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A pre-commercial de novo synthesis of 5a-C-pentyl-4-epi-isofagomine - a powerful pharmacological chaperone for GM1-gangliosidosis

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AFFIDAVIT

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Für meine Eltern und Beate

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Simplicity is the highest goal, achievable when you have overcome all difficulties.

- Frédéric Chopin

Abstract

Lysosomal storage disorders, including G_{M1}-gangliosidosis, are a series of inheritable diseases, which are caused by deficiencies of lysosomal enzymes. Due to genetic mutations, these enzymes often fail to convert their intended substrates, such as glycosphingolipids, properly, which results in dysfunctions of the metabolic pathways. Consequently, the respective metabolites accumulate within the cells and cause various symptoms. A recent therapeutic approach, the chaperone mediated therapy, involves carbohydrate based inhibitors, which are able to promote correct protein folding, because of their strong interactions with the affected enzyme. Iminosugars, which feature a basic nitrogen instead of the endocyclic oxygen, and isoiminosugars, are among the strongest inhibitors of glycosidases, hence, they are considered very promising substances to be used as pharmacological chaperones. The current preparation of *C*-5a-modified isoiminosugars however, is quite challenging as it involves many synthetic steps. Within the scope of this thesis, a *de novo* synthesis was developed, which allows for an easy access to a variety of different *C*-5a-modified isoiminosugars, employing the Morita-Baylis-Hillman reaction.

Kurzfassung

Lysosomale Speicherkrankheiten, wie z.B. G_{M1}-Gangliosidose, sind eine Reihe von erblich bedingten Stoffwechselerkrankungen, die aufgrund von fehlerhaften lysosomalen Enzymen auftreten. Diese Enzyme können aufgrund von genetischen Mutationen die vorgesehenen Substrate, wie etwa Glycosphingolipide, nicht ordnungsgemäß abbauen und es kommt zur Störung des jeweiligen Stoffwechselwegs. Folglich reichern sich die entsprechenden Metaboliten in den betroffenen Zellen an und lösen dadurch unterschiedliche Krankheitsbilder aus. Ein neuartiger Therapieansatz, die Chaperon-Therapie, beruht auf Kohlenhydrat-basierten Inhibitoren, die aufgrund der starken Wechselwirkungen mit den betroffenen Enzymen, diese bei der richtigen Faltung unterstützen können. Iminozucker, bei denen der Ring-Sauerstoff durch einen basischen Stickstoff ersetzt wurde, sowie Isoiminozucker, zählen zu den stärksten Glycosidase-Inhibitoren, und sind somit sehr vielversprechende Substanzen für eine Anwendung als pharmakologische Chaperone. Die Herstellung von *C*-5a-modifizierten Isoiminozuckern ist derzeit jedoch mit großem Aufwand verbunden, weil sie viele synthetische Stufen umfasst. Im Rahmen dieser Arbeit wurde eine *de novo* Synthese entwickelt, die einen einfacheren Zugang zu einer großen Menge an unterschiedlichen *C*-5a-modifizierten Isoiminozuckern mit Hilfe der Morita-Baylis-Hillman Reaktion ermöglicht.

Content

1 Introduction

1.1 Carbohydrates[1–3]

Carbohydrates can be considered nature's most abundant organic compounds, as they account for the largest share of biomass on earth with cellulose being the main product of photosynthesis. Thus, they do not only play a vital role as biological energy source, but are also involved in structural frameworks, cell recognition and communication, and many other regulatory processes. Although the historical formula $C_n(H_2O)_n$ might not indicate much structural diversity at first sight, natural exceptions occur frequently and modified carbohydrates hardly agree to that rule. Due to the high number of stereochemical relations, various possibilities to introduce heteroatoms, different connectivity between multiple carbohydrate units and common linkage to other biomolecules, carbohydrate chemistry provides a challenging playground for synthetic as well as biochemists.

One approach of characterisation can be made by distinguishing between monosaccharides (one carbohydrate unit) di- and oligosaccharides (2 and 3-9 units) and polysaccharides (10 and more units). The latter can be formed either between the same carbohydrate motifs (homo oligo- and homo polysaccharides) or between different monosaccharides (hetero oligo- and hetero polysaccharides).

1.1.1 Monosaccharides

Monosaccharides may be classified according to different criteria, like the amount of carbon atoms (4 C = *tetrose*, 5 C = *pentose*, 6 C = *hexose*, and so on), the position of the carbonyl group (*aldose* at *C*-1, or *ketose* usually at *C*-2), the different configuration of the stereo-centres (for example: D-*gluco*, or D-*galacto* configuration) or the constitution of the formed ring systems.

Glyceraldehyde, for example, the simplest monosaccharide, can be regarded a *triose* (3 C) and an aldose, since its carbonyl group exists in form of an aldehyde at position *C*-1. There are two possible stereoisomers of this *aldotriose*, namely the (*R*)-configured D-glyceraldehyde (**1**) and the (*S*)-configured L-glyceraldehyde. These two epimers served as template for the historic determination of D/L-sugars, in which the configuration at the last (furthest away from the carbonyl group) chiral carbon atom is either on the right (D) or on the left (L) side, when viewed in the Fischer projection. (**Figure 1**) Originally D/L-nomenclature stems from the Latin terms *dexter* (right) and *laevo* (left), as they refer to the observed optical rotation of glyceraldehyde, when

exposed to polarised light. This convention based on rotation just happened to concur with the configuration in Fischer projection by coincidence.

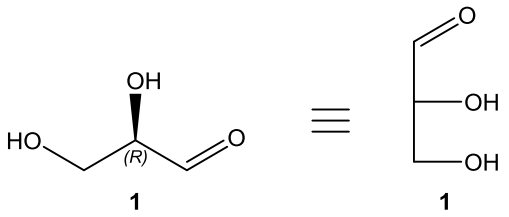


Figure 1. Skeletal formula (*left*) and Fischer projection (*right*) of D-glyceraldehyde (**1**).

An important feature of monosaccharides is the ability to form hemiacetals between the oxygen of a hydroxyl group, and the carbonyl function in an intramolecular fashion. In hexoses, for example, this causes the formation of five- or six-membered ring systems, which are called furanoses and pyranoses, respectively. The cyclisation of such acetals creates a new stereocentre at the former carbonyl carbon position, which is also known as the anomeric carbon atom. The two possible isomers are notated as α- and β-forms. In the simple case of an aqueous solution of D-glucose (**2**), all possible forms of α-and β-furanose and -pyranose rings are in equilibrium with the open-chain monosaccharide, as demonstrated in **Figure 2**.

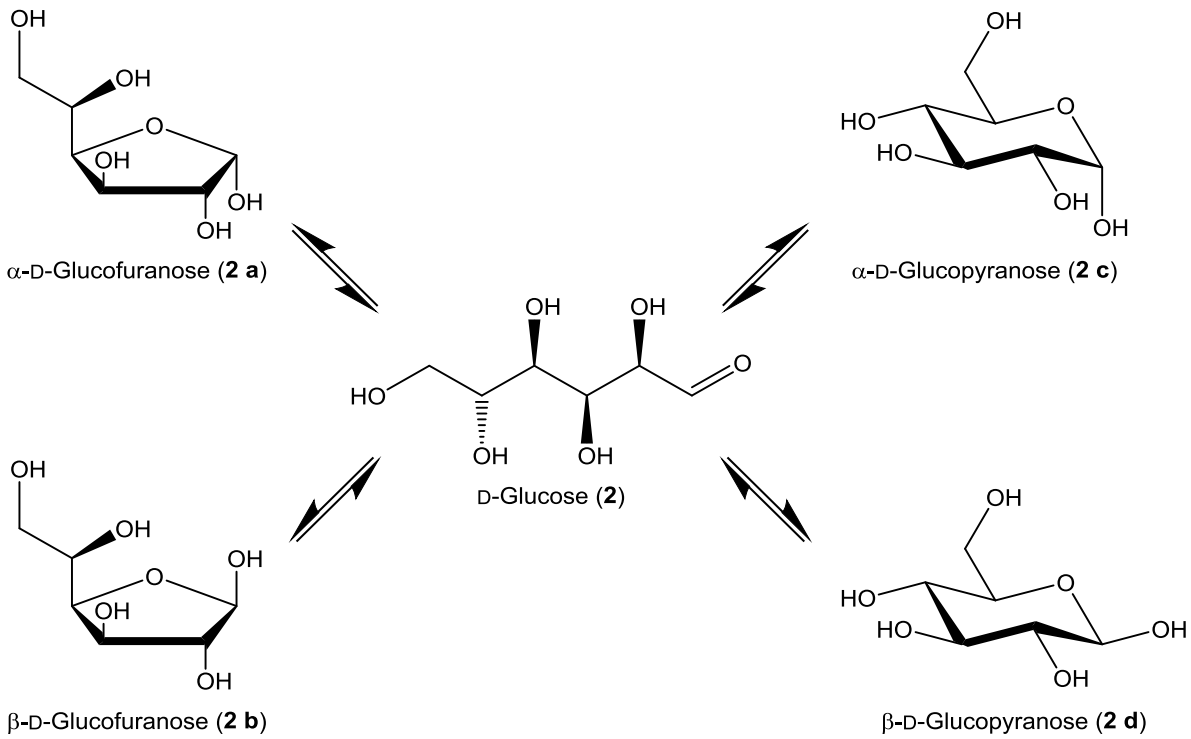


Figure 2. Equilibrium of D-glucose (**2**) in aqueous solution.

1.1.2 Di-, oligo- and polysaccharides

Oligo- and polysaccharides are the oligomers and polymers of monosaccharides, which are not only found as linear chains, but also as branched assemblies or as well defined cycles (cyclodextrines). The number of theoretical arrangements can become astronomically large, if we take into account that even a simple disaccharide, consisting of two identical D-glucopyranose motifs, allows for 11 different constitutions: There are four hydroxyl groups on *C*-2, *C*-3, *C*-4 and *C*-6, which can be linked to the other monosaccharide's hemiacetal position via a so-called *O-glycosidic bond*. In that case, the acetal at the anomeric position can either have α- or β-configuration (α *C*-2, β *C*-2, α *C*-3, β *C*-3, ...) and also form a bond with the anomeric position of the second D-glucose unit, either in form of an α-α, β-β or α-β linkage. One common product from starch degradation, maltose (**3**), is an example for an α(1→4) linked disaccharide, depicted in **Figure 3**, while trehalose (**4**), which was first isolated from an ergot of rye[4], is an α(1→1)α glycosidically linked disaccharide.

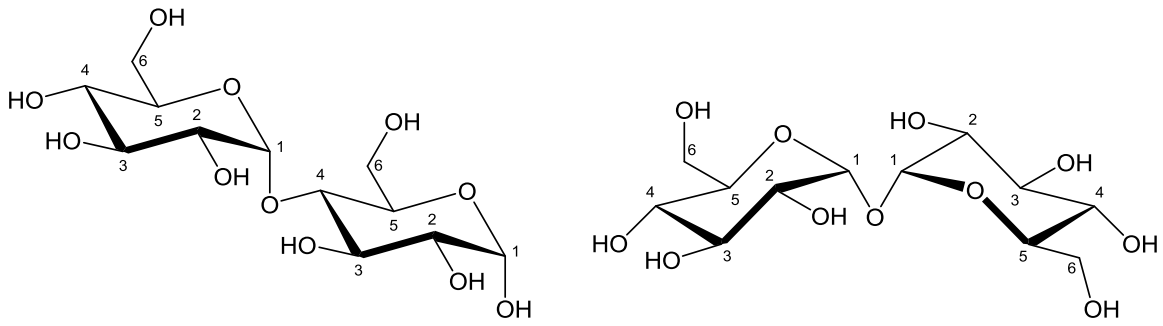


Figure 3. Examples for two different D-glucose disaccharides.

One important difference between the two disaccharides in **Figure 3** is the remaining hemiacetal function in maltose (**3**) as opposed to trehalose (**4**). The aldehyde in **3** can act as a reduction agent, undergoing oxidation to the respective carboxylic acid. This *reducing end* becomes an essential feature, when we want to understand different chemical and biological properties of various carbohydrate assemblies, which can be broken down enzymatically.

1.2 Glycosidases

Carbohydrate processing enzymes are ubiquitous in all living organisms. A large group of these enzymes are made up by glycosyl hydrolases, which are known as glycosidases. Since their main function is the selective cleavage of glycosidic bonds, they are essential for the degradation of di- and oligosaccharides, polysaccharides and glycoconjugates. They can be found within the endoplasmic reticulum and the Golgi apparatus, where they are processing glyco-conjugated proteins. In addition, they are involved in the degradation of oligosaccharides and other glycoconjugates, such as glycosphingolipids or glycosaminoglycans in lysosomes. Their reaction principle is shown in **Figure 4**.

According to the enzyme commission number (EC), they can be divided into *O*- and *S*-glycosidic cleaving hydrolases (EC 3.2.1) and *N*-glycosidic hydrolases (EC 3.2.2). However, this EC system is not fully capable to reflect the structural features of the enzymes. Thus, an alternative system, based on amino acid sequence similarities of glycosyl hydrolases, the GH-classification system, has been established.[5,6]

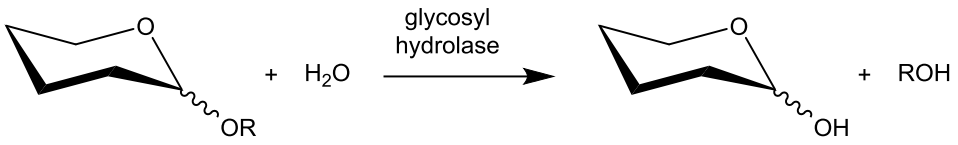


Figure 4. General reaction of glycosyl hydrolases.

1.2.1 Hydrolase mechanisms

The products of glycosyl hydrolase catalysed reactions are the free sugar hemiacetal and the corresponding free alcohol, thiol or amine moiety which was linked via glycosidic bond, the *aglycon*. Such acid-base-catalysed cleavage reactions allow for two different stereochemical outcomes at the anomeric position: If the absolute configuration at the anomeric position remains the same, the respective glycosidase is referred to as *retaining glycosidase*. In case of an inversion of the configuration, the enzyme is considered an *inverting glycosidase*. Both, retaining and inverting glycosidases, generally use two carboxylic acid residues from aspartate or glutamate in their active centre to serve as acid and base, respectively, during the reaction. While the distance between those moieties ranges from 6 – 12 Å in inverting glycosidases, they are found to be closer together in retaining glycosidases with an average distance of 5 Å.[7–9] This difference in size can be explained by looking at the respective reaction mechanisms, which both include the formation of a distorted positively charged oxocarbenium-like transition state.[10]

The mechanism of an inverting α-glucosidase starts with the nucleophilic attack of an activated water molecule of the anomeric position and the protonation of the glycosidic oxygen atom. During the transition state, the water molecule, the prospective aglycon, and the oxo-carbenium species are arranged in some stacked fashion between the two carboxyl motifs.[11–13] After release of the aglycon, the absolute configuration at the anomeric position becomes inverted from α to β or the other way around. In the example in **Figure 5** the hydrolytic cleavage product of the α-D-glucopyranoside **5** is β-D-glucopyranose (**2 d**).

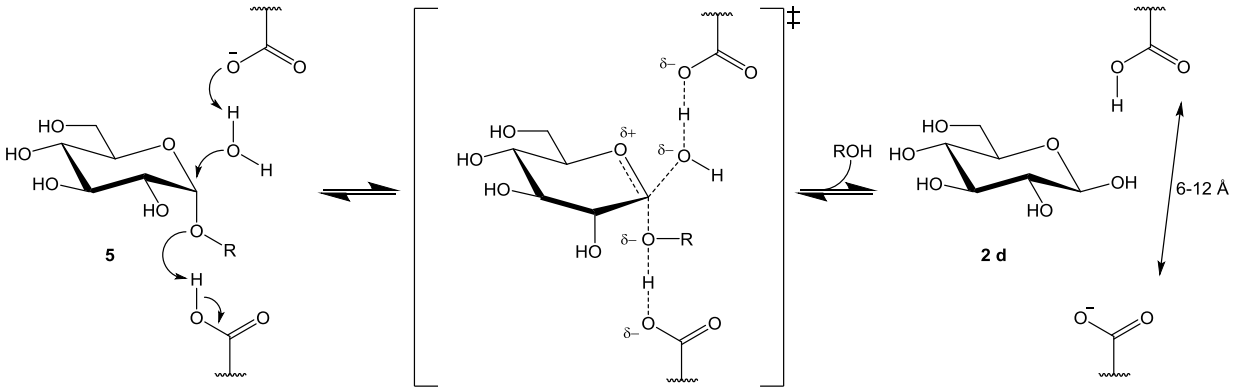


Figure 5. Mechanism of an inverting α-glucosidase.[8]

The retaining glycosidases (**Figure 6**) act according to a double-displacement mechanism, where the anomeric carbon of **5** is first being attacked by the carboxylate residue, while the other one serves as proton source for the glycosidic oxygen moiety. While the protonated aglycon is released, the carbohydrate is bound to the enzyme's active site via a glycosidic bond with inverted configuration at the anomeric position. In the second step an activated water molecule gets added to the carbohydrate, resulting in a second inversion of the anomeric carbon. Thus, the configuration of the released carbohydrate **2 c** equals the original configuration.[11–13] Since the aglycon and the water molecule do not participate in the same transition state, the distance between both carboxylic residues can be much shorter, compared to the inverting glycosidase.

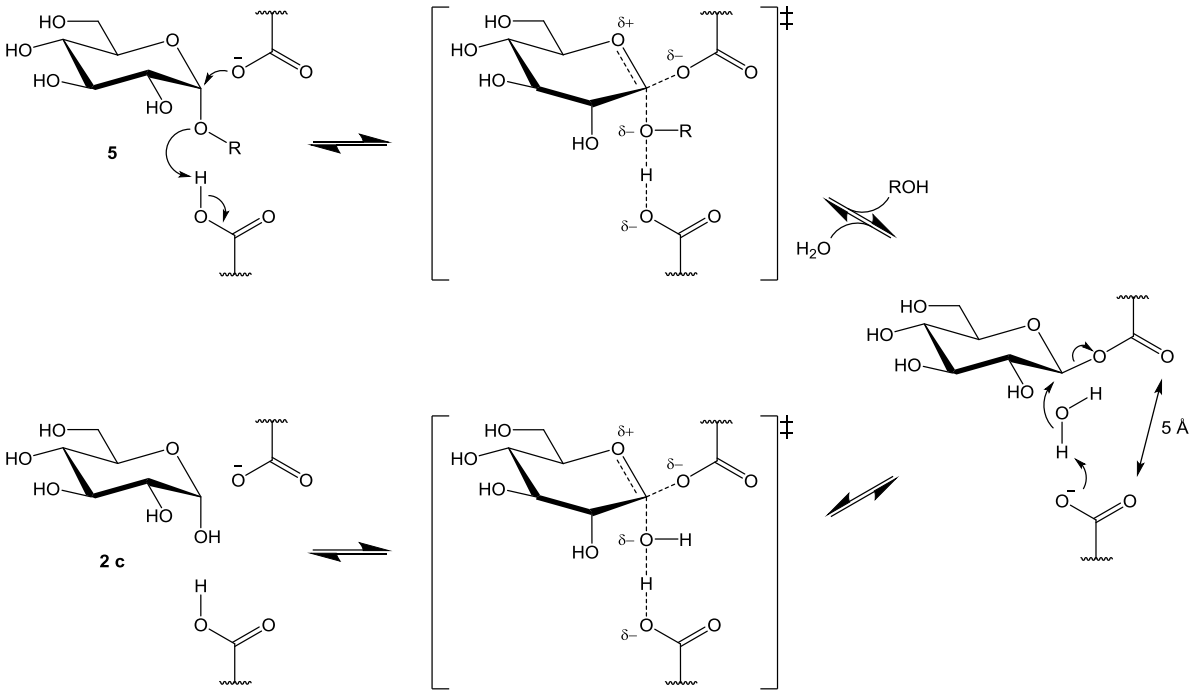


Figure 6. Mechanism of a retaining α-glucosidase.[8]

1.2.2 Glycosidase inhibitors

Depending of their influence on the behaviour of glycosidases and their interference with the catalytic mechanisms, different kinds of inhibitor molecules are known. They can either be natural occurring or designed synthetically, they can act reversibly or irreversibly, and they can either be carbohydrate- or non-carbohydrate based.

