)

Following general procedure C, the crude isomeric mixture of **219 a**,**b** (2.73 g, 10.17 mmol) was treated with Et_3N (5.64 mL, 40.71 mmol) and $(Boc)_2O$ (3.99 g, 18.32 mmol). To remove the excess reagents, the concentrated reaction mixture was quickly passed through silica gel (C/EA 1:1 v/v). The resulting mixture of compound **220 a** and **220 b** (2.91 g, 7.9 mmol, 78.3 %) was immediately taken to the next step.



$C_{16}H_{31}NO_8$

MW: 365.42 g/mol

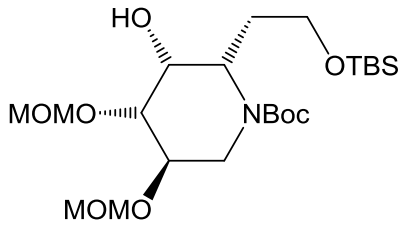
TLC: CAM

R_f: 0.54 ($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)

and 0.55 ($CHCl_3$/MeOH/NH_4OH 8:1:0.01 v/v/v)

N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-Trideoxy-1,5-imino-2,3-di-O-methoxymethylene-D-altro-heptitol (221) and N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-Trideoxy-1,5-imino-2,3-di-O-methoxymethylene-L-galacto-heptitol (222)

Following general procedure D, the isomeric mixture of **220 a** and **220 b** (0,40 g, 1.09 mmol) was treated with imidazole (0.19 g, 2.85 mmol) and TBSCl (0.20 g, 1.31 mmol). Separation of the provided products on silica gel afforded **221** (0.29 g, 0.60 mmol, 55.2%) and **222** (0.10 g, 0.21 mmol, 19.0%).



Compound: **221**

$C_{22}H_{45}NO_8Si$

MW: 479.69 g/mol

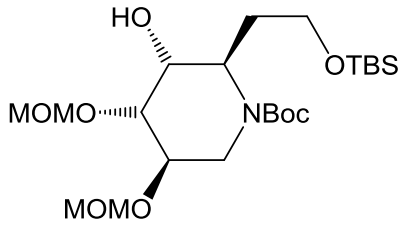
TLC: CAM

R_f: 0.65 (C/EA 1:4 v/v)

SCG: (C/EA 1:1 v/v)

$[a]_D^{20}$ =-12.9 (c 1.41, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.25 (m, 1H, H-5), 4.08-3.95 (m, 2H, H-1eq, H-4), 3.85 (m, 1H, H-2), 3.74 (m, 1H, H-3), 3.71-3.57 (m, 2H, 2x H-7), 3.41 (m, 1H, H-1ax), 2.88, 1.87 (2m, each 1H, 2x H-6).
^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.3 (Boc), 97.9, 94.9 (2x MOM), 79.8 (C-3), 79.7 (Boc), 73.3 (C-2), 66.2 (C-4), 61.9 (C-7), 56.1, 55.7 (2x MOM), 54.0 (C-5), 38.7, 38.5 (C-1), 30.7 (Boc), 26.1, 18.5, -5.3 (TBS).



Compound: **222**

$C_{22}H_{45}NO_8Si$

MW: 479.69 g/mol

TLC: CAM

R_f: 0.55 (C/EA 1:1 v/v)

SCG: (C/EA 6:1 v/v)

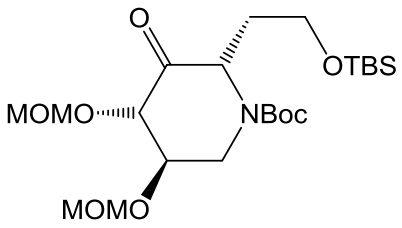
$[a]_D^{20}$ = +6.1 (c 0.915, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.47-4.20 (m, 2H, H-1eq, H-5), 3.98-3.74 (m, 2H, H-2, H-4), 3.61 (m, 3H, H-3, 2x H-7), 2.59 (m, 1H, H-1ax), 1.84-1.57 (m, 2H, 2x H-6).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.7 (Boc), 96.8, 96.5 (2x MOM), 80.1 (Boc), 77.6 (C-3), 73.2 (C-2), 71.2 (C-4), 60.6 (C-7), 56.1, 53.9 (C-5), 42.1, 41.8 (C-1), 33.6 (C-6), 28.5, 28.4 (Boc), 26.0, 18.4, -5.3, -5.4 (TBS).