The natural flavonoid-derived kushenol A (**6**), (-)-kurarinone (**7**) and sophoraflavanone G (**8**), for example, which were found to be reversible glycosidase inhibitors, can be isolated from *Sophora flavescens* together with six other active constituents. (**Figure 7**) The IC_{50} values of **6**, **7** and **8** for α-amylase (from *Bacillus licheniformis*) are 45 µM, 68 µM and 37 µM, and alternatively, 980 µM, 85 µM and 55 µM for β-amylase (from *Barley*).[14]

Another example is based on a group of aromatic urea derivatives, which turned out to be good competitive non-carbohydrate based α-glucosidase inhibitors with K_i = 3.2 µM, 4.6 µM and 7.2 µM for **9**, **10** and **11** respectively.[15]

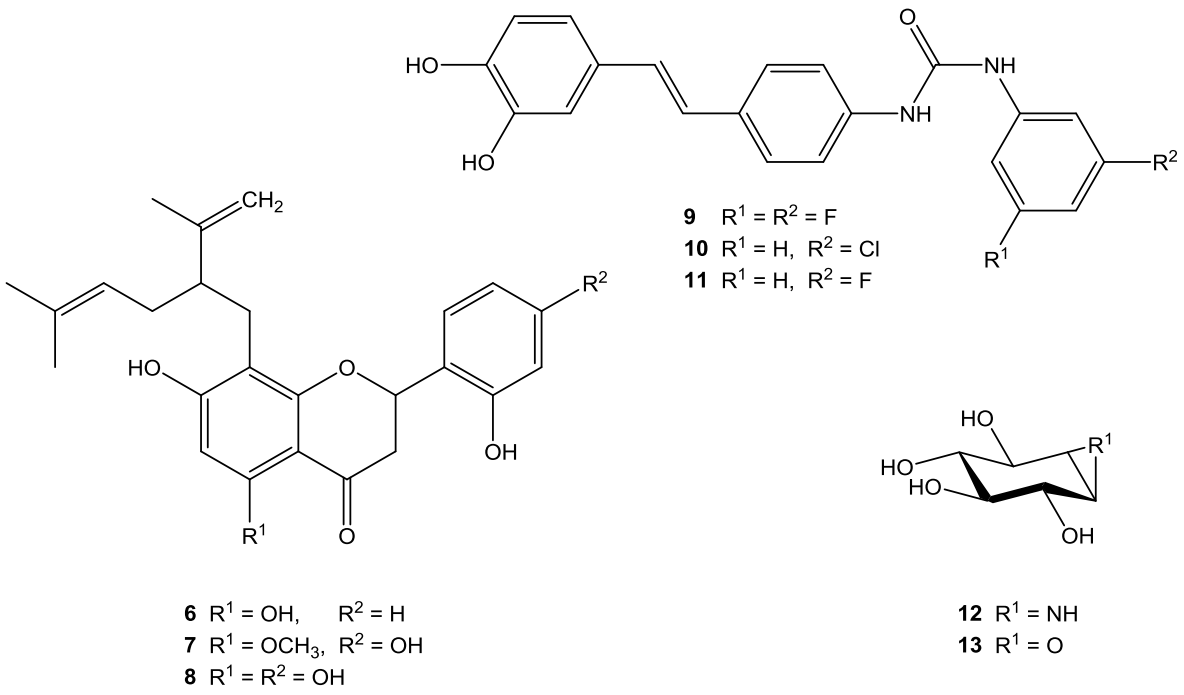


Figure 7. Examples of different glycosidase inhibitors.

As opposed to these "non-sugar" compounds, the aziridine- and epoxide based sugar derivatives **12**[16] and **13**[17,18] are irreversible glycosidase inhibitors. Upon reaction with a carboxylic residue of the enzyme's active site, the strained three-membered ring gets opened through a nucleophilic attack and the inhibitor molecule becomes covalently bound to the respective glycosidase.

Conduritol B-epoxide (**13**), for example, can be used to elucidate the catalytic nucleophile of various glucosidases, for example human lysosomal α-glucosidase.[19] After subsequent degradation of the respective enzyme, the esterified amino acid can be identified. Due to the structurally inherent symmetry, **13** shows biological activity towards α- as well as β-glucosidases, as depicted in **Figure 8**.

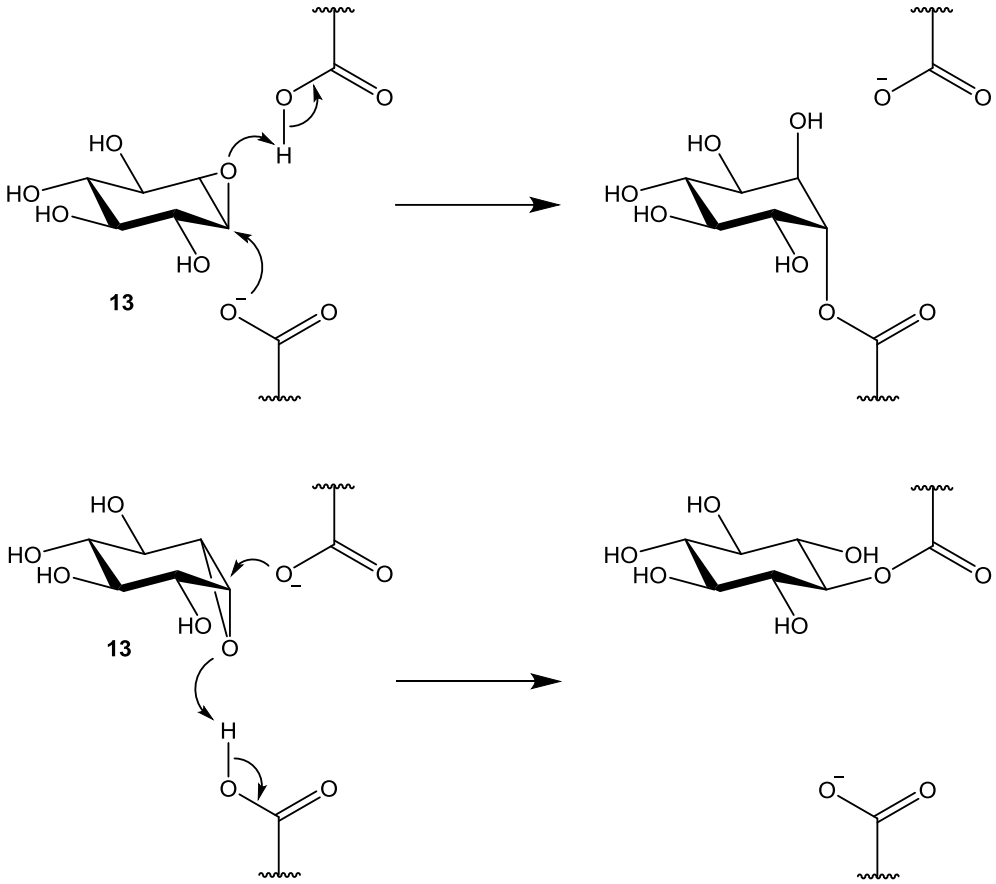


Figure 8. Mechanisms of irreversible inhibitor **13**.[20]

Despite these examples, the biggest group of reversible glycosidase inhibitors consists of analogues of the enzyme's natural substrates featuring incorporated nitrogen atoms. The reason for their outstanding biological activities originates from the strong ionic interactions between the negatively charged carboxylic acid residue at the enzyme's active site and the positively charged protonated nitrogen. This protonation can occur in two different ways: Either as a consequence of the environmental pH-value in aqueous solution, whereupon the positively charged inhibitor molecule interacts with the enzyme, or the nitrogen-bearing sugar gets to the active site as free base and becomes protonated by one of the glycosidase's carboxylic residues.[21]

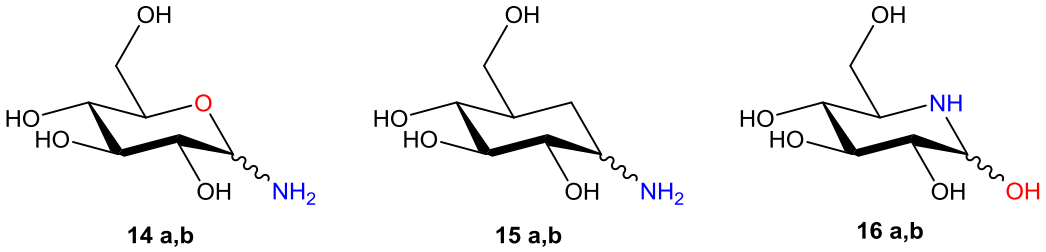


Figure 9. Nitrogen containing analogues of D-glucopyranose

If this nitrogen occurs as a glycosidically linked amine group like in glucosylamine (**14 a,b**) (**Figure 9**), these carbohydrates are referred to as aminoglycosides.[22] The class of carbasugars, like validamine[23] (**15 a,b**), are cyclitols with a cycloalkane scaffold bearing an exocyclic nitrogen moiety. Such carbasugars were first isolated from different bacteria.[24,25]

If the endocyclic oxygen is replaced by a nitrogen atom, like in the well-known nojirimycin[26] (**16 a,b**), the respective sugar analogues are considered iminosugars. This compound class is probably the most prominent one among all types of reversible glycosidase inhibitors, and will be further discussed in the following chapter.

1.3 Iminosugars

1.3.1 A large family potential glycosidase inhibitors

During the discovery period of the first iminosugars in the early 1960's, the new properties arising from the trivalent nitrogen were initially not easy to predict.[27–29] In 1976, ten years after the first synthesis of 1-deoxynojirimycin (DNJ) (**17**) by Paulsen,[30] this compound was found to be a potent inhibitor of α-glucosidases. Besides pyranosidic analogues such as DNJ (**17**), furanosidic analogues such as 2,5-dideoxy-2,5-imino-D-mannitol (**18**) and bicyclic ring systems were also found to be potent glycosidase inhibitors.[31–33] The bicyclic iminosugars depicted in **Figure 10** could be isolated from naturally occurring sources. For example, castanospermine (**19**) was isolated from the seeds of *Castanospermum australe*, calystegine C_1 (**20**) from the roots of *Calystegia sepium*, and swainsonine (**21**) from the legume *Swaisona canescens*.[31] (**Figure 10**)

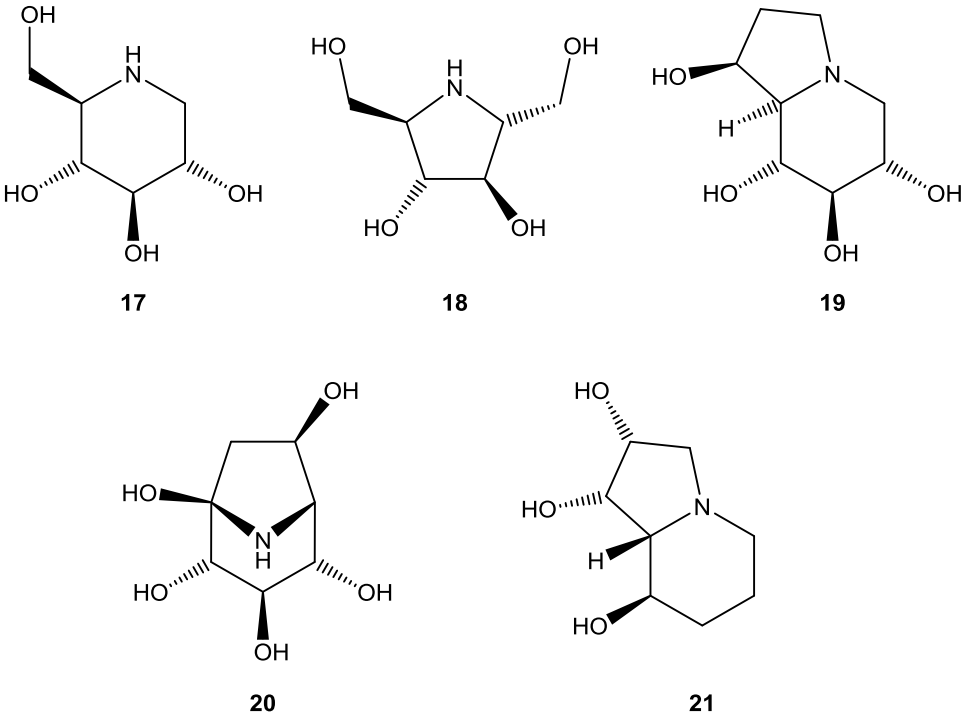


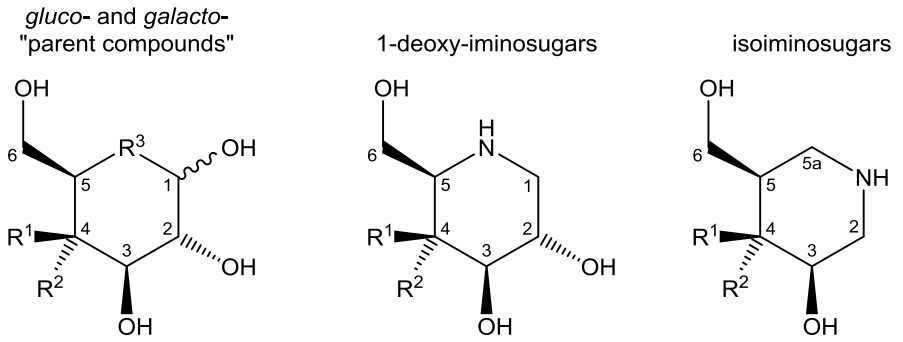
Figure 10. Important representatives of natural iminosugars.

1.3.2 Structural correlations between isoiminosugar and iminosugars

Nojirimycin (**16**) (**Figure 9**) owes its name to its early isolation from the bacteria *Streptomyces nojiriensis*.[34] Since its stereo configuration corresponds to D-glucose (**2**), this nomenclature has been applied to other iminosugars like galactonojirimycin (**23**) or mannojirimycin, for the respective D-*galacto*-, or D-*manno*-configured analogues.

Derivatives of the D-*gluco*-configured **16** without a hydroxyl function at *C*-1 position, like deoxynojirimycin (**17**) were found to be glucosidase inhibitors (for example **17** K_i = 25 µM, α-D-glucosidase from yeast). The D-*galacto*-configured deoxygalactonojirimycin (DGJ) (**24**) (**Figure 11**), on the other hand, is a powerful galactosidase inhibitor (**24** K_i = 0.016 µM, α-D-galactosidase from green coffee beans).[35]

With the synthesis of isofagomine (IFG) (**25**) in 1994, by Bols, Lundt and coworkers, which was the first synthetic iminosugar derivative bearing the nitrogen atom at the former anomeric position, the compound class of isoiminosugars was established.[36,37] This movement of the nitrogen dramatically increased inhibitory effects towards β-glucosidases. DNJ (**17**) shows an inhibition constant K_i = 47 µM for β-glucosidase from almonds. In comparison, the respective isoiminosugar **25** features a K_i value of 0.11 µM for the same glucosidase, which corresponds to more than a 400-fold enhanced binding affinity.[38]



2 c,d R^1 = H, R^2 = OH, R^3 = O
16 a,b R^1 = H, R^2 = OH, R^3 = NH
22 a,b R^1 = OH, R^2 = H, R^3 = O
23 a,b R^1 = OH, R^2 = H, R^3 = NH

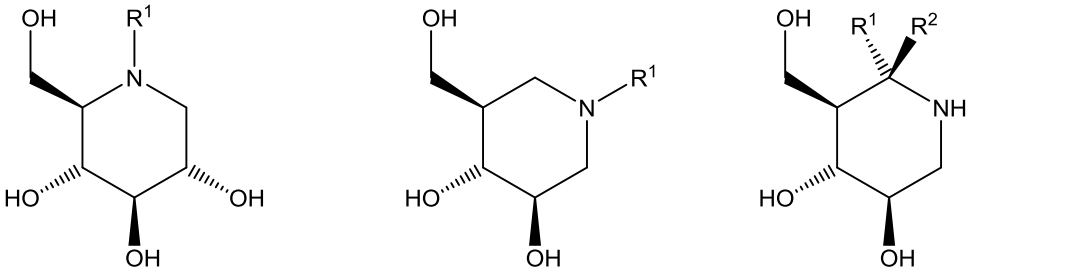
17 R^1 = H, R^2 = OH
24 R^1 = OH, R^2 = H

25 R^1 = H, R^2 = OH
26 R^1 = OH, R^2 = H

Figure 11. Structural relations between imino- (**16**, **17**, **23**, **24**), isoimino- (**25**, **26**), and conventional "sugars" (**2**, **22**).

1.3.3 Lipophilic derivatives of iminosugars

In order to obtain even more potent carbohydrate based inhibitors, the introduction of hydrophobic residues in iminosugars turned out to be a suitable approach. Particularly in the case glycosyl hydrolases with lipophilic substrates, such as glucocerebrosidase (GCase), much better interactions could be observed after functionalisation with alkyl moieties such as in **Figure 12**.



17 R^1 = H
27 R^1 = CH_3
28 R^1 = $(CH_2)_3CH_3$
29 R^1 = $(CH_2)_5CH_3$
30 R^1 = $(CH_2)_8CH_3$
31 R^1 = $(CH_2)_9CH_3$
32 R^1 = $(CH_2)_{11}CH_3$
33 R^1 = $(CH_2)_5OCH_2$-adamantanyl

25 R^1 = H
34 R^1 = $(CH_2)_3CH_3$
35 R^1 = $(CH_2)_3Ph$
36 R^1 = $(CH_2)_8CH_3$

25 R^1 = R^2 = H
37 R^1 = R^2 = $(CH_2)_2CH_3$
38 R^1 = $(CH_2)_2CH_3$, R^2 = H
39 R^1 = $(CH_2)_3OH$, R^2 = H
40 R^1 = $(CH_2)_3CH_3$, R^2 = H
41 R^1 = $(CH_2)_5CH_3$, R^2 = H
42 R^1 = $(CH_2)_6CH_3$, R^2 = H
43 R^1 = $(CH_2)_8CH_3$, R^2 = H

Figure 12. Different lipophilic substitution patterns for DNJ (**17**) and IFG (**25**).

Since the early 1980's it is possible to obtain *N*-substituted derivatives of DNJ (**17**), via acidic reductive amination of amino-furanoses.[39] While the inhibitory potential already significantly

increases with the introduction of a simple methyl group (**27**), further elongation of the alkyl moiety enhances the inhibitor's potential even more. In case of recombinant human acid β-glucosidase, switching from an *N*-butyl **28** to an *N*-nonyl **30** chain, for example, increased the inhibition potential almost by the factor of 400. (**Table 1**)

Table 1. Biological activity of *N*-modified DNJ (**17**).

	***N* residue**	**K_i [nM]**	**Enzyme**
17	-H	1000	α-Glucosidase 1 from calf liver[40]
27	-methyl	70	α-Glucosidase 1 from calf liver[40]
28	-butyl	90	α-Glucosidase 1 from calf liver[40]
29	-hexyl	130	α-Glucosidase 1 from calf liver[40]
17	-H	210000	Cytosolic β-glucosidase from calf liver[41]
31	-decyl	8200	Cytosolic β-glucosidase from calf liver[41]
32	-dodecyl	3800	Cytosolic β-glucosidase from calf liver[41]
28	-butyl	116000	Recombinant human acid β-glucosidase[42]
30	-nonyl	300	Recombinant human acid β-glucosidase[42]
33	$-(CH_2)_5OCH_2-$ adamantanyl	1	Membrane β-glucosidase 2 from Gaucher spleen[43]

In contrast to iminosugars, alkylation of the nitrogen in isoiminosugars did not turn out to have the same tremendously increasing effect on the biological activity. The introduction of a 3-phenylpropyl group in **35** hardly resulted in a 1.5-fold better K_i-value for rabbit-muscle glycogen phosphorylase than IFG (**25**). On the other hand, an *N*-nonyl chain in the IFG derivative **36** almost caused the entire loss of its inhibiting effect for human β-glucocerebrosidase. (**Table 2**)

Table 2. Biological activity of *N*-modified IFG (**25**).

	***N* residue**	**K_i [nM]**	**Enzyme**
25	-H	1200	Rabbit-muscle glycogen phosphorylase[44]
35	$-(CH_2)_3Ph$	850	Rabbit-muscle glycogen phosphorylase[44]
25	-H	56	Human β-glucocerebrosidase[45]
34	-butyl	44000	Human β-glucocerebrosidase[45]
36	-nonyl	>100000	Human β-glucocerebrosidase[45]

According to these very different effects, observed for *N*-alkyl moieties in imino- and isoiminosugars, it can be assumed, that the relative position of the alkyl chain, as well as the position of the nitrogen, play quite an important role. Whereas the "enhancing" alkyl group is linked to the former endocyclic *C*-5 oxygen position in iminosugars, the nitrogen connects the "decreasing" side chains to the prior anomeric *C*-1 carbon position in isoiminosugars. The obvious solution to that problem –moving the side chain back to the former endocyclic *C*-5 oxygen position (which will be named *C*-5a position in isoiminosugars, despite any differences in literature), has raised some synthetic challenges. It was as late as 2005, when (5a*S*)-5a-*C*-nonyl-isofagomine (**43**) was reported to be one of the most powerful β-glucosidase inhibitors with IC_{50} = 0.6 nM for human β-glucocerebrosidase.[45] (**Table 3**)

Table 3. Biological activity of *C*-5a-modified IFG (**25**).

	***C*-5a residue**	**IC_{50} [nM]**	**K_i [nM]**	**Enzyme**
38	-(*S*)-propyl		610	Human β-glucocerebrosidase pH5.5[46]
37	2x -propyl		600	Human β-glucocerebrosidase pH5.5[46]
40	-(*S*)-butyl	160	120	Human β-glucocerebrosidase[45]
39	-(*S*)-$(CH_2)_3OH$		100	Human β-glucocerebrosidase pH5.5[46]
25	2x -H	56	25	Human β-glucocerebrosidase[45]
41	-(*S*)-hexyl	4.2		Human β-glucocerebrosidase[45]
42	-(*S*)-heptyl	1.8		Human β-glucocerebrosidase[45]
43	-(*S*)-nonyl	0.6	0.2	Human β-glucocerebrosidase[45] / pH5.5[46]

Remarkably, the introduction of short alkyl chains, such as *C*-5a-butyl **40**, does not increase the binding affinity, compared to parent compound IFG (**25**). Neither does a second alkyl chain at the same position, having axial orientation as in *C*-5a-di-propyl **37**. However, an appropriate terminal modification of the alkyl chain might enhance inhibition abilities, according to *C*-5a-3-hydroxy-propyl **39** compared to *C*-5a-propyl **38**.

After analysing all different inhibition constants from **Table 1** - **Table 3**, there is reason to assume, that the family of *C*-5a-modified isoiminosugars provides some of the most promising compounds in order to develop efficient glycosidase inhibitors with intriguing biological activities. The derivatisation with lipophilic substituents allows for a large chemical diversity and for structural tuning.[46] Thus, target orientated synthesis of isoiminosugars provides a suitable basis for the manipulation and evaluation of carbohydrate processing enzymes, such as human lysosomal glycosidases, which degrade glycoproteins and glycolipids.

1.4 Glycosphingolipids

Sphingolipids (SLs) and glycosphingoslipids (GSLs) are essential constituents of mammalian cell walls, as was first reported in 1884, when they were discovered in ethanolic brain extracts.[47] The structural basis of GSLs is composed of a mono- or oligosaccharide and an *O*-glycosidically linked, fatty acid *N*-acylated, sphingosine residue. The setup of the general structure, as in glucosylceramide (**44**), for example, is described in **Figure 13**.

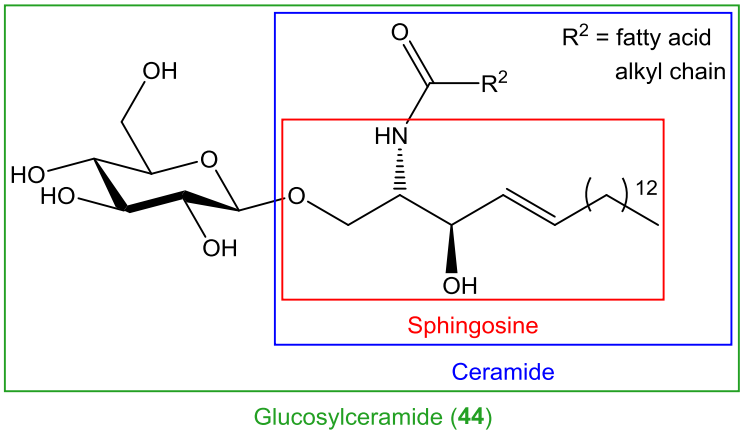


Figure 13. Structure of glucosylceramide (**44**).

While the fatty acid at the *N*-acyl position allows for structural variation regarding chain length and saturation, sphingosine usually features a C_{18} chain, as its biosynthesis begins with L-serine and palmitoyl-CoA. The ceramide unit is formed over four steps, which take place at the endoplasmic reticulum (ER). The primary hydroxyl group can undergo phosphorylation to yield ceramide-1-phosphate, or ceramide can be converted into sphingomyelin by addition of a phosphorylcholine group.[48,49] This addition takes place on the luminal side of the trans-Golgi membrane, or on the surface of the plasma membrane. Functionalisation with one monosaccharide transforms ceramide either into galactosylceramide at the ER,[50,51] or into glucosylceramide (**44**) at the cytosolic side of the cis-Golgi apparatus.[52,53] The latter reaction, which is catalysed by glucosylceramide synthase (GCS), uses UDP-glucose as glycoside source and is essential for the biosynthesis of more complex GSLs. The formation of gangliosides or globosides for example, depends on GCS, since glucosylceramide (**44**) is the key intermediate for their anabolic pathways.

1.4.1 Biological significance of glycosphingolipids

Due to their high variability and structural complexity, SLs and GSLs are considered to be involved in a series of fundamental biological processes. Besides being "regular" membrane-constituents. As a consequence of their distinct carbohydrate residue, GSLs are able to arrange themselves in the form of so-called lipid-rafts on the surface of plasma membranes.[54] These lipid rafts serve as recognition sites for lectins or other GSLs, in terms of cell-cell communication, or cell-substrate interactions.

The highest concentration of GSLs can be found within the brain tissue, where they are a major constituent of the outer lipid membrane with up to 25 %. Their biosynthesis and composition was found to change with ongoing age. Thus, the heterogeneity of GSLs rises, while their total amount was found to decrease in adult brains.[55]

As ceramide is the main component of epidermal stratum corneum, it prevents the skin of terrestrial mammals from dehydration.[56] Glucosylceramide was identified to play an important role in the biochemical pathways of ceramide regulation and distribution. Therefore, any dysfunction within the glucosylceramide (**44**) equilibrium can cause disturbances in the epidermic barrier and result in skin disorders, as they can be observed from Gaucher disease patients.[57]

The membrane bound GSLs in digestive and respiratory systems are not only essential for favoured cell interactions, but provide also an attractive binding site for pathogens like viruses and bacteria. Surface proteins of the influenza virus family for example, include lectins, which specifically attach to terminal *N*-acetylneuraminic acid (NANA) moieties on host cells.[58] The bacterium *vibrio cholerae* uses a sialidase to cleave more complex GSLs on intestinal cell surfaces like G_{D1a} down to G_{M1}, the preferred binding domain of the cholera toxin enzyme, which can consequently enter the host cell via endocytosis.[59,60]

1.4.2 Glycosphingolipid degradation

The majority of GSL degradation takes places in endosomes and lysosomes and is catalysed by a series of glycosidases, as depicted in **Figure 14**. They act as exo-glycosidases, as each enzyme can cleave one carbohydrate moiety starting from the non-reducing end. The common product of this degradation process is ceramide (**50**), which can further be broken down into sphingosine and the respective fatty acid.[61,62]

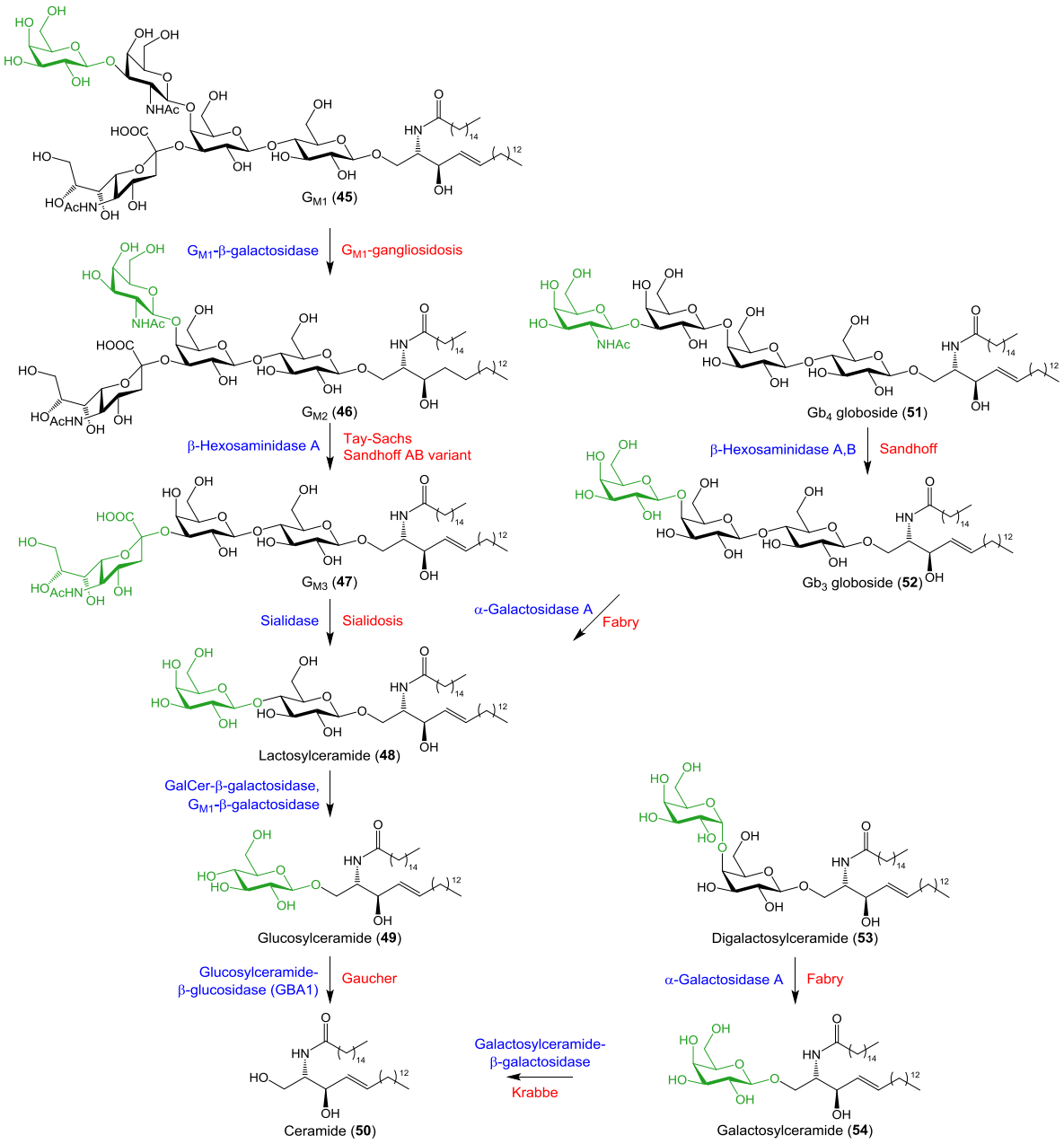


Figure 14. Selected GSL degradation cascade with glycosidases (blue), cleaved carbohydrate moieties (green) and the observed disease in case of malfunctioning glycosidases (red). *Adapted from Kolter and Sandhoff*.[61]

If one of these glycosidases in **Figure 14** fails to convert their respective substrate, these metabolites accumulate in the cell and can cause different diseases.

1.5 Lysosomal storage disorders

Lysosomal storage disorders (LSDs) are a series of inheritable metabolic diseases, which are caused by deficiencies of lysosomal enzymes due to mutations in the genes, which encode them. Consequently, the catabolic degradation cascades get interrupted and the substrates of the defect hydrolases accumulate in an uncontrolled manner. Based on the large variety of lysosomal metabolites, such as glycoproteins or GSLs, and their processing enzymes, LSDs occur in over 40 different known types.[63,64] Although the most frequent LSD, Gaucher disease type I, has an average incidence of 1 among 50,000–200,000 births as a single disease,[65,66] the combined prevalence of LSDs is estimated to be 1 in 6,000–8,000 births.[67,68]

Depending on the kind of mutation, the progression of the particular disorder might be very different, which makes an effective diagnosis especially challenging. While the symptoms can seem harmless and might stay undetected in adult stage, they tend to be very severe at infant age. As the diseases proceed, they can affect skin and organs, skeletal growth or neurological development, since all their functions rely on an unimpaired GSL metabolism.[69,70]

The relations between mutant enzymes and their respective substrates are described for some of the most prevalent LSDs in **Figure 14**. Several therapeutic approaches have been made in order to deal with the accumulated metabolites caused by the different disorders.

1.5.1 Present treatment strategies of lysosomal storage disorders

Present treatments of LSDs include enzyme replacement therapy (ERT), where a recombinant version of the defective enzyme is administered to the patient intravenously on a regular basis. The enzyme can be taken up by the cell via receptor mediated endocytosis and start to reduce the amount of accumulated metabolites.[71,72] Different enzymes have been approved for ERT, including *Cerezyme®* for the treatment of type 1 Gaucher disease with annual costs of approx. EUR 200,000[73] and *Replagal®* for Fabry disease patients which costs around EUR 300,000 p.a..[74] Despite those advances, the inability of the recombinant enzyme to cross the blood-brain barrier limits the application of ERT to patients without neurological dysfunctions.[75]

A different strategy to deal with LSDs is offered by the substrate reduction therapy (SRT), which aims on inhibiting the biosynthesis of accumulated GSLs.[76–78] The iminosugar *N*-butyl-1-deoxynojirimycin (**28**), for example, is used as a reversible inhibitor of glucosylceramide synthase and disrupts the biosynthesis of glucosylceramide (**44**). Therefore, **28**, also known as miglustat, is primarily used for the treatment of type 1 Gaucher disease under the trade name *Zavesca®*, with annual costs of approx. EUR 120,000.[79]

The first approach to treat LSDs was made by hematopoietic stem cell transplantation (HSCT), with stem cells from a healthy donor. These hematopoietic stem cells are intended to repopulate within the patient's body and replace deficient tissues, and additionally, express functioning hydrolases, which can be distributed via blood stream.[70,80] However, the application of HSCT is only limited to a few cases. Other methods consist of bone marrow transplantation and gene therapy.[81,82]

1.5.2 Chaperone mediated therapy

Most recent efforts to treat LSDs are based on the chaperone mediated therapy (CMT), which utilises small molecules to serve as pharmacological chaperones (PCs). Due to their strong interactions with the defective enzyme's active site, these PCs are capable to promote correct protein folding within the endoplasmic reticulum. Selective, reversible inhibitors, such as iminosugars, turned out to be suitable molecules for that purpose, when applied in sub-inhibitory concentrations. The first principle of a pharmacological chaperone was described by Fan and collaborators in 1999. They reported a stabilisation of α-galactosidase A in cells of Fabry patients, when exposed to deoxygalactonojirimycin (**24**) in sub-inhibitory concentrations.[83] This iminosugar **24** was recently approved by the European Union for the treatment of Fabry patients in May 2016 and is known under the trade name *Galafold*™.[84]f

In healthy patients, natural chaperone proteins like calnexin or calreticulin are able to assist in folding and assembling of macromolecular structures within the ER. These correctly folded proteins can subsequently be transported to their next cellular destination like the Golgi apparatus. (**Figure 15 a**) If, however, a structural failure occurs within the translated protein because of an inherited mutation, the protein cannot be folded correctly by natural chaperones, and is therefore forced to undergo ER associated degradation (ERAD). The mutant protein gets transported outside of the ER, where it is degraded into small peptide segments by proteasomes. (**Figure 15 b**) When LSD patients are treated with pharmacological chaperone molecules, these PCs are able to reach the ER. While the chaperone interacts with the active site of the malfunctioning enzyme, it assists the natural chaperones to achieve improved folding of the mutant protein, thus preventing it from ERAD. As these pharmacological chaperones are predominantly strong reversible inhibitors of the respective protein, they can occupy the active site, while the PC-protein complex is transported to the Golgi apparatus, and consequently to the lysosomes via intracellular trafficking. Since the lysosomes are generally more acidic (pH 4.5-5.5) than the ER (pH 6.5-7),[46] the PC-protein complex dissociates much easier, and the proteins can start to process their intended substrates with increased activity. (**Figure 15 c**)

For cellular application, a "washout" period, turned out to be useful in order to obtain better enzymatic activity, since the remaining PCs still exhibit strong affinities for their corresponding proteins. Glucocerebrosidase from Gaucher patients, for example, showed a further increase in activity after incubation in isofagomine (IFG) -free media, when they were activated with IFG (**25**) as a PC.[85]

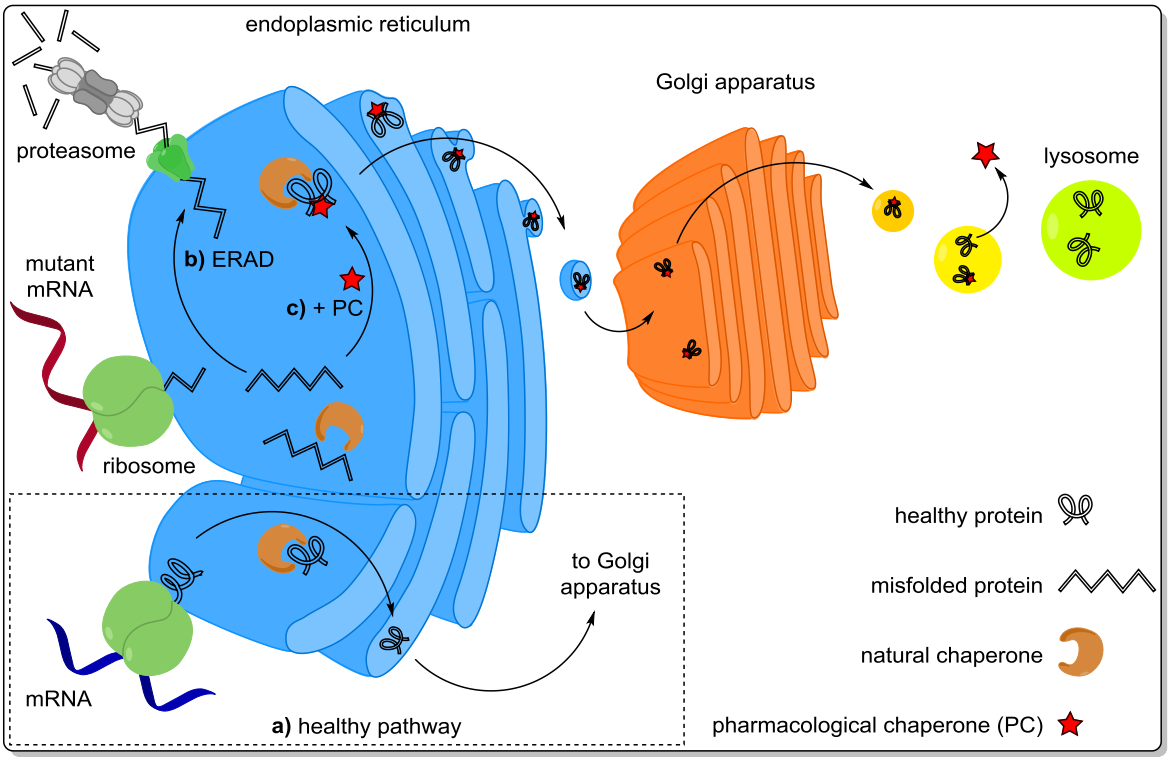


Figure 15. **a)** Healthy protein pathway: natural chaperones assist in the correct folding of translated proteins in the ER, before they get transported to the Golgi apparatus. **b)** Misfolded proteins are translated because of mutant mRNA and undergo ER-associated degradation (ERAD) since they cannot be folded correctly by natural chaperones. **c)** Pharmacological chaperones (PCs) strongly interact with the misfolded proteins' active site, thus helping them to (re)gain their intended structure. The protein-PC complex gets transported to the Golgi apparatus, and further to the lysosomes, where the complex dissociates due to the changed pH-value.[86]

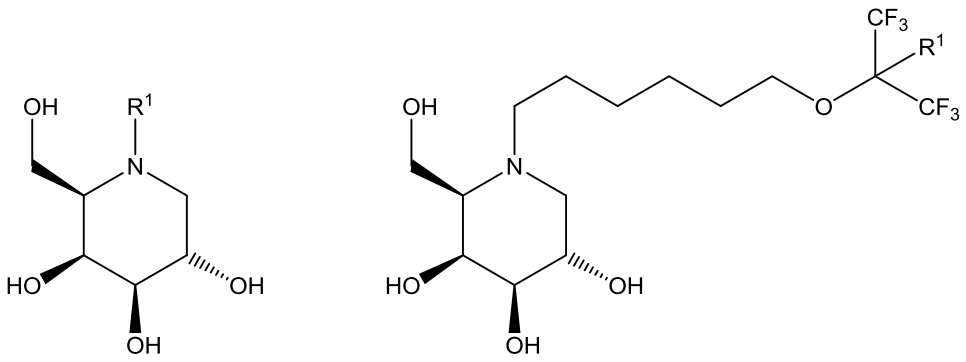
The stabilising effect of PCs on their respective enzyme could easily be demonstrated by melting temperature (T_m) determination of the enzyme in presence of a chaperone. The powerful β-glucosidase inhibitor (5a*S*)-5a-*C*-nonyl-isofagomine (**43**), for instance, is capable of raising the T_m of the respective complex with glucocerebrosidase to 67 °C, compared to a T_m of 47 °C in the absence of a pharmacological chaperone.[46]

Major advantages of CMT compared to ERT, are the PC's ability to cross the blood-brain barrier, good cell permeability, oral administration of the drug, and the possibility to reduce production costs of the small pharmacological molecules by effective synthetic approaches.

1.5.3 G_{M1}-Gangliosidosis

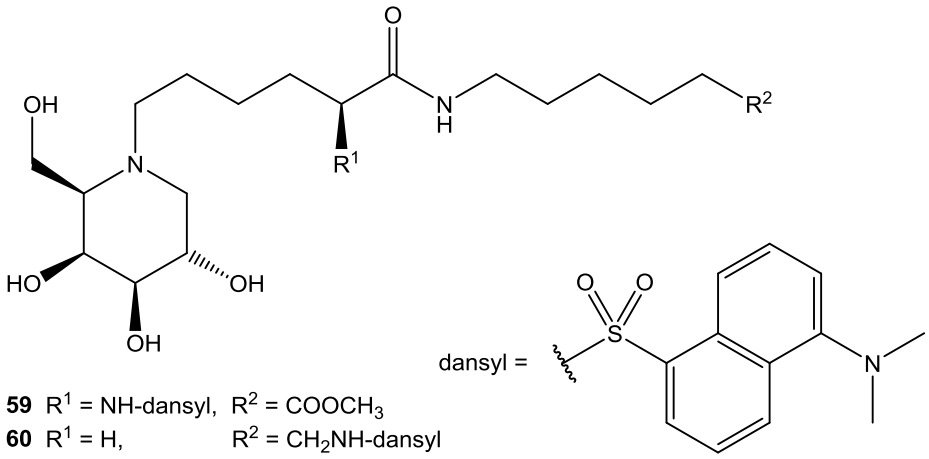
G_{M1}-Gangliosidosis (GM1) is a neurodegenerative LSD, resulting from a defective human β-galactosidase (EC 3.2.1.23), which converts the ganglioside G_{M1} into G_{M2} by removal of the outer β-galactopyranosyl unit. (**Figure 14**) Among GM1 patients, over 160 different alternations in the *GLB1* gene were found to cause dysfunctions of the encoded lysosomal enzyme.[87] The total prevalence is estimated between 1:100,000-200,000 among live births, and was found to be distinctly higher for populations in Malta, Brazil and Cyprus. GM1 can be divided into three different types, depending on the age of onset: type 1 occurs at infantile age within the first 6 months, type 2 can be observed in late infantile, early juvenile age between 6 months and 3 years, and the chronic form with late onset, type 3, affects patients from 3 to 30 years of age.[88]

Due to the neuropathic progression of the disease, present treatments such as ERT or gene therapy cannot be used for most GM1 patients, since their application is limited by their inability to cross the blood-brain barrier. Therefore, SRT and especially CMT are considered the best methods to overcome these problems.



24 R^1 = H
55 R^1 = $(CH_2)_3CH_3$
56 R^1 = $(CH_2)_8CH_3$

57 R^1 = CF_3
58 R^1 = Ph



59 R^1 = NH-dansyl, R^2 = $COOCH_3$
60 R^1 = H, R^2 = CH_2NH-dansyl

Figure 16. DGJ (**24**) and its *N*-substituted derivatives as pharmacological chaperones for GM1.