N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-di-O-methoxymethylene-L-xylo-hept-4-ulose (223)

Following general procedure E, alcohol **221** (0.29 g, 0.60 mmol) was treated with Dess-Martin periodinane (0.31 mg, 0.73 mmol). Purification on silica gel afforded **223** (0.25 g, 0.52 mmol, 86.6%) which was immediately employed in the next step without further characterisations.



$C_{22}H_{43}NO_8Si$

MW: 477.67 g/mol

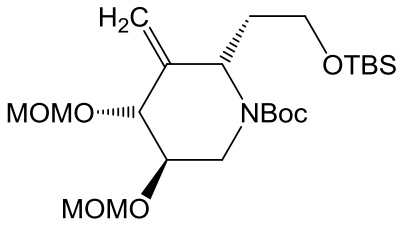
TLC: CAM

R_f: 0.75 (C/EA 2:1 v/v)

SCG: (C/EA 5:1 v/v)

N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-di-O-methoxymethylene-4-C-methylene-L-ribo-heptitol (225)

Following general procedure G, ulose **223** (0.25 mg, 0.52 mmol) was treated with Tebbe´s reagent (0.5 M in toluene, 1.3 mL, 0.65 mmol), Et_2O (10 mL) and NaOH (3 N, 0.7 mL). Purification on silica gel provided compound **225** (150 mg, 0.32 mmol, 60.3%).



$C_{23}H_{45}NO_7Si$

MW: 475.70 g/mol

TLC: CAM

R_f: 0.75 (C/EA 2:1 v/v)

SCG: (C/EA 10:1 v/v)

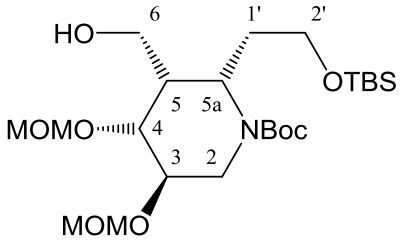
$[a]_D^{20}$ = -50.4 (c 0.88, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.22, 5.08 (2s, each 1H, *exo*-methylene), 4.79 (m, 1H, H-5), 4.28-3.98 (m, 2H, H-1eq, H-3), 3.77 (m, 1H, H-2), 3.68-3.42 (m, 2H, 2x H-7), 3.41 (m, 1H, H-1ax), 1.97, 1.78 (2m, each 1H, 2x H-6).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 155.2 (Boc), 139.4 (C-4), 119.2 (*exo*-methylene), 98.4, 93.1 (2x MOM), 79.8, 79.6 (Boc), 75.2 (C-3), 72.3 (C-2), 60.9, 60.3 (C-7), 55.6 (2x MOM), 54.5, 53.4 (C-5), 37.9, 37.4, 36.9, 36.8 (C-1, C-6), 28.55 (Boc), 26.1, 18.4, -5.2, -5.3 (TBS).

(5aS)-N-tert-Butyloxycarbonyl-3,4-di-O-methoxymethylene-5a-C-(tert-butyldimethylsilyloxy)ethyl-5-epi-isofagomine (227) *and* *(5aS)-N-tert-Butyloxycarbonyl-3,4-di-O-methoxymethylene-5a-C-(tert-butyldimethylsilyloxy)ethyl-isofagomine (228)*

Following general procedure I (variant 2), compound **225** (100 mg, 0.21 mmol) was treated with 9-BBN (0.5 M in THF, 2 mL), H_2O (3 mL), NaOH (3 N, 5 mL) and H_2O_2 (33%, 5 mL) to provide a mixture of crude alcohols **227** and **228** which were immediately taken to the next step.