In order to develop suitable PCs for the treatment of GM1, the iminosugars DGJ (**24**) as well as *N*-*n*-butyl-DGJ (**55**) were found to have an impact on β-galactosidase activity in cell lines with GM1-mutations. Cells with R201H mutation (causing adult GM1) showed a 2.6 and 2.1-fold increase in activity, when cultured in the presence of 500 µM **24** and **55** respectively. The β-galactosidase activity in R201C mutant cell lines (juvenile GM1) increased by a factor of 5.4 and 4.8 under the same conditions.[89] When two different human GM1 patient-derived R201H fibroblast lines were treated with 1.2 µM of *N*-*n*-nonyl-DGJ (**56**) (K_i = 0.18 µM) for five days, a 4.9-fold and a 7.3-fold enhanced residual activity of mutant β-galactosidases were determined.[90]

Modification of the *N*-alkyl chain by attachment of the fluorescent dansyl group, afforded the strong β-galactosidase inhibitors DLHex-DGJ (**59**) and its derivative **60**. They exhibit K_i values of 0.6 µM and 0.7 µM towards β-galactosidase. The methyl ester terminated DLHex-DGJ (**59**) caused 5-6-fold enhanced activity at 20 µM, and an 18-fold increase at a concentration of 500 µM for R201C and R201H mutant cell lines.[91]

The dansyl terminated **60** was able to induce nearly a 6-fold increase in β-galactosidase activity at a concentration of 10 µM.[92] (**Figure 16**)

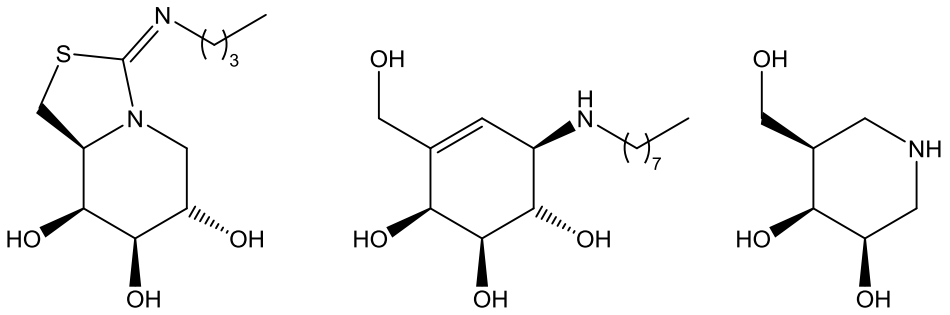
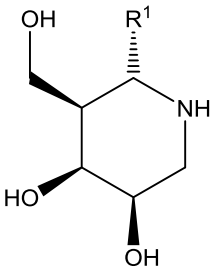


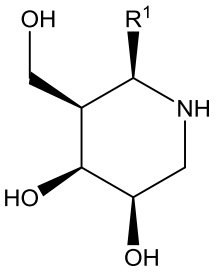
Figure 17. Selected isothiourea-iminosugar **61**, carbasugar **62** and isoiminosugar **26** as PCs for GM1.

The isothiourea-iminosugar derivative **61** turned out to be capable of increasing β-galactosidase activity by a factor of 6 in R201C GM1 fibroblasts with concentrations above 100 µM.[93,94] The unsaturated carbasugar *N*-octyl-4-*epi*-β-valienamine (NOEV) (**62**), one of the most powerful β-galactosidase inhibitors (IC_{50} = 0.125 µM), is also a very prominent pharmacological chaperone.[95,96] Already at a concentration of 0.2 µM, NOEV (**62**) causes a 4.5-fold (R201H) and a 5.1-fold (R201C) increase in β-galactosidase activity for GM1 fibroblasts.[97]

The *galacto*-configured isoiminosugar 4-*epi*-isofagomine (4-*epi*-IFG) (**26**), also a powerful β-galactosidase inhibitor (IC_{50} = 0.4 µM), was recently reported to promote 2.7-fold enhancement in R201C mutant GM1 fibroblasts at a concentration of 10 µM.[98,99] (**Figure 17**)

Various modifications at the *C*-5a position of this 4-*epi*-IFG parent compound (**26**) were carried out by Stütz and co-workers in order to elucidate the influence of different side chains on the interaction potential of the resulting β-galactose inhibitor.[100,101]





63 a $R^1 = (CH_2)_2OH$, IC_{50} = 30 **63 b** $R^1 = (CH_2)_2OH$, IC_{50} = 2.4
64 a $R^1 = (CH_2)_4NH$-dansyl, IC_{50} = 2.7 **64 b** $R^1 = (CH_2)_4NH$-dansyl, IC_{50} = 0.21
65 a $R^1 = (CH_2)_6NH$-dansyl, IC_{50} = 0.38 **65 b** $R^1 = (CH_2)_6NH$-dansyl, IC_{50} = 0.094

Figure 18. Different modifications of the 4-*epi*-IFG parent compound with their respective IC_{50}-values in [µM] (human lysosomal β-galactosidase). Left side: (5a*S*)-series; Right side: (5a*R*)-series.[102]

Remarkably, the 4-*epi*-IFG derivatives with (5a*R*)-linked side chains generally appear to be more potent inhibitors of β-galactosidase, than their (5a*S*)-configured counterparts. (**Figure 18**). For these six isoiminosugars a strong correlation between their inhibition strengths and their enhancing effects as pharmacological chaperones could be observed. The three most promising inhibitors (5a*S*)-C_6 **65 a**, (5a*R*)-C_4 **64 b** and (5a*R*)-C_6 **65 b** are able to cause a 3-fold to 8-fold increase of R201C mutant β-galactosidase activity at a concentration between 0.02 µM and 0.04 µM, and allow for more than a 10-fold enhanced activity at 0.2 µM. This corresponds to more than double the potential of the NOEV (**62**) benchmark chaperone at the same concentration.[102]

However, the current synthetic access to *C*-5a-elongated 4-*epi*-IFGs, such as **64 a,b** or **65 a,b** is quite challenging, regarding costs and time. It is based on an approximately 20 step-synthesis, starting from commercially available 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose (**66**). (**Figure 19**) The key steps of this approach include a reductive amination of furanose **72**, to the iminosugar derivative **73**, a hydroboration-oxidation to the isoiminosugar **77**, as well as elongation of the carbon side chain.

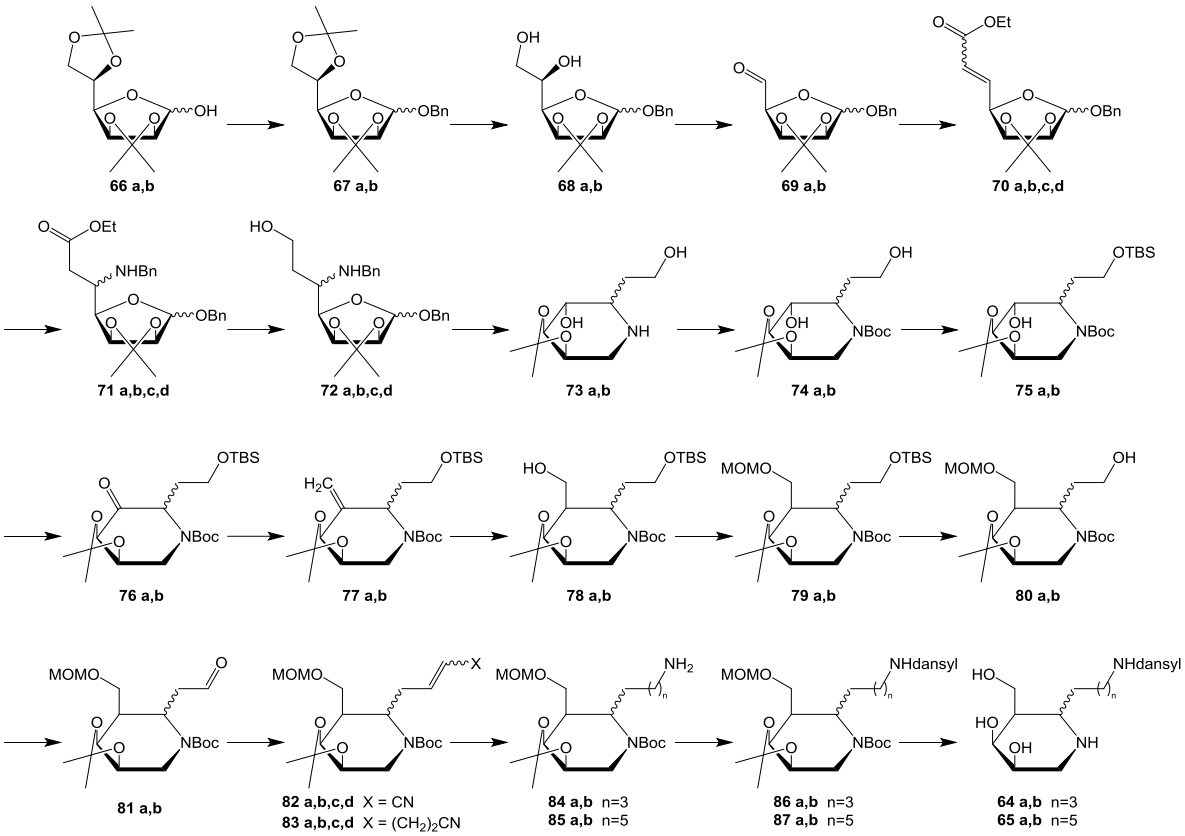


Figure 19. Current synthetic access to powerful *C*-5a-modified 4-*epi*-IFG derivatives.

2 Problem description and aim of this work

Based on the findings, that *C*-5a-elongated 4-*epi*-IFGs are the strongest reversible inhibitors of lysosomal β-galactosidases so far, they are considered very promising candidates to be used as pharmacological chaperones for the treatment of LSDs, such as G_{M1}-gangliosidosis. Biological evaluation of these isoiminosugars provided by Stütz and collaborators,[100,101] clearly demonstrated the potential to exceed the well-known benchmark chaperone NOEV (**62**).

For that reason, any efforts to abbreviate and facilitate the currently challenging synthetic access[102] to *C*-5a-modified 4-*epi*-IFGs would be desirable, in order to make a larger variety of these compounds available for chemical and biological testing. Consequently, a new approach employing the so-called Morita-Baylis-Hillman (MBH) reaction has been investigated in context with this thesis. The successful novel synthesis of a *C*-5a-modified isoiminosugar via MBH is considered the best "proof of concept" for this approach and was the final aim of this work.

2.1 Synthetic approach

The MBH reaction, established by Ken-ichi Morita in 1968,[103] Anthony B. Baylis and Melville E. D. Hillman in 1972,[104] was intended as starting point of the new synthetic approach. This carbon-carbon bond forming reaction can generally occur between the α position of an activated alkene (an electron withdrawing group (EWG) adjacent to a vinyl residue) and a carbonyl group. For our purpose, the reaction between an α,β-unsaturated carbonyl **89** and an aldehyde **88** was especially interesting. (**Figure 20**) The product obtained from the MBH reaction provides an ideal structural frame for the further synthesis of *C*-5a-modified isoiminosugars, as it is demonstrated in the retrosynthesis in **Figure 21**.

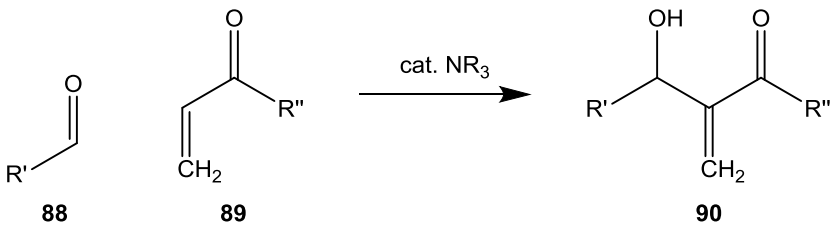


Figure 20. MBH reaction principle between an aldehyde (**88**) and an α,β-unsaturated carbonyl (**89**).

Given the retrosynthetic considerations in **Figure 21**, the *C*-5a-modified isoiminosugar **A** can be obtained from selective oxidation of the exocyclic methylene group in compound **B**. The iminosugar **B** is the cyclisation product of **C**, after its leaving group (LG) could be introduced at the primary position from alcohol **D**. The secondary amine compound **D** can be afforded by exchanging the secondary alcohol for an amine at the *C*-5 position in alcohol **E**. This alcohol is the reduction product of ketone **F**, which is the MBH reaction product of D-glyceraldehyde **G** and the α,β-unsaturated carbonyl **H**.

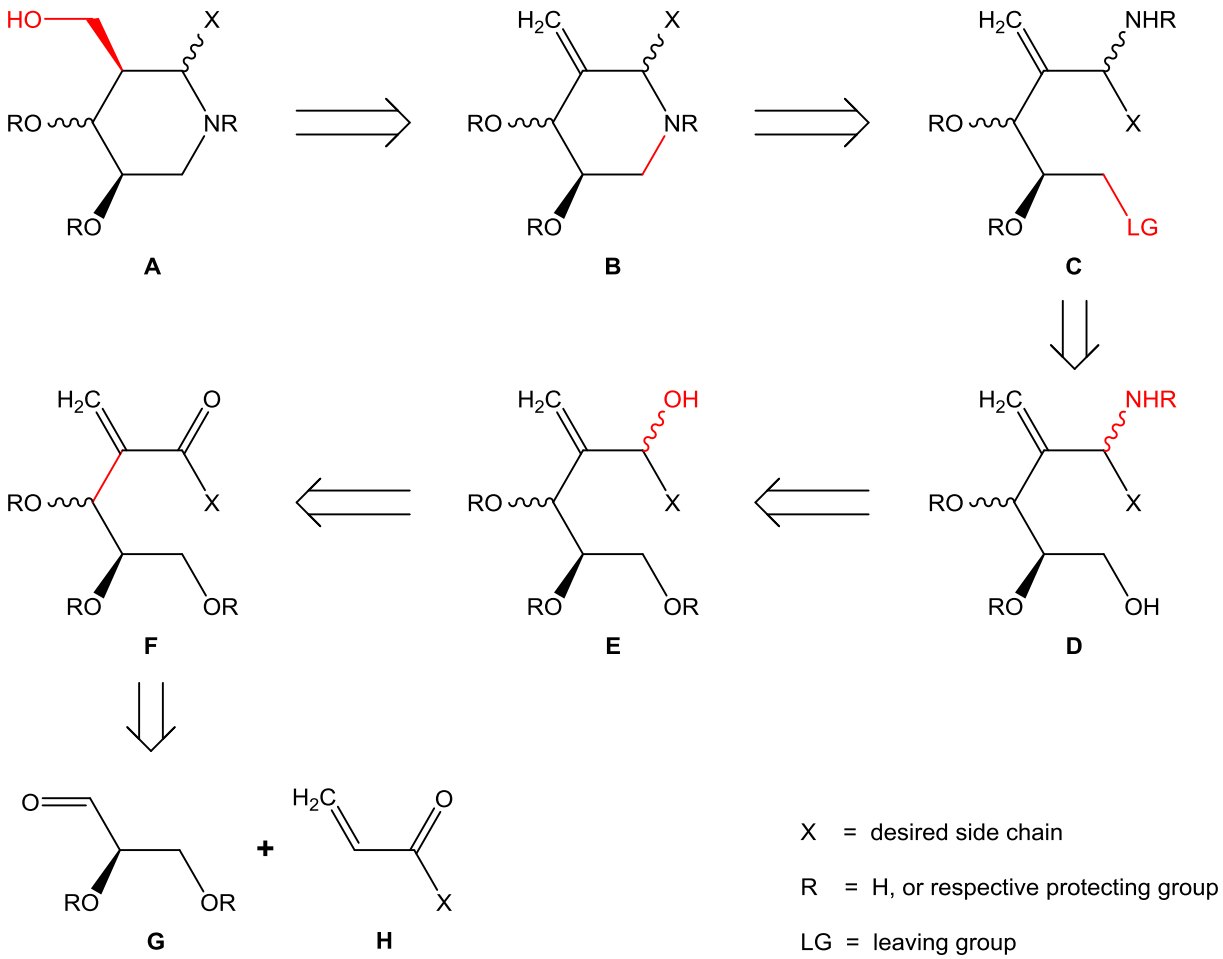


Figure 21. Retrosynthetic considerations for the synthesis of *C*-5a-modified isoiminosugar (**A**).

3 Results and discussion

3.1 Morita-Baylis-Hillman reaction

3.1.1 Reagent synthesis

The C-C bond forming MBH reaction between a carbonyl group and an activated alkene was the first step of the *de novo* synthesis of this *C*-5a-modified IFG from readily available starting products.

The isopropylidene protected D-glyceraldehyde **93** is considered the ideal carbonyl constituent for the MBH reaction, to build up the structural carbon frame of the prospective isoiminosugar. Since **93** is prone to epimerise at its *C*-2 position due to possible deprotonation of the α hydrogen, in-situ preparation of D-glyceraldehyde is the best way to prevent such problems.

D-glyceraldehyde can easily be obtained from the reduced hexose, D-mannitol (**91**). First, **91** can be protected at its outer hydroxyl groups selectively, to afford 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**92**). In the second step, the C-C bond between the unprotected inner vicinal 3,4-diol can be cleaved in an oxidative fashion, using $NaIO_4$. One special feature of the protected mannitol **92**, is the inherent C_2-rotational symmetry (no C_{2h}, as the 2-dimensional Fischer projection might accidently imply). Its symmetry axis, depicted as black dot in **Figure 22**, bisects the bond between *C*-3 and *C*-4, which is subject to the oxidative cleavage. Because of this symmetry, the two cleavage products are identical molecules and comply with two equivalents of aldehyde **93**. This special property not only entails a high atom efficiency of the reaction, but is also very favourable for the application of this cleavage reaction, as there are practically no side products formed in the reaction, which need to be removed.

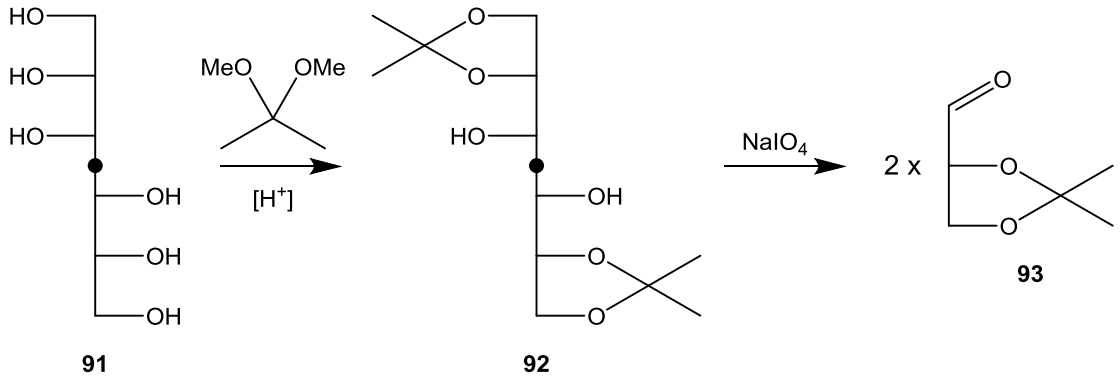


Figure 22. Synthesis of protected D-glyceraldehyde **93**. The C_2 symmetry axes of **91** and **92** are depicted as black dots.

3.1.2 Scope of the MBH reaction

Three different vinyl compounds were tested as the second reactant in the MBH reaction, which generally consists of an alkene activated by an EWG. The widespread polymer precursors acrylonitrile (**95**) and methyl acrylate (**98**) were suitable candidates to evaluate the scope of the MBH reaction, as they possess either a nitrile group or a methyl ester as EWG. (**Figure 23**)

Generally, this addition of a vinyl compound to an aldehyde causes the formation of a new stereocentre, as the former carbonyl atom changes its hybridisation from planar sp^2 to tetragonal sp^3 in the MBH adduct. Both **95** and **98** afforded a mixture of epimers **96**, **97** and epimers **99**, **100** respectively, when they were used in the MBH reaction.

A common drawback of the MBH reaction however, the relative slow reaction rate, could partially be overcome by performing the reaction in an aqueous solvent system, while using the tertiary amine catalyst 1,4-diazabicyclo[2.2.2]octane (DABCO) (**94**) in stoichiometric amounts.[105] Full conversion could be observed between 15-45 h, and yields of up to 85 % regarding aldehyde **93**, could be achieved.