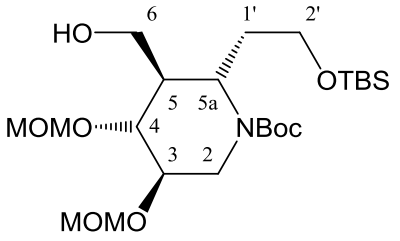


Compound: **227**

$C_{23}H_{47}NO_8Si$

MW: 493.71 g/mol

TLC: CAM



Compound: **228**

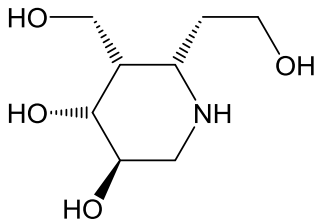
$C_{23}H_{47}NO_8Si$

MW: 493.71 g/mol

TLC: CAM

(5aS)-5a-C-(2-Hydroxy)ethyl-5-epi-isofagomine (229) and (5aS)-5a-C-(2-Hydroxy)ethyl-isofagomine (230)

Following general procedure O, the mixture of crude compounds **227** and **228** were converted into crude **229** and **230**. Purification on silica gel afforded polyols **229** (22 mg, 0.12 mmol, 54.7 % starting from **225**) and **230** (7 mg, 0.04 mmol, 17.4 % starting from **225**).



Compound: **229**

$C_8H_{17}NO_4$

MW: 191.23 g/mol

TLC: CAM, NIP

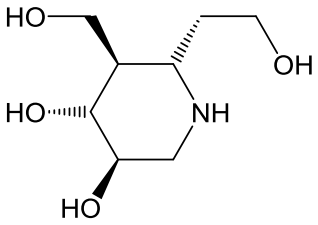
R_f: 0.45 ($CHCl_3$/MeOH/NH_4OH 4:4:1 v/v/v)

SCG: ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

^{1}H NMR (300 MHz, D_2O) (hydrochloride): δ = 4.06 (m, 1H, H-3), 3.92 (dd, 1H, J5,6a 3.2 Hz, J6a,6b 11.6 Hz, H-6a), 3.84-3.59 (m, 4H, H-4, H-6b, 2x H-2´), 3.54 (m, 1H, H-5), 3.42 (dd, 1H, J2ax,2eq 12.8 Hz, J2eq,3 4.7 Hz, H-2eq), 2.83 (dd, 1H, J2ax,3 9.3 Hz, H-2ax), 2.18 (dt, 1H, J 8.3 Hz, J 4.3 Hz, H-5), 2.03 (m, 1H, H-1´), 1.92 (m, 1H, H-1´).

^{13}C NMR (75.5 MHz, D_2O) (hydrochloride): δ = 71.1 (C-4), 65.6 (C-3), 58.7 (C-2´), 56.3, 56.1 (C-5a, C-6), 45.2 (C-2), 39.9 (C-5), 30.2 (C-1´).

^{13}C NMR (75.5 MHz, D_2O) (free base): δ = 73.6 (C-4), 67.4 (C-3), 59.0 (C-2´), 56.6 (C-6), 55.1 (C-5a), 47.6 (C-2), 43.0 (C-5), 32.5 (C-1´).



Compound: **230**

$C_8H_{17}NO_4$

MW: 191.23 g/mol

TLC: CAM, NIP

R_f: 0.40 ($CHCl_3$/MeOH/NH_4OH 4:4:1 v/v/v)

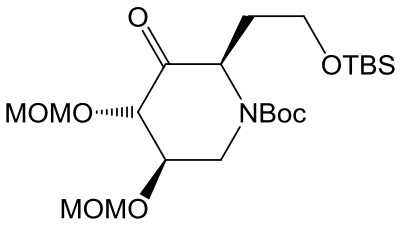
SCG: ($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

^{1}H NMR (300 MHz, D_2O) (hydrochloride): δ = 4.02 (dd, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 3.81-3.65 (m, 4H, H-3, H-6b, 2x H-2´), 3.63 (t, 1H, $J_{3,4}$ = $J_{4,5}$ 9.9 Hz, H-4), 3.50 (dd, 1H, $J_{2eq,3}$ 5.1 Hz, H-2eq), 3.46 (ddd, 1H, $J_{5,5a}$ 11.4 Hz, $J_{5a,1'}$9.1 Hz, $J_{5a,1'}$3.8 Hz, H-5a), 2.89 (t, 1H, $J_{2ax,2eq}$ = $J_{2ax,3}$ 12.0 Hz, H-2ax), 2.25-1.82 (m, 2H, 2x H-1´), 1.74 (m, 1H, H-5).