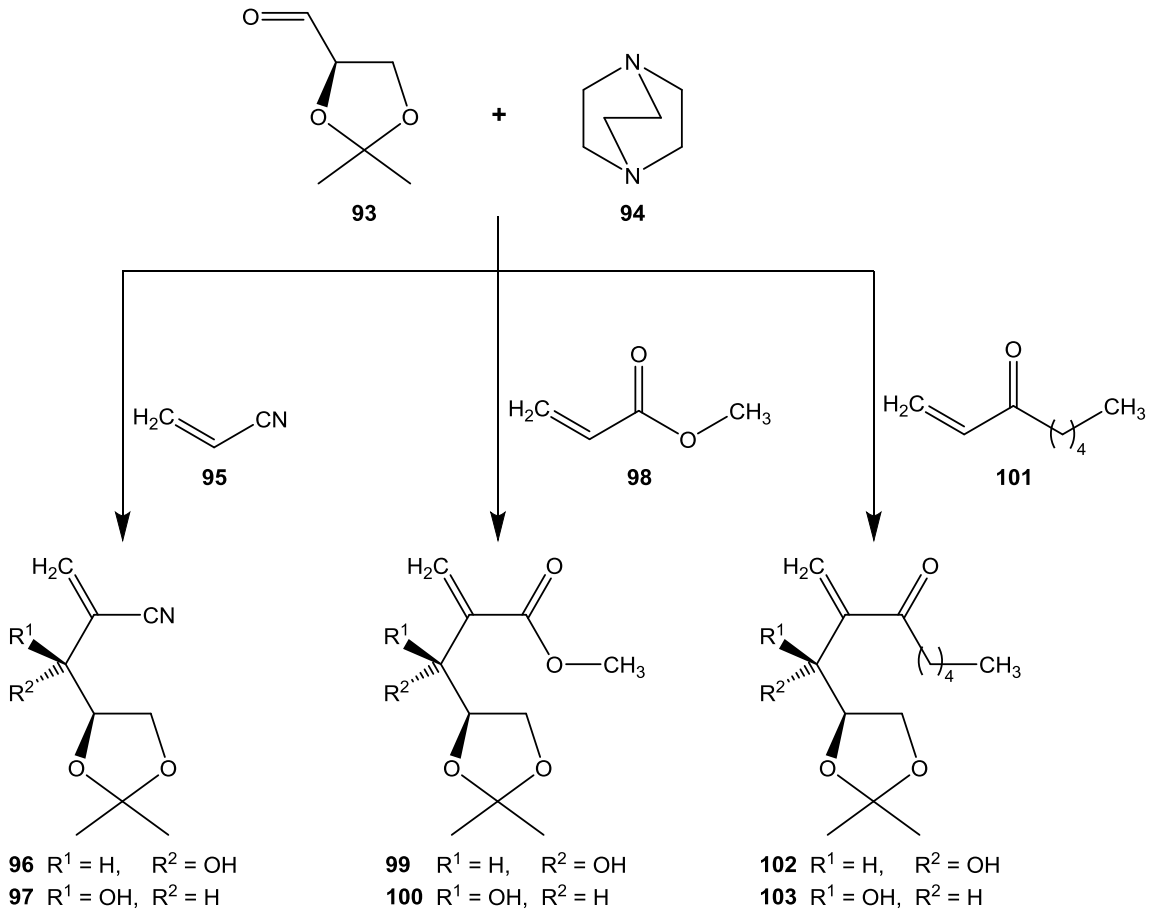


Figure 23. Use of different activated alkenes for the MBH reaction.

Based on the biological effects of lipophilic *C*-5a-linked side chains in IFG, as discussed in chapter 1.3.3, we decided to aim for an *n*-pentyl side chain to prove the applicability of this synthetic approach. In order to implement this pentyl chain, the α,β-unsaturated ketone 1-octen-3-one (**101**), which can incidentally be recognised by its persistent mushroom-like odour, was used as the second MBH reaction partner.

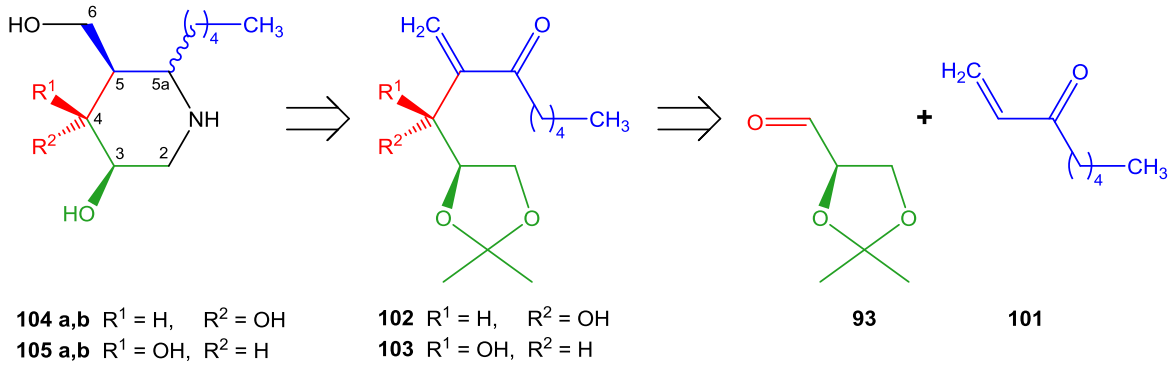


Figure 24. Structural correlation between the MBH reaction and the desired IFG derivatives.

In context with the proposed IFG synthesis, the newly formed hydroxyl groups in **102** and **103** (**Figure 24**) correspond to the *C*-4 alcohol in the final IFG compound **104** and **105**. Depending on the stereochemical outcome of the MBH reaction, this *C*-4 hydroxyl group determines, if the modified IFG features either D-*gluco*- or D-*galacto*-configuration. As the MBH reaction is generally non-stereospecific, both (*R*)- and (*S*)-configuration (*gluco* and *galacto* respectively) are expected. This unselective progress is not necessarily a disadvantage. It can rather be beneficial in order to get access to a larger variety of different IFG and 4-*epi*-IFG derivatives. Consequently, this approach is suitable to get access to the *gluco*- and the *galacto*- series by the same approach.

However, instead of a diastereomeric mixture of (*R*)-**102** and (*S*)-**103**, the epimers were isolated with an approximate ratio of 1:3, with (*S*)-configured **103** (= *galacto*) being the major product. This observed stereoselectivity might be induced by the chiral substituted 1,3-dioxolane ring, which is formed between the 2,3-diol of aldehyde **93** and its isopropylidene protecting group. In combination with the larger 1-octen-3-one (**101**) it can favour the formation of one epimer over the other during the transition state. Interestingly, this preference towards the formation of one epimer could not be observed for the smaller acrylonitrile (**95**) constituent.

Consequently, we decided to follow the reaction pathway of the major D-*galacto*-configured product and focussed on the synthesis of *C*-5a-elongated-4-*epi*-IFG, which is anticipated to be more active towards G_{M1} β-galactosidase, than its *gluco*-analogue, as discussed in chapter 1.5.2.

3.2 *Galacto*-configured series

With the highly functionalised D-*galacto*-adduct **103**, formed in the MBH reaction between octenone **101**, and the protected D-glyceraldehyde **93**, the basis towards *C*-5a-elongated-4-*epi*-IFG was established. The first reactions on the new structural frame **103**, included protection of the new hydroxyl group and the selective reduction of the α,β-unsaturated ketone, at the carbonyl position. (**Figure 25**)

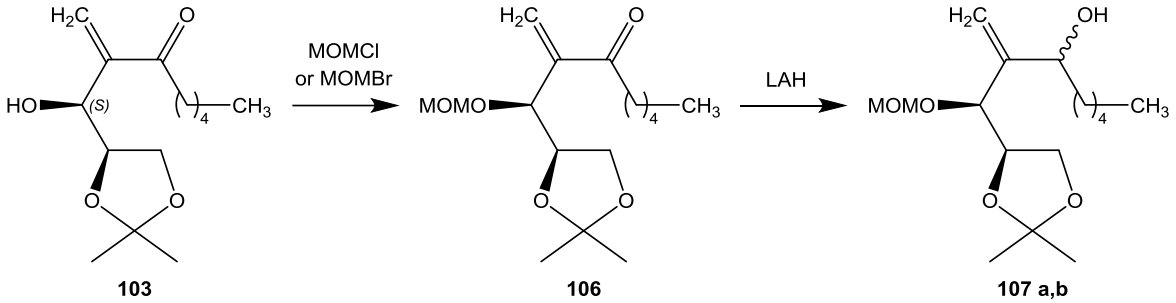


Figure 25. Protection and reduction reactions performed on the MBH adduct **103**.

To be able to cleave multiple acid-labile protecting groups under acidic hydrolysis in the last step, we decided to use an acid-labile methoxymethyl (MOM) group. The (*S*)-configured free hydroxyl group of **103** was protected, using the respective chloroalkyl or bromoalkyl ether and Hünig's base (*N*,*N*-diisopropylethylamine).

The selective reduction of the ketone group in **106** was carried out by using lithium aluminium hydride (LAH). The conjugated double bond was not reduced, since this functionality was necessary in a later stage of the synthesis. Due to the absence of any adjacent stereo-inducing group, a 1:1 mixture of **107 a** and **107 b** was obtained.

3.2.1 Introduction of the nitrogen

The next steps in the synthesis were determined by the introduction of a nitrogen atom, and its respective protection. All these reactions could be performed with both epimers simultaneously. A Mitsunobu reaction including phthalimide as nucleophile, was our method of choice. During this reaction, a highly reactive oxyphosphonium intermediate is formed between the alcohol moiety and triphenylphosphine (PPh_3). In form of an S_N2 reaction step, the carbon atom of the activated alcohol gets attacked by the deprotonated phthalimide with the release of triphenylphosphine oxide. Thus, the configuration of the attacked carbon in **108 a,b** becomes inverted.

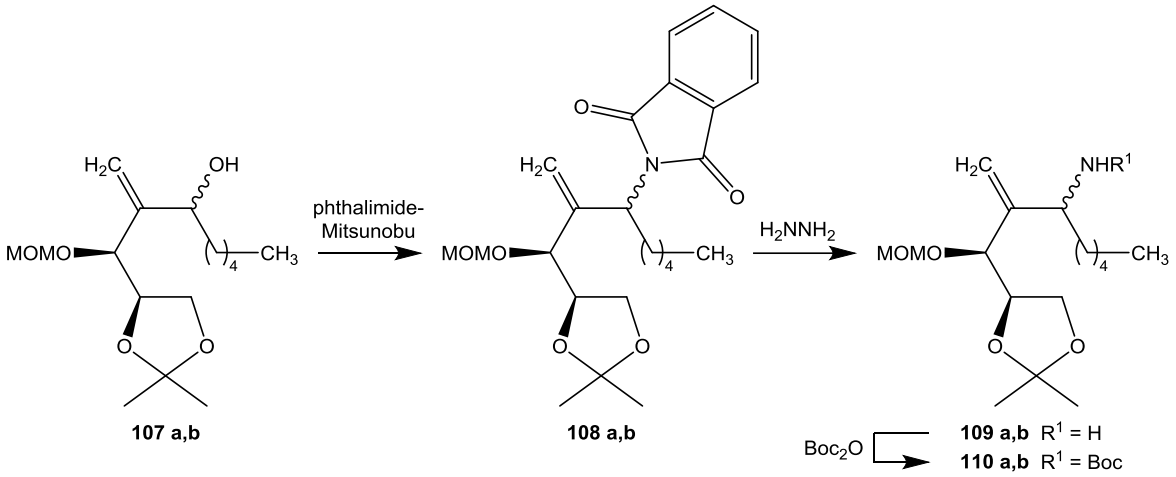


Figure 26. Introduction of the nitrogen via phthalimide Mitsunobu.

The phthalimide residue could be removed with the aid of hydrazine (H_2NNH_2), which was available as the monohydrate. The hydrazine forms phthalhydrazide, upon reaction with the phthalimide residue, affording an unprotected secondary amine, as in **109 a,b**. (**Figure 26**) Separation of this phthalhydrazide and the free amines **109 a,b** by silica gel flash column chromatography (FCC) was not efficient, and, if at all, only possible to some extend by filtration of the phthalhydrazide precipitate in ethanol. Thus, **109 a,b** were not isolated as pure compounds, but immediately used for the next reaction step.

This step was the protection of the free amine by a *tert*-butyloxycarbonyl (Boc) protecting group. The Boc group was chosen, since its cleavage is possible under acidic conditions in the last step, together with the MOM group. It was introduced by the di-*tert*-butyl dicarbonate (Boc_2O) reagent. After the Boc-protection step, purification of **110 a,b** was again possible to a fully satisfying extend.

3.2.2 Cyclisation reaction

To allow for the desired cyclisation reaction, a suitable leaving group had to be introduced at the primary alcohol position. Beforehand, removal of the isopropylidene protecting group from this alcohol was necessary. Although the MOM-, as well as the Boc-group are also sensitive to acidic conditions, careful treatment with *p*-toluenesulfonic acid (pTSA) allowed for selective removal of the primary isopropylidene group from *C*-1 and *C*-2.

Fortunately, the presence of the secondary unprotected alcohol at *C*-2 did not interfere with the exclusive conversion of the hydroxyl group at position *C*-1 into an LG. The primary alcohol **111 a**

was selectively converted to the corresponding bromodeoxy compound **112 a**, using triphenylphosphine dibromide (PPh_3Br_2). **112 a** however, was less prone to be attacked by the nitrogen, than initially assumed.

Careful treatment of the bromodeoxy species in the (5a*R*)-series with a base, such as Na_2CO_3 or NaH, caused the formation of an intermediate, instead of the intended iminosugar scaffold. When this intermediate was further treated with NaH, the nitrogen was able to attack the *C*-1 position of the presumably reactive intermediate, and the desired ring closing reaction occurred. (**Figure 27**)

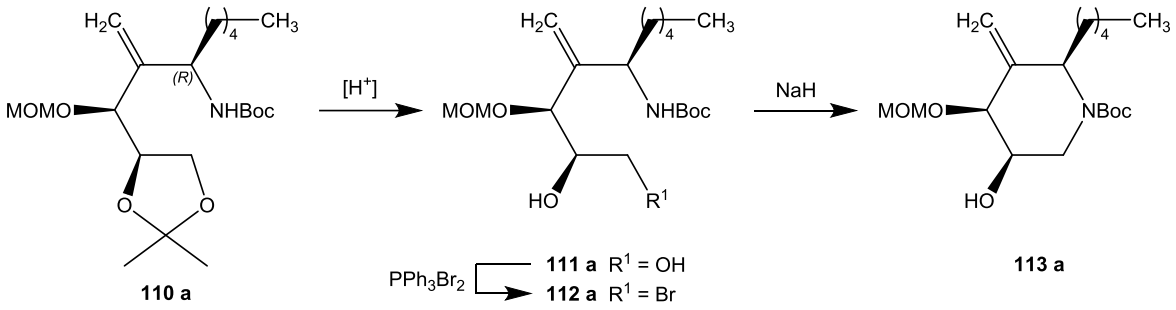


Figure 27. Halogenation and cyclisation in the (5a*R*)-series.

The progress of the successful cyclisation could be observed by NMR. At first, a distinct change of the chemical ^{13}C shift from the diol to the bromodeoxy species occurred. In the (5a*S*)-series for example, the ^{13}C signal of the primary alcohol (δ = 64.3 ppm) migrates in high-field direction after the halogenation (δ = 36.6 ppm). The proton signals however, almost remain unaffected between 3.9 – 3.6 ppm; depicted as blue signals in the (heteronuclear single quantum coherence) HSQC spectrum in **Figure 28**. Both ^{13}C and ^{1}H shifts at positions 2, 3 and 5 (red signals in the HSQC), also remain unchanged during that reaction.

After the cyclisation, the proton signals of the former primary alcohol became very distinctive, as they split up by more than 1 ppm. They can be found around 4.2 ppm and 3.1 ppm respectively. This huge gap is caused by their different axial or equatorial orientation on the six-membered iminosugar scaffold. The ^{13}C signal of *C*-1 changes from 36.6 ppm in the bromide, to around 44.2 ppm in the corresponding amine.

These signals were found to be poorly resolved, because of conformational changes, caused by the tertiary nitrogen in the ring. The effects of the ring flip interconversions become less severe after removal of the bulky Boc protecting group.

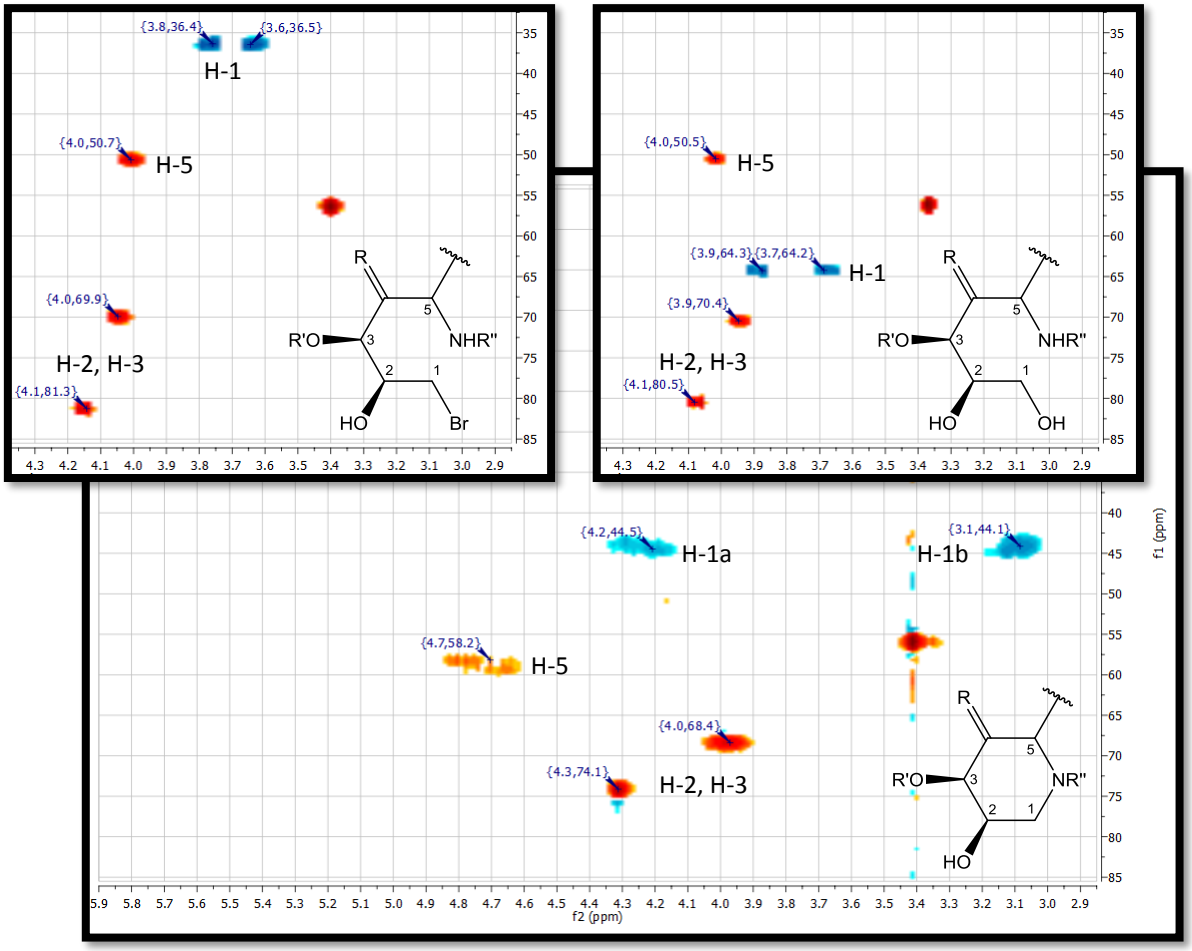


Figure 28. HSQC NMR spectra of the bromide **112 b**, diol **111 b** and cyclisation product **113 b** in the (5a*S*)-series. ^{1}H shifts are represented on the x-axis, ^{13}C shifts on the y-axis, in [ppm], CH and CH_3 signals are shown in red and CH_2 signals in blue.

The synthesis of the (5a*S*)-configured counterparts was performed analogously to the (5a*R*)-series. At first, the isopropylidene protecting group of **110 b** was removed selectively by carefully applying acidic conditions employing pTSA. The resulting diol **111 b** was halogenated at the primary position, using PPh_3Br_2, to afford the bromodeoxy derivative **112 b**.

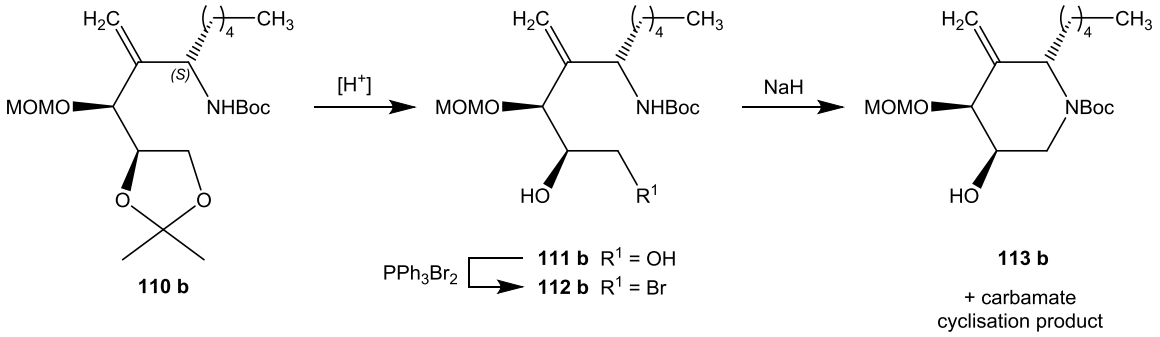


Figure 29. Halogenation and cyclisation in the (5a*S*)-series.

In order to perform the desired cyclisation reaction, the bromide species **112 b** was treated with sufficient amounts of NaH. However, the main product of this reaction in the (5a*S*)-series turned out to be a different, unexpected product. According to the pieces of information obtained by NMR, we assume the formation of a cyclic carbamate species, by nucleophilic attack of the Boc carbonyl group. Compared to the NMR spectra of the bromodeoxy compound **112 b**, the ^{13}C signals of the quaternary Boc carbon (Cq.) (81.4 ppm) and of the three methyl groups (28.4 ppm) were missing in the isolated cyclisation product. (**Figure 30**) The carbonyl signal around 160 ppm however, was still present. This means, that only the *t*-butyl moiety of the Boc group was cleaved off. The proton spectrum also clearly confirms the loss of the Boc's *t*-butyl group.