^{13}C NMR (75.5 MHz, D_2O) (hydrochloride): δ = 70.4 (C-4), 68.4 (C-3), 58.3 (C-2´), 56.1, 55.9 (C-5a, C-6), 46.3 (C-2), 44.9 (C-5), 31.0 (C-1´).

N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-di-O-methoxymethylene-D-arabino-hept-4-ulose (224)

Following general procedure E, alcohol **222** (0.8 g, 1.67 mmol) was treated with Dess-Martin periodinane (1.3 g, 3.00 mmol) to provide crude **224** which was immediately employed in the next step.



$C_{22}H_{43}NO_8Si$

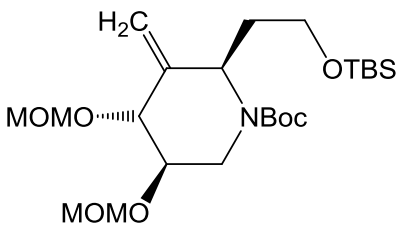
MW: 477.67 g/mol

TLC: CAM

R_f: 0.55 (C/EA 2:1 v/v)

N-tert-Butyloxycarbonyl-7-O-tert-butyldimethylsilyl-1,5,6-trideoxy-1,5-imino-2,3-di-O-methoxymethylene-4-C-methylene-D-arabino-heptitol (226)

Following general procedure G, crude ulose **224** was treated with Tebbe´s reagent (0.5 M in toluene, 4.7 mL, 2.33 mmol), Et_2O (20 mL) and NaOH (1.5 N, 1.7 mL). Purification on silica gel provided compound **226** (250 mg, 0.53 mmol, 31.5% starting from **222**).



$C_{23}H_{45}NO_7Si$

MW: 475.70 g/mol

TLC: CAM

R_f: 0.55 (C/EA 2:1 v/v)

SCG: (C/EA 10:1 v/v)

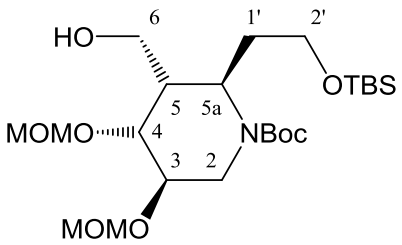
$[\alpha]_D^{20}$ = +4.5 (c 0.935, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.19, 5.04 (2s, each 1H, *exo*-methylene), 4.82 (m, 1H, H-5), 4.41 (m, 1H, H-1eq), 4.20 (m, 1H, H-3), 3.70-3.42 (m, 3H, H-2, 2x H-7), 2.88 (m, 1H, H-1ax), 1.84 (m, 2H, 2x H-6).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 154.5 (Boc), 142.7 (C-4), 110.7 (*exo*-methylene), 96.8, 96.5 (MOM), 80.2 (Boc), 78.5, 78.0 (C-2), 77.4, 76.9 (C-3), 60.4, 59.9 (C-7), 56.4, 55.7 (C-5), 56.0 (MOM), 43.7, 42.6 (C-1), 33. 6 (C-6), 28.5 (Boc), 26.1, 18.4, -5.3, -5.2 (TBS).

(5aS)-N-tert-Butyloxycarbonyl-3,4-di-O-methoxymethylene-5a-C-(tert-butyldimethylsilyloxy)ethyl-5-epi-isofagomine (231)

Following general procedure I (variant 2), compound **226** (250 mg, 0.53 mmol) was treated with 9-BBN (0.5 M in THF, 6.3 mL), H_2O (3 mL), NaOH (3 N, 5 mL) and H_2O_2 (33%, 5 mL) to provide crude alcohol **231** which was immediately taken to the next step.