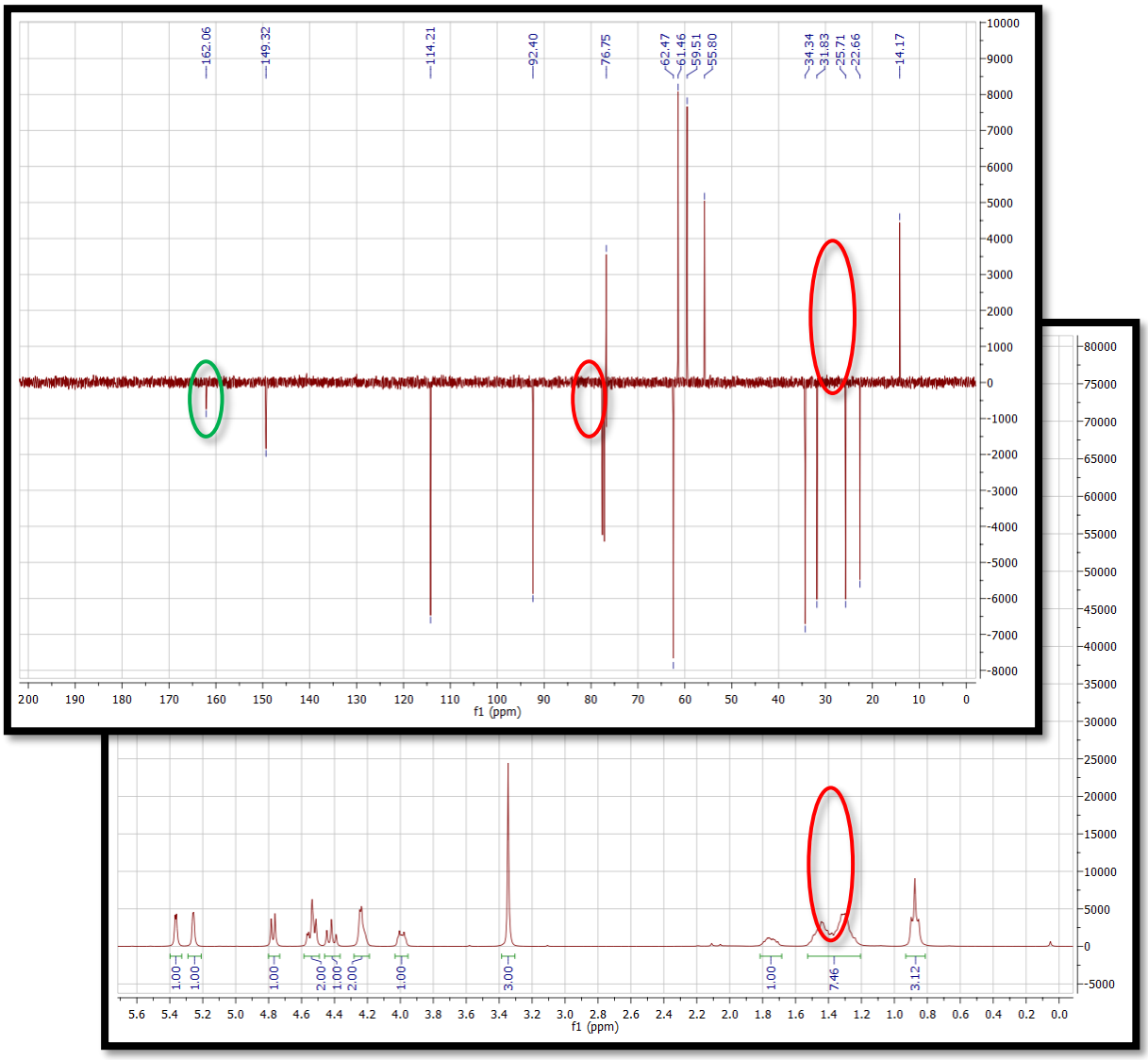


Figure 30. APT and ^{1}H NMR spectra of the main cyclisation product in the (5a*S*)-series. Matching (green) and missing (red) signals of the Boc group are highlighted by ellipsoids.

Despite these difficulties, we were still able to isolate the desired cyclisation product **113 b**, the (*S)*-configured analogue of **113 a** in smaller amounts (approximately 15 %). An alternative strategy, which includes protection of the free secondary alcohol in **112 b** before the crucial cyclisation step might help to avoid these side reactions and will be subject to prospective work.

3.2.3 Hydroboration–oxidation

The last key step in our synthesis of *C*-5a-elongated-4-*epi*-IFG is the introduction of the *C*-6 hydroxyl group. This preferably stereoselective reaction can be performed at the exocyclic methylene group by hydroboration-oxidation.

Before the hydroboration step, the remaining free hydroxyl group in **113 a** was protected with a MOM group to afford **114 a** in the (5a*R*)-series. (**Figure 31**) Thonhofer and Stütz[102] demonstrated, that a stereoselective oxidation of the exocyclic methylene group was possible in analogues of **114 a** in the (5a*R*)-series, by using a sterically demanding borane species. The product of this reaction using 9-borabicyclo[3.3.1]nonane (9-BBN), was a (5*R*)-configured primary alcohol. According to these experiences, we used the same bulky 9-BBN reagent for our hydroboration-oxidation of alkene **114 a**.

Eventually, we were able to synthesise **115 a**, the desired (5*R*)-configured primary alcohol with D-*galacto*-configuration, selectively. After removing of the Boc- and both MOM-protecting groups under acidic conditions, (5a*R*)-5a-*C*-pentyl-4-*epi*-isofagomine (**105 a**) was obtained as the final product.

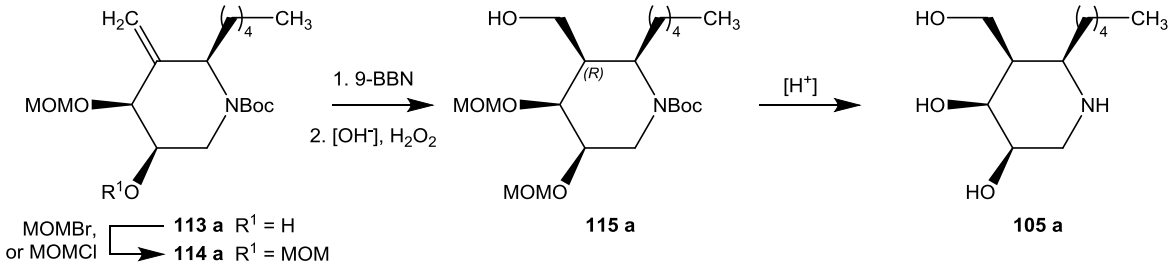


Figure 31. Hydroboration-oxidation to give the final compound **105 a** in the (5a*R*)-series.

Contrary to the (5a*R*)-series, the sterically demanding 9-BBN was not necessary to perform the intended selective hydroboration-oxidation in the (5a*S*)-series. Consequently, a BH_3 – THF complex solution was used as reagent for the hydroboration-oxidation of **114 b**, instead of 9-BBN,

and resulted in the formation of the desired (5*R*)-configured primary alcohol **115 b** in the (5a*S*)-series. (**Figure 32**) In the last step, all protecting groups were removed under acidic conditions, to obtain the final compound of the (5a*S*)-series, (5a*S*)-5a-*C*-pentyl-4-*epi*-isofagomine (**105 b**).

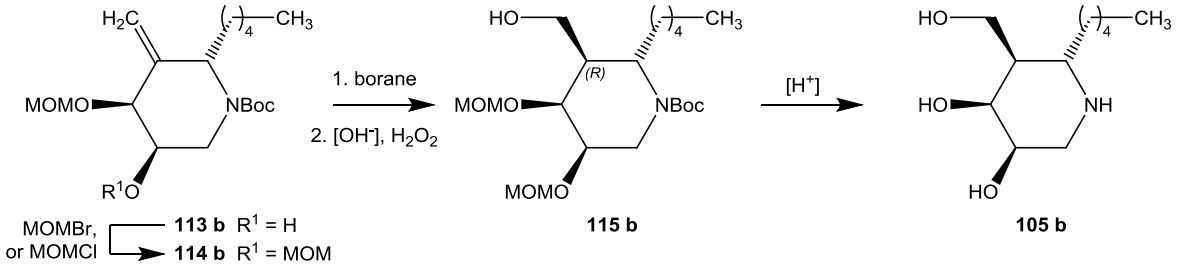


Figure 32. Hydroboration-oxidation to give the final compound **105 b** in the (5a*S*)-series.

4 Conclusion and Outlook

4.1 Application of the MBH reaction

Within the scope of this thesis a novel approach for the *de novo* synthesis of *C*-5a-elongated-isofagomine derivatives has been demonstrated. This alternative synthetic pathway is based on the C-C bond forming Morita-Baylis-Hillman reaction, which can generally occur between a carbonyl species and an activated alkene, in presence of an amine catalyst. The application of this MBH reaction has been tested with three different activated alkenes: acrylonitrile, methyl acrylate, and the α,β-unsaturated ketone 1-octen-3-one **101**. In all three cases, the easily available isopropylidene protected D-glyceraldehyde **93** was used as carbonyl constituent in the MBH reaction. Common problems associated with the MBH, such as low yields and slow reaction rates could largely be overcome by using the tertiary amine DABCO **94** in stoichiometric amounts in an aqueous solvent system.

Against initial expectations, the MBH reaction between **93** and **101** did not afford a diastereomeric 1:1 mixture of *C*-3 alcohols, but preferably caused the formation of the (3*S*)-configured adduct **103** with an approximate ratio of 3:1. (**Figure 33**) This might be the result of asymmetric induction by the chiral ring system, formed between the 2,3-diol moiety of **93** and its isopropylidene protecting group. Thus, we focused on the synthesis of D-*galacto*-configured 4-*epi*-IFG derivatives, which are expected to be powerful inhibitors of β-galactosidases.

The reduction of the ketone group in the MBH adduct **103** afforded a 1:1 ratio of two diastereomers, which were the origin of the later (5a*R*) and (5a*S*) side chains. Hydroboration-oxidation of the exocyclic methylene group to the primary alcohol was possible in both (5a*R*) and (5a*S*) series with the desired outcome. The final products, (5a*R*) and (5a*S*)-5a-*C*-pentyl-4-*epi*-isofagomine **105 a,b**, were the best proof for the practicability of the new developed synthesis.

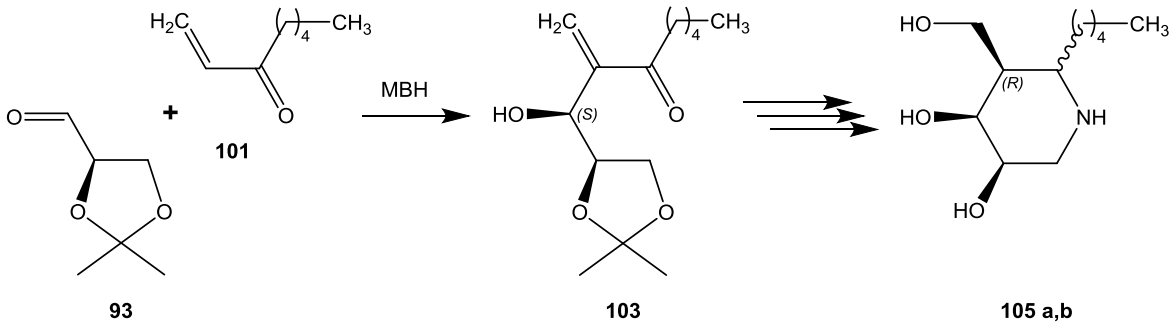


Figure 33. Application of the MBH reaction for the synthesis of *C*-5a-elongated 4-*epi*-IFGs.

4.2 Outlook

The synthetic approach towards *C*-5a-modified isoiminosugars, which was developed during this work is not considered to be limited to the pentyl-chain featuring derivatives **105 a,b**. It is much more intended to serve as basis for the generation of a large variety of different *C*-5a-modified isoiminosugars, by using different building blocks in the MBH reaction. By using different α,β-unsaturated carbonyl constituents, the length of the alkyl chain is variable, as in **J**, and also subject to possible modification, as in **H**, to yield isoiminosugars, such as **I** and **A**. (**Figure 34**) Furthermore, it would be interesting to test cyclic substrates like 2-cyclopenten-1-one **116**, for their applicability in this MBH synthesis. A successful implementation of **116** would afford isoiminosugar derivatives of bicyclic castanospermine (**19**), such as **K**.

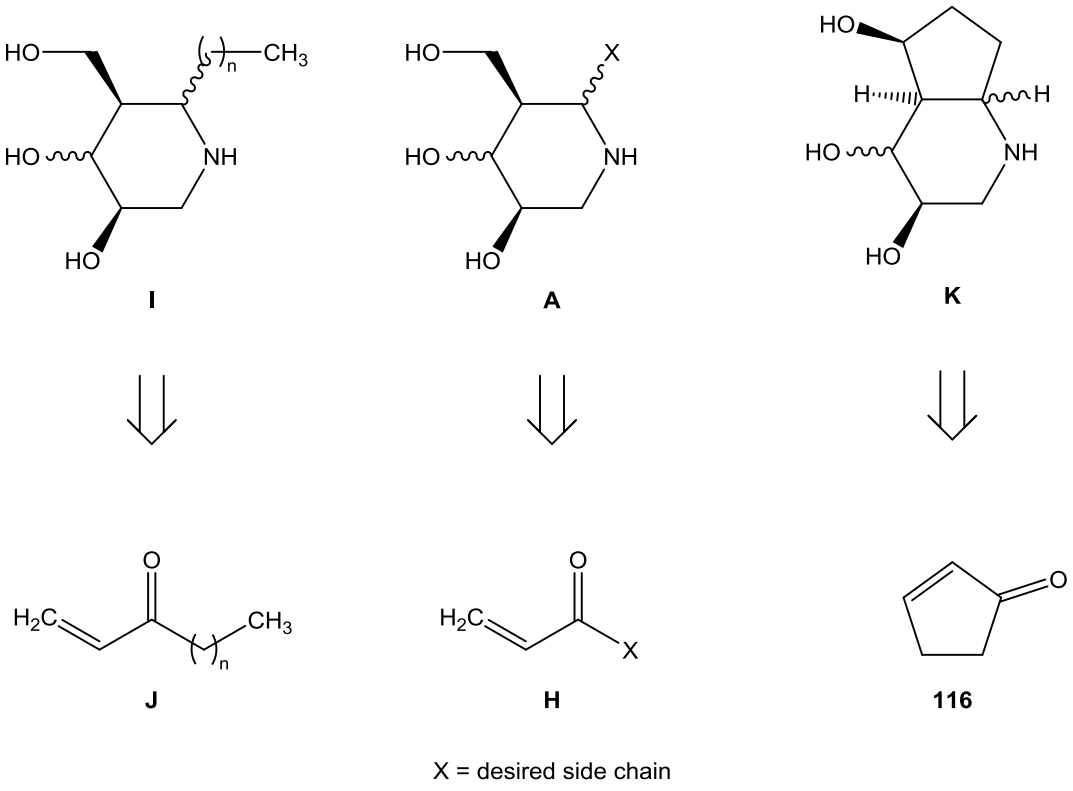


Figure 34. Possible scope of isoiminosugars, by the new synthetic approach.

4.2.1 Biological evaluation

The biological evaluation of the 5a-*C*-pentyl-4-*epi*-isofagomine **105 a,b** is currently in progress, and the discussion of their results will be subject of prospective work.

5 Experimental

5.1 General methods

Materials and methods

All commercially available chemicals, from Sigma Aldrich, Roth, VWR or Thermo Fisher Scientific, were used in the described reactions without further purification, unless stated otherwise.

Thin layer chromatography

To control conversion and reaction progress, analytical thin layer chromatography (TLC) using silica gel 60 F_{254} coated alumina sheets (Merck), was performed. Different solvent mixtures were used as eluents, which are described together with the respective product (v/v). Compounds were detected either under UV-light (254 nm) and/or by the use of one of the following staining reagents and subsequent heating with a heat gun.

CAM: *Ceric sulfate, ammonium molybdate*: Ammonium molybdate (100 g) dissolved in 10 % sulfuric acid (1000 mL), ceric sulfate (8 g) in sulfuric acid (80 mL) - both solutions were mixed subsequently.

VAN: *Vanillin, sulfuric acid*: A solution of water (30 mL), concentrated sulfuric acid (4 mL), ethanol (25 mL) and vanillin (0.3 g).

Silica gel flash column chromatography

In order to purify obtained reaction products, silica gel flash column chromatography (FCC) was used. Silica gel 0.035-0.070 mm, 60 A (ACROS ORGANICS) was used as stationary phase, while the eluent was a solvent mixture, with either constant ratio or increasing polar gradient, as reported with the respective reaction (v/v).

Nuclear magnetic resonance

Nuclear magnetic resonance spectroscopy (NMR) was performed on a Bruker Ultrashield 300 ™ spectrometer. Chemical shifts (δ) are reported in [ppm] and coupling constants (*J*) in [Hz]. ^{1}H spectra were recorded at 300.36 MHz in $CDCl_3$ (δ = 7.26) or CD_3OD (centre line δ = 3.31) and referenced to the respective solvent residual peaks. ^{13}C Spectra were measured at 75.53 MHz and referenced to the solvent signals in $CDCl_3$ (centre line δ = 77.16) or CD_3OD (centre line δ = 49.00).[106]

s	(singlet)	bs	(broad signal)	m	(multiplet)
d	(doublet)	dd	(doublet of doublets)	ddd	(doublet of dd)
t	(triplet)	td	(triplet of doublets)	q	(quartet)

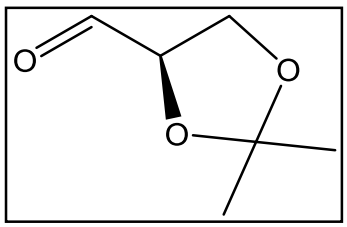
Specific rotation

The specific rotation $[\alpha]_D^{20}$ of chiral compounds was determined at 20 °C on a Perkin Elmer 341 polarimeter, with a path length of 10 cm, operating at a wave length of 589 nm. The concentration *c* of the measured compound is reported in [g/100 mL].

5.2 Morita-Baylis-Hillman reaction

2,3-*O*-Isopropylidene-D-glyceraldehyde (93)

A solution of commercially available 1,2:5,6-di-*O*-isopropylidene-D-mannitol (**92**) (648 mg, 2.47 mmol) in CH_2Cl_2 (50 mL) was mixed with H_2O (10 mL) under ambient conditions. Upon vigorous stirring small amounts of $NaIO_4$ were added to the colourless emulsion, until full conversion of the substrate was observed. After 45 min the organic phase was extracted with $NaHCO_3$ (sat.), dried over Na_2SO_4 and concentrated under reduced pressure to yield a colourless syrup (410 mg, 3.15 mmol, 64 %).



$C_6H_{10}O_3$

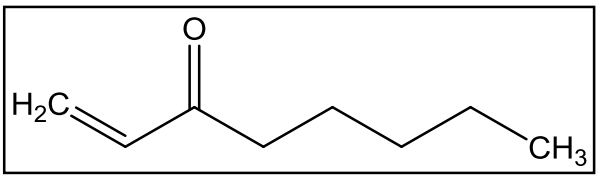
MW: 130.14 g/mol

TLC: CAM

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

Oct-1-en-3-one (101)

To a solution of oxalyl chloride (8.00 mL, 93.3 mmol) in CH_2Cl_2 (400 mL), DMSO (7.90 mL, 112 mmol) was added dropwise at -78 °C. A solution of commercially available 1-octen-3-ol (racemic mixture, 9.60 mL, 62.1 mmol) in CH_2Cl_2 (20 mL) was added to the reaction mixture, followed by triethylamine (35.0 ml, 218 mmol) after 20 min. The reaction was stirred for 3 h at -78 °C until complete conversion and was warmed up to ambient temperature. The organic phase was extracted with HCl (2 M) and $NaHCO_3$ (sat.), dried over Na_2SO_4 and removed under reduced pressure. The remaining yellow syrup (15.6 g, max. 93.3 mmol, >100 %) was used for in the following step without further purification.



$C_8H_{14}O$

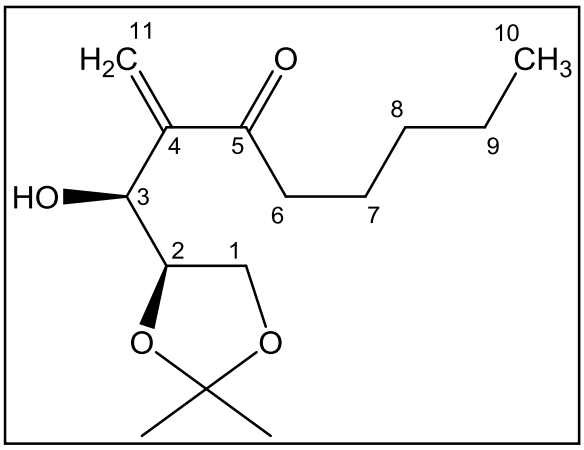
MW: 126.20 g/mol

TLC: UV, VAN

R_f: 0.7 (C/EA 3:1 v/v)

(2*R*,3*S*)-1,2,3-Trihydroxy-1,2-*O*-isopropylidene-4-*C*-methylene-decan-5-one (103)

A solution of the α,β-unsaturated ketone **101** (1.01 g, 8.00 mmol) in 1,4-dioxane, H_2O (3 + 1 mL) was treated with DABCO (880 mg, 7.80 mmol) under ambient conditions. After 15 min, a 50 % solution (w/v) of aldehyde **93** (410 mg, 3.15 mmol) in 1,4-dioxane was added dropwise. When full conversion was observed after ca. 15 h, all solvents were removed under reduced pressure and the crude product was dissolved in CH_2Cl_2. The organic phase was extracted with HCl (2 M) and $NaHCO_3$ (sat.), dried over Na_2SO_4 and concentrated under reduced pressure. After purification of the residue using FCC, the desired compound was obtained as colourless syrup (396 mg, 1.54 mmol, 49 %).