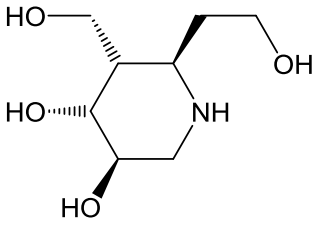
$C_{23}H_{47}NO_8Si$

MW: 493:71 g/mol

TLC: CAM

(5aS)-5a-C-(2-Hydroxy)ethyl-5-epi-isofagomine (232)

Following general procedure P, crude compound **231** was converted into title compound **232**. Purification on silica gel afforded polyol **232** (128 mg, 0.67 mmol, 40.1 % starting from **226**).



$C_8H_{17}NO_4$

MW:	191.23 g/mol
TLC:	CAM, NIP
R_f:	0.45 ($CHCl_3$/MeOH/NH_4OH 4:4:1 v/v/v)
SCG:	($CHCl_3$/MeOH/NH_4OH 8:4:1 v/v/v)

^{1}H NMR (300 MHz, D_2O) (hydrochloride): δ = 3.98 (dd, 1H, $J_{5,6a}$ 5.4 Hz, $J_{6a,6b}$ 11.9 Hz, H-6a), 3.92-3.69 (m, 6H, H-3, H-4, H-5a, H-6b. 2x H-2´), 3.45 (dd, 1H, $J_{2ax,2eq}$ 13.3 Hz, $J_{2eq,3}$ 3.6 Hz, H-2eq), 3.17 (dd, 1H, $J_{2ax,3}$ 7.8 Hz, H-2ax), 2.06 (m, 1H, H-5), 1.92 (m, 2H, 2x H-1´).

^{13}C NMR (75.5 MHz, D_2O) (hydrochloride): δ = 68.7, 67.2 (C-3, C-4), 59.1 (C-6), 58.8 (C-2´), 53.2 (C-5a), 43.5 (C-2), 43.2 (C-5), 27.5 (C-1´).

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M. Zoidl, B. Müller, C. Hojnik, M. Schalli, M. Thonhofer, A. E. Stütz, A. Siriwardena, and T.M. Wrodnigg;
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“Synthesis of 2,5-Dideoxy-2,5-imino-D-Hexitol Derivatives: Potential Inhibitors of Lysosomal α-Galactosidase?“,
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“Synthesis of 2,5-Dideoxy-2,5-imino-D-Hexitol Derivatives: Potential Inhibitors of Lysosomal α-Galactosidase?“,
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16th European Carbohydrate Symposium, 03.-07. July **2011**, Sorrento, Italy.

Curriculum Vitae

Martin Simon Thonhofer, geboren am 22. April 1982 in Linz als Sohn von Dr. Kurt Thonhofer und Mag. Gertrude Thonhofer, geborene Payr.

1988-1992	Besuch der Volksschule 2, Linz
1992-1996	Besuch des BRG Auhof, Linz
1996-2001	Besuch der HTBLA 2 Paul-Hahn (Zweig für Maschinenbau)
Juni 2001	Reifeprüfung
2001-2002	Ableistung eines einjährigen Präsenzdienstes
WS 2002	Beginn des Studiums der Mechatronik an der Johannes Kepler Universität Linz
WS 2003	Beginn des Studiums der Technischen Chemie an der Technischen Universität Graz
Dezember 2009	Erste Diplomprüfung; Fortsetzung des Studiums in der Studienrichtung „Allgemeine Technische Chemie“
Februar 2011-2012	Durchführung der Diplomarbeit mit dem Thema: „C-5 Modifications in the Hexose Series“ am Institut für Organische Chemie der Technischen Universität Graz
09.08.2012	Zweite Diplomprüfung mit „Auszeichnung“ bestanden
Ab Sep. 2012	Beginn der Dissertation mit dem Thema: „Probes and Potential Drugs for Lysosomal Diseases and Alzheimer´s“ unter der Leitung von Herrn Prof. Arnold E. Stütz am Institut für Organische Chemie der Technischen Universität Graz