$C_{14}H_{24}O_4$

MW: 256.34 g/mol

TLC: UV, CAM

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

FCC: (C/EA 15:1 → 5:1 v/v)

$[\alpha]_D^{20}$: +3.5 ° (c = 1.09, $CHCl_3$)

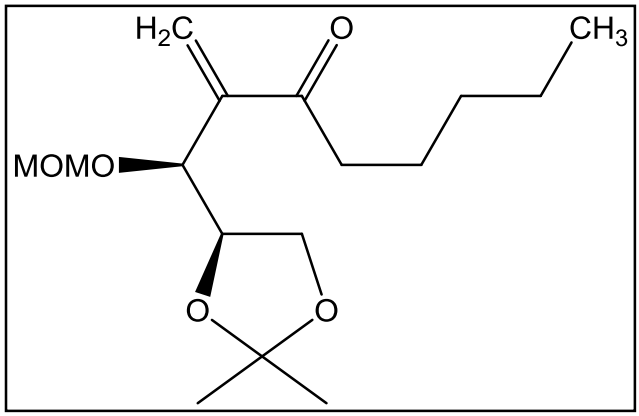
^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.12, 6.05 (2s, 2H, H-11), 4.46 (d, $J_{2,3}$=5.5, 1H, H-3), 4.17 (dd, $J_{1,2}$=6.2, 1H, H-2), 3.79 (d, 2H, H-1), 3.22 (bs, 1H, 3-OH), 2.61 (t, $J_{6,7}$=7.4, 2H, H-6), 1.52 (m, 2H, H-7), 1.33, 1.24 (2s, 6H, iPr), 1.22 (m, 4H, H-8, H-9), 0.81 (t, J=6.7, 3H, H-10).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 202.59 (C-5), 146.21 (C-4), 126.32 (C-11), 109.54 (iPr-Cq), 76.72 (C-2), 70.78 (C-3), 65.23 (C-1), 38.11 (C-6), 31.35 (C-8), 26.48, 25.07 (2C, iPr-CH_3), 23.95 (C-7), 22.41 (C-9), 13.86 (C-10).

5.3 Parallel reactions for (5a*R*) and (5a*S*)-series

(2*R*,3*S*)-1,2,3-Trihydroxy-1,2-*O*-isopropylidene-3-*O*-methoxymethyl-4-*C*-methylene-decan-5-one (106)

To a solution of the Morita-Baylis-Hillman reaction adduct **103** (396 mg, 1.54 mmol) in CH_2Cl_2 (15 mL), Hünig's Base (0.79 mL, 4.63 mmol) and MOMCl (0.16 mL, 2.16 mmol) were added. The solution was heated to reflux over 24 h, while more Hünig's Base (3.20 mL, 18.5 mmol) and MOMCl (0.66 mL, 8.64 mmol), were added until full conversion was observed. The resulting brown reaction mixture was extracted with HCl (2 M) and $NaHCO_3$ (sat.), the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by FCC afforded the MOM protected compound **106** as colourless syrup (411 mg, 1.37 mmol, 88 %).



$C_{16}H_{28}O_5$

MW: 300.40 g/mol

TLC: UV, CAM

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

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

$[\alpha]_D^{20}$: +12.2 ° (c = 1.05, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.11, 5.98 (2s, 2H, H-11), 4.64 (d, $J_{2,3}$=5.3, 1H, H-3), 4.50 (2d, *J*=10.7, 2H, MOM-CH_2), 4.10 (dd, $J_{1,2}$=6.1, 1H, H-2), 3.89 – 3.74 (m, 2H, H-1), 3.25 (s, 3H, MOM-CH_3), 2.59 (2t, $J_{6,7}$=7.2, 2H, H-6), 1.51 (m, 2H, H-7), 1.28, 1.21 (2s, 6H, iPr), 1.19 (m, 4H, H-8, H-9), 0.79 (t, *J*=6.7, 3H, H-10).

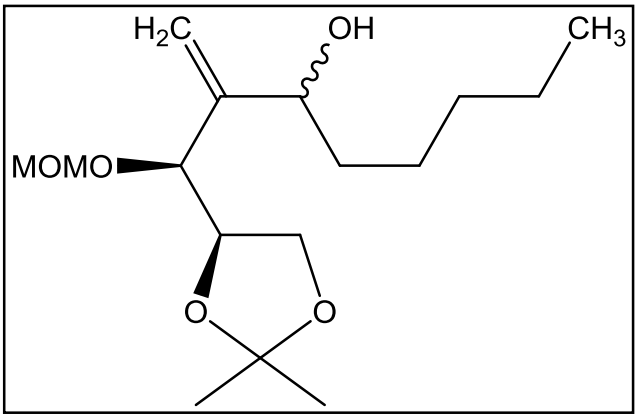
^{13}C NMR (76 MHz, $CDCl_3$): δ = 201.05 (C-5), 146.63 (C-4), 125.35 (C-11), 109.39 (iPr-Cq), 95.44 (MOM-CH_2), 77.59 (C-2), 73.57 (C-3), 65.67 (C-1), 55.73 (MOM-CH_3), 38.12 (C-6), 31.34 (C-8), 26.17, 25.21 (2C, iPr-CH_3), 23.93 (C-7), 22.40 (C-9), 13.83 (C-10).

(2*R*,3*S*,5*S*)-1,2-*O*-Isopropylidene-3-*O*-methoxymethyl-4-*C*-methylene-decane-1,2,3,5-tetraol (107 a)

and

(2*R*,3*S*,5*R*)-1,2-*O*-Isopropylidene-3-*O*-methoxymethyl-4-*C*-methylene-decane-1,2,3,5-tetraol (107 b)

A solution of compound **106** (3.85 g, 12.8 mmol) in THF (70 mL) was cooled to 0 °C and treated with LAH (730 mg, 19.2 mmol). When full conversion was observed after 5 min, H_2O (2.1 mL) and NaOH (3 M, 2.1 mL) were added carefully. The dark grey suspension was allowed to reach ambient temperature and was stirred for additional 2 h, until a white flocculation occurred. This precipitate was filtered off and washed with THF. The filtrate was dried over Na_2SO_4 and concentrated under reduced pressure. After purification by FCC both epimers **107 a** and **107 b** were obtained as colourless syrup (3.45 g, 11.4 mmol, 89 %). Due to their similar R_f values, separation between the two epimers by FCC was only possible to some extend for **107 a**.



$C_{16}H_{30}O_5$

MW: 302.41 g/mol

TLC: CAM

R_f: **107 a**: 0.45 (C/EA 2:1 v/v)

107 b: 0.40 (C/EA 2:1 v/v)

FCC: (C/EA 20:1 → 10:1 v/v)

$[\alpha]_D^{20}$: **107 a**: +77.4 ° (c = 0.90, $CHCl_3$)

107 a: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.29, 5.25 (2s, 2H, H-11), 4.59, 4.46 (2d, *J*=6.7, 2H, MOM-CH_2), 4.22 – 4.07 (m, 4H, H-1a, H-2, H-3, H-5), 3.95 – 3.87 (m, 1H, H-1b), 3.34 (s, 3H, MOM-CH_3), 3.02 (bs, 1H, 5-OH), 1.71 – 1.46 (m, 2H, H-6), 1.42, 1.33 (2s, 6H, iPr), 1.28 (m, 6H, H-7, H-8, H-9), 0.87 (t, *J*=5.9, 3H, H-10).

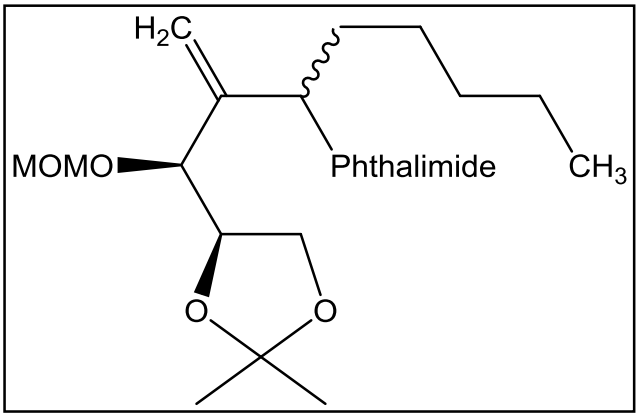
107 a: ^{13}C NMR (76 MHz, $CDCl_3$): δ = 149.39 (C-4), 115.76 (C-11), 109.88 (iPr-Cq), 93.96 (MOM-CH_2), 78.63, 75.92, 73.03 (3C, C-2, C-3, C-5), 68.14 (C-1), 55.92 (MOM-CH_3), 36.06 (C-6), 31.84, 25.71, 22.76 (3C, C-7, C-8, C-9), 26.38, 25.28 (2C, iPr-CH_3), 14.15 (C-10).

(2*R*,3*S*,5*R*)-1,2-*O*-Isopropylidene-3-*O*-methoxymethyl-4-*C*-methylene-5-phthalimido-decane-1,2,3-triol (108 a)

and

(2*R*,3*S*,5*S*)-1,2-*O*-Isopropylidene-3-*O*-methoxymethyl-4-*C*-methylene-5-phthalimido-decane-1,2,3-triol (108 b)

A solution of the epimers **107 a** and **107 b** (2.11 g, 6.98 mmol) in THF (45 mL) was treated with PPh_3 (3.66 g, 14.0 mmol) at 0 °C. Diethyl azodicarboxylate (DEAD) (2.20 mL, 14.0 mmol) and phthalimide (2.05 g, 14.0 mmol) were added, whereupon an intense yellow solution was formed, which was allowed to warm up to ambient temperature after 1.5 h. The reaction was stirred for additional 17 h. THF was removed under reduced pressure. Remaining reagents and impurities were precipitated from Et_2O, C at -18 °C and filtered off. The filtrate was concentrated under reduced pressure and the residue was purified by FCC, to obtain a colourless syrup (2.41 g, 5.58 mmol, 80 %). Contrary to **108 a**, **108 b** was not purified as single epimer at this stage, and was therefore not further characterised.



$C_{24}H_{33}NO_6$

MW: 431.53 g/mol

TLC: UV, CAM

R_f: **108 a**: 0.56 (C/EA 2:1 v/v)

108 b: 0.52 (C/EA 2:1 v/v)

FCC: (C/EA 15:1 → 10:1 v/v)

$[\alpha]_D^{20}$: **108 a**: +91.0 ° (c = 1.00, $CHCl_3$)

108 a: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (ddd, *J*=33.3, 5.3, 3.1, 4H, Phthalimide-CH), 5.63, 5.44 (2s, 2H, H-11), 4.83 (dd, *J*=10.3, 4.9, 1H, H-5), 4.54, 4.20 (2d, *J*=6.7, 2H, MOM-CH_2), 4.22 (m, 1H, H-2), 4.05 (dd, $J_{1a,1b}$=8.4, $J_{1a,2}$=5.9, 1H, H-1a), 4.02 (d, $J_{2,3}$=7.9, 1H, H-3) 3.80 (dd, 1H, H-1b), 3.23 (s, 3H, MOM-CH_3), 2.46 – 2.27, 2.04 – 1.86 (2m, 2H, H-6), 1.26, 1.22 (2s, 6H, iPr), (m, 6H, H-7, H-8, H-9), 0.84 (t, *J*=6.9, 3H, H-10).

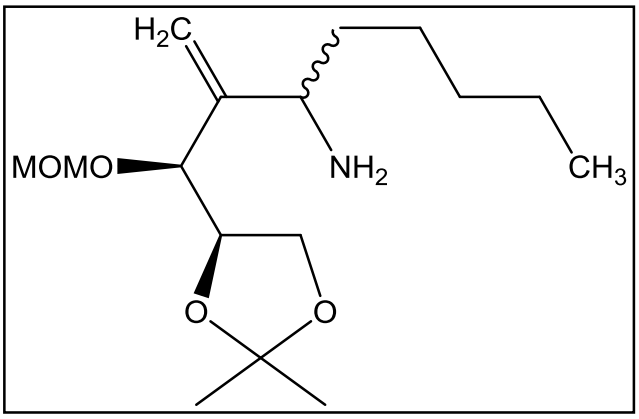
108 a: ^{13}C NMR (76 MHz, $CDCl_3$): δ = 168.52 (2C, Phthalimide-CO), 142.98 (C-4), 133.92 (2C, Phthalimide-CH), 132.11 (2C, Phthalimide-Cq), 123.18 (2C, Phthalimide-CH), 119.40 (C-11), 109.58 (iPr-Cq), 93.78 (MOM-CH_2), 79.71 (C-3), 76.60 (C-2), 67.76 (C-1), 55.85 (MOM-CH_3), 51.66 (C-5), 31.49, 26.35, 22.61 (3C, C-7, C-8, C-9) 30.01 (C-6), 26.49, 25.44 (2C, iPr-CH_3), 14.09 (C-10).

(2*R*,3*S*,5*R*)-5-Amino-1,2-*O*-isopropylidene-3-*O*-methoxymethyl-4-*C*-methylene-decane-1,2,3-triol (109 a)

and

(2*R*,3*S*,5*S*)-5-Amino-1,2-*O*-isopropylidene-3-*O*-methoxymethyl-4-*C*-methylene-decane-1,2,3-triol (109 b)

To a 5 % solution (w/v) of compound **108 a** (668 mg, 1.59 mmol) in EtOH, hydrazine monohydrate (0.10 mL, 1.91 mmol) was added and the reaction was heated to reflux. After 4 h, when full conversion was observed, the solvent was removed under reduced pressure and the crude product was immediately used for the next step. The same procedure was used to obtain **109 b** from **108 b**.



$C_{16}H_{31}NO_4$

MW: 301.43 g/mol

TLC: CAM

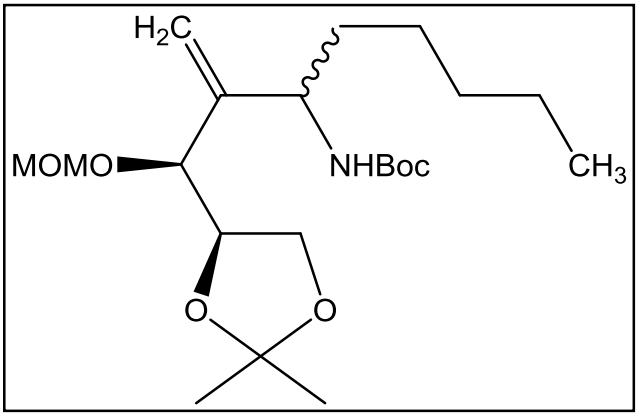
R_f: 0.48 ($CHCl_3$/MeOH/NH_3 8:1:0.01 v/v/v)

(2*R*,3*S*,5*R*)-*N*-tert-Butyloxycarbonyl-5-amino-1,2-*O*-isopropylidene-3-*O*-methoxymethyl-4-*C*-methylene-decane-1,2,3-triol (110 a)

and

(2*R*,3*S*,5*S*)-*N*-tert-Butyloxycarbonyl-5-amino-1,2-*O*-isopropylidene-3-*O*-methoxymethyl-4-*C*-methylene-decane-1,2,3-triol (110 b)

A solution of the crude product **109 a** in MeOH was treated with Et_3N (5.10 mL, 36.7 mmol) and Boc_2O (5.20 g, 23.9 mmol) under ambient conditions. When full conversion was observed after 15 h, the reaction mixture was concentrated under reduced pressure and dissolved in CH_2Cl_2. The organic phase was extracted with HCl (2 M) and $NaHCO_3$ (sat.), dried over Na_2SO_4 and removed under reduced pressure. Further purification by FCC yielded a colourless syrup (514 mg, 1.28 mmol, 80 % over 2 steps). The same procedure was used to obtain the *N*-Boc protected of **110 b** from the free amine **109 b**.



$C_{21}H_{39}NO_6$

MW: 401.54 g/mol

TLC: CAM, VAN

R_f: 0.65 (C/EA 2:1 v/v)

FCC: (C/EA 10:1 → 5:1 v/v)

$[\alpha]_D^{20}$: +67.9 ° (c = 0.89, $CHCl_3$)

110 a ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.23, 5.16 (2s, 2H, H-11), 4.71 (bs, 1H, NH), 4.62, 4.56 (2d, *J*=6.7, 2H, MOM-CH_2), 4.27 (m, 1H, H-2), 4.13 (d, $J_{2,3}$=5.5, 1H, H-3), 4.08 – 3.88 (m, 3H, H-1, H-5), 3.35 (s, 3H, MOM-CH_3), 1.73 – 1.60 (m, 1H, H-6a), 1.41 (bs, 13H, H-6b, Boc, iPr), 1.34 (s, 3H, iPr), 1.27 (m, 6H, H-7, H-8, H-9), 0.87 (t, *J*=6.7, 3H, H-10).

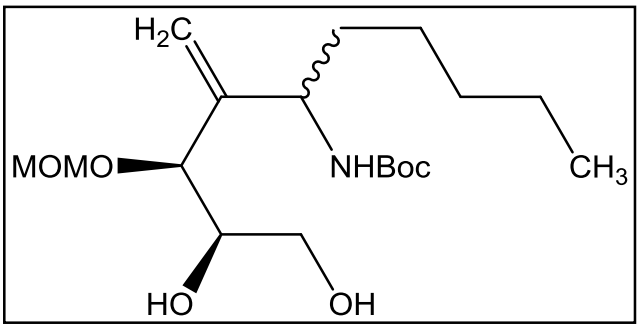
110 a ^{13}C NMR (76 MHz, $CDCl_3$): δ = 155.28 (Boc-CO), 148.53 (C-4), 113.12 (C-11), 109.57 (iPr-Cq), 94.59 (MOM-CH_2), 79.29 (Boc-Cq), 77.36 (C-2), 76.95 (C-3), 66.12 (C-1), 55.86 (MOM-CH_3), 52.55 (C-5), 34.99 (C-6), 31.72, 25.85, 22.70 (3C, C-7, C-8, C-9), 28.51 (3C, Boc-CH_3), 26.51, 25.54 (2C, iPr-CH_3), 14.13 (C-10).

(2*R*,3*S*,5*R*)-*N*-tert-Butyloxycarbonyl-5-amino-3-*O*-methoxymethyl-4-*C*-methylene-decane-1,2,3-triol (111 a)

and

(2*R*,3*S*,5*S*)-*N*-tert-Butyloxycarbonyl-5-amino-3-*O*-methoxymethyl-4-*C*-methylene-decane-1,2,3-triol (111 b)

A 10 % solution (w/v) of **110 a** (220 mg, 0.54 mmol) in CH_2Cl_2 was treated with a few drops of H_2O and MeOH (to prevent phase separation) under ambient conditions, then pTSA was added, until pH = 2 was reached. Full conversion was observed after 22 h. The reaction was neutralised using solid $NaHCO_3$. After removal of CH_2Cl_2 and MeOH under reduced pressure, the aqueous reaction mixture was extracted with CH_2Cl_2, the organic layer dried over Na_2SO_4 and concentrated under reduced pressure. Purification using FCC afforded the desired compound **111 a** as colourless syrup (191 mg, 0.53 mmol, 97%). The epimer **111 b**, could be prepared analogously, by deprotection of **110 b**.



$C_{18}H_{35}NO_6$

MW: 361.48 g/mol

TLC: CAM

R_f: **111 a**: 0.70 (C/EA 1:2 v/v)

111 b: 0.62 (C/EA 1:2 v/v)

FCC: (C/EA 10:1 → 2:1 v/v)

111 a: $[\alpha]_D^{20}$: +74.9 ° (c = 0.84, $CHCl_3$)

111 b: $[\alpha]_D^{20}$: +15.1 ° (c = 0.76, $CHCl_3$)

111 a: **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 5.27, 5.21 (2s, 2H, H-11), 4.88 (bs, 1H, NH), 4.57, 4.47 (2d, *J*=6.6, 2H, MOM-CH_2), 3.94 – 3.61 (m, 5H, H-1, H-2, H-3, H-5), 3.36 (s, 3H, MOM-CH_3), 1.82 – 1.64 (m, 1H, H-6a), 1.42 (s, 10H, H-7a, Boc), 1.36 – 1.17 (m, 6H, H-6b, H-7b, H-8, H-9), 0.88 (t, *J*=6.1, 3H, H-10).

111 a: **^{13}C NMR** (76 MHz, $CDCl_3$): δ = 156.41 (Boc-CO), 151.15 (C-4), 110.01 (C-11), 93.82 (MOM-CH_3), 80.53 (Boc-Cq), 76.52, 75.42 (2C, C-2, C-3), 64.07 (C-1), 55.87 (MOM-CH_2), 54.74 (C-5), 33.85 (C-6), 31.66, 22.66 (2C, C-8, C-9), 28.47 (3C, Boc-CH_3), 26.01 (C-7), 14.13 (C-10).

111 b: **^{1}H NMR** (300 MHz, $CDCl_3$): δ = 5.31, 5.21 (2s, 2H, H-11), 4.92 (bs, 1H, NH), 4.59, 4.43 (2d, *J*=6.5, 2H, MOM-CH_2), 4.12 – 3.84 (m, 4H, H-1a, H-2, H-3, H-4), 3.73 – 3.62 (m, 1H, H-1b), 3.37 (s, 3H, MOM-CH_3), 1.74 – 1.56 (m, 1H, H-6a), 1.41 (s, 10H, H-7a, Boc), 1.37 – 1.13 (m, 6H, H-6b, H-7b, H-8, H-9), 0.88 (t, *J*=6.3, 3H, H-10).

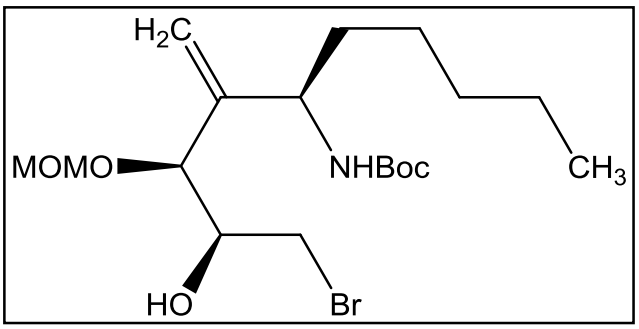
111 b: **^{13}C NMR** (76 MHz, $CDCl_3$): δ = 156.43 (Boc-CO), 146.91 (C-4), 117.83 (C-11), 93.82 (MOM-CH_2), 80.62 (C-3), 80.54 (Boc-Cq), 70.40 (C-2), 64.29 (C-1), 56.18 (MOM-CH_3), 50.65 (C-5), 35.73 (C-6), 31.62, 22.63 (2C, C-7, C-9), 28.47 (3C, Boc-CH_3), 25.97 (C-7), 14.14 (C-10).

5.4 (5a*R*)-Series ("cis" side chain)

(2*R*,3*S*,5*R*)-*N*-tert-Butyloxycarbonyl-5-amino-1-bromo-3-*O*-methoxymethyl-4-*C*-methylene-decane-2,3-diol (112 a)

To a 1 % solution (w/v) of **111 a** (247 mg, 0.68 mmol) in CH_2Cl_2, were added pyridine (0.55 mL, 6.83 mmol) and PPh_3Br_2 (0.50 g, 1.18 mmol). The reaction was heated to reflux temperature for 3 h. Additional PPh_3Br_2 (0.30 g, 0.68 mmol) was added and the reaction was stirred for 18 h at ambient conditions. After full conversion, the reaction was quenched by addition of EtOH, extracted with HCl (2 M) and $NaHCO_3$ (sat.), and the organic layer was dried over Na_2SO_4. After

removing CH_2Cl_2 under reduced pressure, the crude product was dissolved in Et_2O and cooled to -18 °C, to precipitate impurities. The desired compound **111 a** was obtained after purification of the concentrated filtrate by FCC as colourless syrup (226 mg, 0.53 mmol, 78 %).



$C_{18}H_{34}BrNO_5$

MW: 424.38 g/mol

TLC: CAM

R_f: 0.70 (C/EA 2:1 v/v)

FCC: (C/EA 10:1 → 6:1 v/v)

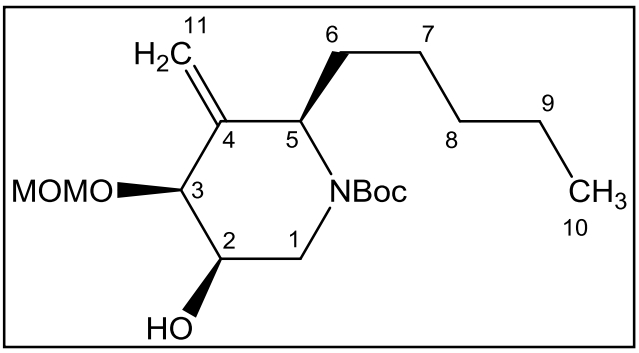
$[\alpha]_D^{20}$: +43.6 ° (c = 1.13, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.26, 5.23 (2s, 2H, H-11), 4.57, 4.47 (2d, *J*=6.7, 2H, MOM-CH_2), 3.95 – 3.84 (m, 2H, H-3, H-5), 3.80 – 3.61 (m, 3H, H-1, H-2), 3.37 (s, 3H, MOM-CH_3), 1.79 – 1.66 (m, 1H, H-6a), 1.39 (s, 10H, H-7a, Boc), 1.23 (m, 6H, H-6b, H-7b, H-8, H-9), 0.86 (t, *J*=6.4, 3H, H-10).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 156.19 (Boc-CO), 150.37 (C-4), 110.50 (C-11), 93.81 (MOM-CH_2), 80.41 (Boc-Cq), 76.21 (C-3), 75.60 (C-2), 56.16 (MOM-CH_3), 54.57 (C-5), 36.72 (C-1), 33.64 (C-6), 31.58, 22.61 (2C, C-8, C-9), 28.40 (3C, Boc-CH_3), 25.82 (C-7), 14.09 (C-10).

(5*R*)-*N*-tert-Butyloxycarbonyl-1,4,5-trideoxy-1,5-imino-3-*O*-methoxymethyl-4-*C*-methylene-5-*C*-pentyl-L-*erythro*-pentitol (113 a)

Under inert conditions (N_2 atmosphere), a 5 % solution (w/v) of **112 a** (270 mg, 0.64 mmol) in DMF/THF (1:1 v/v) was treated with NaH (77.0 mg of a 60 % dispersion in mineral oil, 1.91 mmol) at ambient temperature. When full conversion was observed after 1 h, the reaction was cooled to 0 °C and quenched by addition of H_2O (0.5 mL). The reaction mixture was extracted with HCl (2 M) and $NaHCO_3$ (sat.), the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. After purification by FCC a colourless syrup (145 mg, 0.42 mmol, 66 %) was obtained.



$C_{18}H_{33}NO_5$

MW: 343.46 g/mol

TLC: CAM

R_f: 0.64 (C/EA 1:1 v/v)

FCC: (C/EA 10:1 → 5:1 v/v)

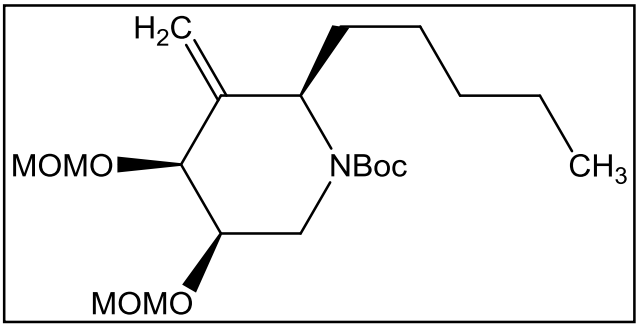
$[\alpha]_D^{20}$: +37.8 ° (c = 1.36, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): Major conformer: δ = 5.10, 5.06 (2s, 2H, H-11), 4.73, 4.55 (2d, *J*=6.6, 2H, MOM-CH_2), 4.55 (m, 1H, H-5), 4.24 (d, $J_{2,3}$=3.8, 1H, H-3), 3.98 (m, 1H, H-1eq), 3.60 (m, 1H, H-2), 3.38 (s, 3H, MOM-CH_3), 2.95 (m, 1H, H-1ax), 1.82 – 1.56 (m, 2H, H-6), 1.43 (s, 9H, Boc), 1.36 – 1.13 (m, 6H, H-7, H-8, H-9), 0.91 – 0.80 (bs, 3H, H-10).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 154.57 (Boc-CO), 146.52 – 140.63 (C-4), 119.04 – 111.55 (C-11), 93.35 (MOM-CH_2), 80.68, 79.98 (Boc-Cq), 77.36 (C-3), 68.70 (C-2), 57.25 (C-5), 55.92 (MOM-CH_3), 40.69, 39.45 (C-1), 35.24, 32.32 (C-6), 31.80 – 22.64 (3C, C-7, C-8, C-9), 28.52 (3C, Boc-CH_3), 14.12 (C-10).

(5*R*)-*N*-tert-Butyloxycarbonyl-1,5-imino-1,4,5-trideoxy-2,3-di-*O*-methoxymethyl-4-*C*-methylene-5-*C*-pentyl-L-*erythro*-pentitol (114 a)

A solution of **113 a** (145 mg, 0.42 mmol) in CH_2Cl_2 (15 mL) was treated with Hünig's Base (0.44 mL, 2.53 mmol), MOMBr (0.10 mL, 1.27 mmol) and was heated to reflux temperature. After 18 h, when full conversion was observed, the reaction was quenched by addition of MeOH. The brown solution was extracted with HCl (2 M), $NaHCO_3$ (sat.), the organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by FCC afforded **114 a**, a colourless syrup (111 mg, 0.29 mmol, 68 %).



$C_{20}H_{37}NO_6$

MW: 387.52 g/mol

TLC: CAM

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

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

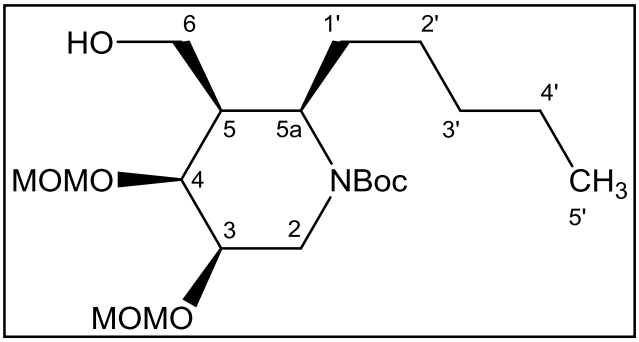
$[\alpha]_D^{20}$: +33.1 ° (c = 1.17, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): Major conformer: δ = 5.08, 5.04 (2s, 2H, H-11), 4.80 – 4.50 (m, 5H, H-5, MOM-CH_2), 4.34 (d, $J_{2,3}$=3.3, 1H, H-3), 4.13 (dd, $J_{1ax,1eq}$=12.8, $J_{1eq,2}$=5.0, 1H, H-1eq), 3.61 (m, 1H, H-2), 3.40, 3.38 (2s, 6H, MOM-CH_3), 3.13 (m, 1H, H-1ax), 1.78 – 1.47 (m, 2H, H-6), 1.44 (s, 9H, Boc), 1.25 (bs, 6H, H-7, H-8, H-9), 0.86 (bs, 3H, H-10).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 154.79, 154.60 (Boc-CO), 141.35, 140.68 (C-4), 118.44, 117.79 (C-11), 94.96, 93.02 (2C, MOM-CH_2), 79.97 (Boc-Cq), 75.05 (C-3), 73.56, 73.26 (C-2), 57.65, 56.54 (C-5), 55.83 – 55.49 (2C, MOM-CH_3), 38.39, 37.01 (C-1), 32.86, 32.40 (C-6), 31.99 – 22.73 (3C, C-7, C-8, C-9), 28.53 (3C, Boc-CH_3), 14.15 (C-10).

(5a*R*)-*N*-tert-Butyloxycarbonyl-3,4-di-*O*-methoxymethyl-5a-*C*-pentyl-4-*epi*-isofagomine (115 a)

A solution of **114 a** (166 mg, 0.43 mmol) in dry THF (10 mL) was cooled to 0 °C and treated with 9-BBN (4.28 mL of a 0.5 M solution in THF, 2.14 mmol) under inert conditions (N_2 atmosphere). The reaction mixture was allowed to warm up to ambient temperature and was stirred for 72 h when additional 9-BBN (6.42 mL, 3.21 mmol) was added. When full conversion was observed after 96 h, the reaction was worked up by the consecutive addition of H_2O (5 mL), NaOH (3 M, 8 mL) and H_2O_2 (30 %, 8 mL). After dilution with Et_2O, the organic layer was extracted with H_2O and dried over Na_2SO_4. After removal of the solvent under reduced pressure and purification by FCC the desired compound **115 a** was obtained as colourless syrup (70 mg, 0.17 mmol, 40 %).



$C_{20}H_{39}NO_7$

MW: 405.53 g/mol

TLC: CAM

R_f: 0.30 (C/EA 1:1 v/v)

FCC: (C/EA 10:1 → 1:1 v/v)

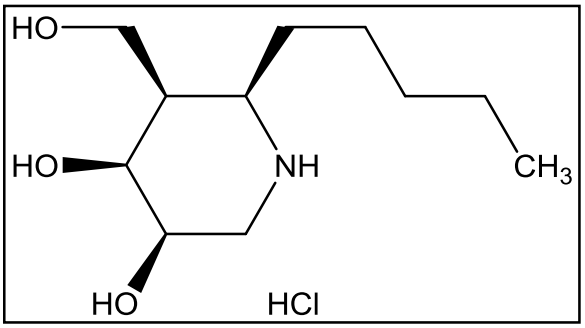
$[\alpha]_D^{20}$: -39.6 ° (c = 0.87, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): Major conformer: δ = 4.68 – 4.59 (m, 4H, MOM-CH_2), 4.21 – 3.81 (m, 3H, H-2eq, H-4, H-5a), 3.72 (m, 1H, H-6a), 3.58 – 3.47 (m, 2H, H-3, H-6b), 3.42, 3.37 (2s, 6H, MOM-CH_3), 3.07 – 2.81 (m, 1H, H-2ax), 2.63 (bs, 1H, 6-OH), 1.99 – 1.80 (m, 2H, H-5, H-1'a), 1.43 (s, 9H, Boc), 1.33 – 1.05 (m, 7H, H-1'b, H-2', H-3', H-4'), 0.93 – 0.77 (m, 3H, H-5').

^{13}C NMR (76 MHz, $CDCl_3$): δ = 155.04 (Boc-CO), 98.97, 95.28 (2C, MOM-CH_2), 79.92 (Boc-Cq), 74.58 (C-4), 74.14 (C-3), 60.29 (C-6), 56.18, 55.75 (2C, MOM-CH_3), 51.75, 50.78 (C-5a), 45.21 (C-5), 38.16, 36.73 (C-2), 31.82 – 22.85 (3C, C-2', C-3', C-4'), 28.51 (3C, Boc-CH_3), 28.17 (C-1'), 14.16 (C-5').

(5a*R*)-5a-*C*-Pentyl-4-*epi*-isofagomine hydrochloride (105 a)

To a 1 % solution (w/v) of **115 a** (19 mg, 47 µmol) in MeOH, a few drops of H_2O and HCl (conc.) were added and the reaction was stirred for 48 h between ambient temperature and 45 °C. After full conversion, the reaction mixture was concentrated under reduced pressure, treated with NH_3 (25 % aq.), and purified by FCC to afford the desired product as free base (8.5 mg, 39 µmol, 83 %). Treatment with HCl (gaseous) afforded the respective hydrochloride **105 a**.



$C_{11}H_{24}ClNO_3$

MW: 253.77 g/mol

TLC: CAM

R_f: 0.24 ($CHCl_3$/MeOH/NH_3 3:1:0.01 v/v/v)

FCC: ($CHCl_3$/MeOH/NH_3 3:1:0.01 v/v/v)

$[\alpha]_D^{20}$: -8.3 ° (c = 1.04, MeOH)

^{1}H NMR (300 MHz, CD_3OD): δ = 4.02 – 3.91 (m, 3H, H-3, H-4, H-6a), 3.84 (dd, $J_{6a,6b}$=11.8, $J_{5,6b}$=4.4, 1H, H-6b), 3.35 (m, 1H, H-5a), 3.28 (dd, $J_{2ax,2eq}$=13.2, $J_{2eq,3}$=3.5, 1H, H-2eq), 3.21 (dd, $J_{2ax,3}$=12.8, 1H, H-2ax), 2.15 (m, 1H, H-5), 1.99 – 1.74 (m, 2H, H-1'), 1.52 – 1.26 (m, 6H, H-2', H-3', H-4'), 0.93 (t, *J*=6.7, 3H, H-5').

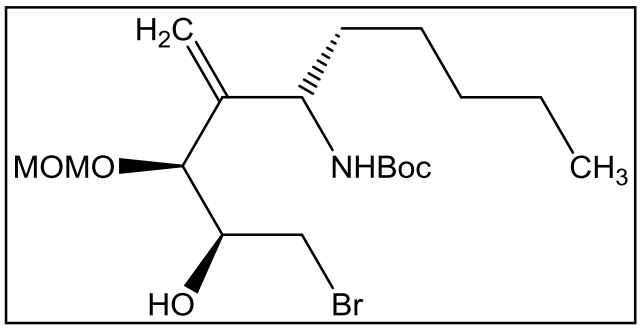
^{13}C NMR (76 MHz, CD_3OD): δ = 69.39 (C-4), 66.68 (C-3), 58.86 (C-5a), 57.33 (C-6), 48.15 (C-2), 42.46 (C-5), 32.64, 23.43 (2C, C-3', C-4'), 30.30 (C-1'), 26.47 (C-2'), 14.28 (C-5').

5.5 (5a*S*)-Series ("trans" side chain)

(2*R*,3*S*,5*S*)-*N*-tert-Butyloxycarbonyl-5-amino-1-bromo-3-*O*-methoxymethyl-4-*C*-methylene-decane-2,3-diol (112 b)

To a 5 % solution (w/v) of **111 b** (363 mg, 1.00 mmol) in CH_2Cl_2, pyridine (0.81 mL, 10.0 mmol) and PPh_3Br_2 (1.31 g, 3.10 mmol) were added, and the mixture was heated to reflux temperature for 18 h. The reaction was allowed to reach ambient temperature and EtOH was added. The organic

phase was extracted with HCl (2 M) and $NaHCO_3$ (sat.), dried over Na_2SO_4 and concentrated under reduced pressure. Purification by FCC afforded the desired compound **112 b** as colourless syrup (338 mg, 0.80 mmol, 80 %).



$C_{18}H_{34}BrNO_5$

MW: 424.38 g/mol

TLC: CAM

R_f: 0.60 (C/EA 2:1 v/v)

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

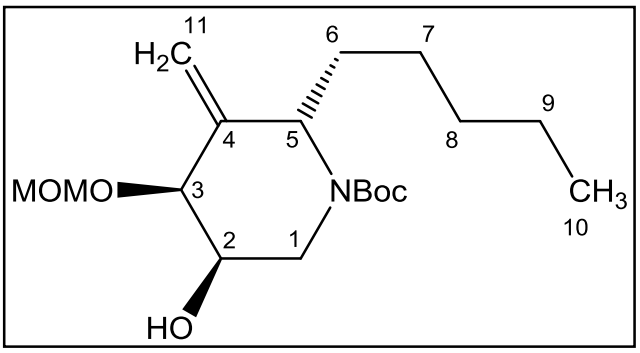
$[\alpha]_D^{20}$: +32.3 ° (c = 1.10, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.29, 5.21 (2s, 2H, H-11), 4.93 (bs, 1H, NH), 4.49 (2d, *J*=6.5, 2H, MOM-CH_2), 4.11 (d, *J*=8.5, 1H, H-3), 4.05 – 3.91 (m, 2H, H-2, H-5), 3.66 (ddd, *J*=15.2, 10.4, 3.7, 2H, H-1), 3.36 (s, 3H, MOM-CH_3), 1.67 – 1.53 (m, 1H, H-6a), 1.35 (s, 10H, H-7a, Boc), 1.31 – 1.11 (m, 6H, H-6b, H-7b, H-8, H-9), 0.83 (t, *J*=6.3, 3H, H-10).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 156.38 (Boc-CO), 146.54 (C-4), 118.23 (C-11), 93.94 (MOM-CH_2), 81.37 (Boc-Cq), 80.63 (C-3), 69.79 (C-2), 56.39 (MOM-CH_3), 50.78 (C-5), 36.55 (C-1), 35.69 (C-6), 31.60, 22.60 (2C, C-8, C-9), 28.43 (3C, Boc-CH_3), 25.95 (C-7), 14.11 (C-10).

(5*S*)-*N*-tert-Butyloxycarbonyl-1,4,5-trideoxy-1,5-imino-3-*O*-methoxymethyl-4-*C*-methylene-5-*C*-pentyl-L-*erythro*-pentitol (113 b)

Under inert conditions (N_2 atmosphere), a 5 % solution (w/v) of **112 b** (383 mg, 0.90 mmol) in DMF/THF (1:1 v/v) was treated with NaH (110 mg of a 60 % dispersion in mineral oil, 2.70 mmol) at ambient temperature. When full conversion was observed after 1 h, the reaction mixture was quenched through the addition of H_2O (0.5 mL) at 0 °C and extracted with HCl (2 M) and $NaHCO_3$ (sat.). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The desired cyclisation product **113 b**, which turned out to be the minor product of the reaction, was obtained by FCC as colourless syrup (49 mg, 0.14 mmol, 16 %).



$C_{18}H_{33}NO_5$

MW: 343.46 g/mol

TLC: CAM

R_f: 0.40 (C/EA 1:1 v/v)

FCC: (C/EA 10:1 → 6:1 v/v)

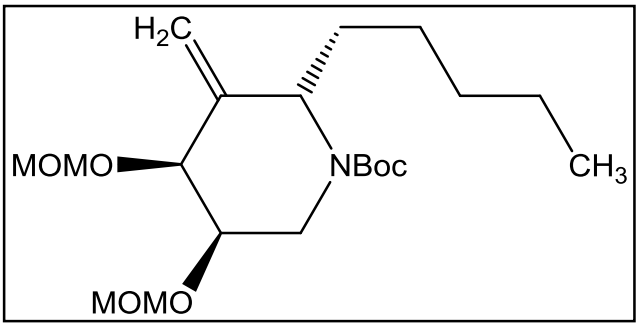
$[\alpha]_D^{20}$: +8.7 ° (c = 1.10, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.26 – 5.05 (m, 2H, H-11), 4.77, 4.69 (2d, *J*=6.8, 2H, MOM-CH_2), 4.79 – 4.57 (m, 1H, H-5), 4.37 – 3.89 (m, 2H, H-2, H-3), 4.23 – 4.10 (m, 1H, H-1eq), 3.41 (s, 3H, MOM-CH_3), 3.20 – 2.98 (m, 1H, H-1ax), 1.67 – 1.48 (m, 2H, H-6), 1.44 (s, 9H, Boc), 1.36 – 1.13 (m, 6H, H-7, H-8, H-9), 0.86 (t, *J*=6.9, 3H, H-10).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 155.48 (Boc-CO), 140.58 (C-4), 112.62 (C-11), 95.29 (MOM-CH_2), 80.16 (Boc-Cq), 74.29, 68.29 (2C, C-2, C-3), 59.75, 58.41 (C-5), 55.90 (MOM-CH_2), 44.76, 43.72 (C-1), 31.46, 26.01, 22.67 (3C, C-7, C-8, C-9), 31.01 (C-6), 28.49 (3C, Boc-CH_3), 14.07 (C-10).

(5*S*)-*N*-tert-Butyloxycarbonyl-1,5-imino-1,4,5-trideoxy-2,3-di-*O*-methoxymethyl-4-*C*-methylene-5-*C*-pentyl-L-*erythro*-pentitol (114 b)

A solution of **113 b** (43 mg, 0.13 mmol) in CH_2Cl_2 (5 mL) was treated with Hünig's base (0.22 mL, 1.25 mmol) and MOMCl (0.05 mL, 0.63 mmol), and was stirred under ambient conditions for 72 h. After full conversion, excess reagents were quenched by addition of EtOH and the organic phase was extracted with HCl (2 M) and $NaHCO_3$ (sat.), dired over Na_2SO_4 and concentrated under reduced pressure. Purification by FCC afforded the desired compound **114 b** (44 mg, 0.11 mmol, 91 %) as colourless syrup.



$C_{20}H_{37}NO_6$

MW: 387.52 g/mol

TLC: CAM

R_f: 0.76 (C/EA 1:1 v/v)

FCC: (C/EA 10:1 → 5:1 v/v)

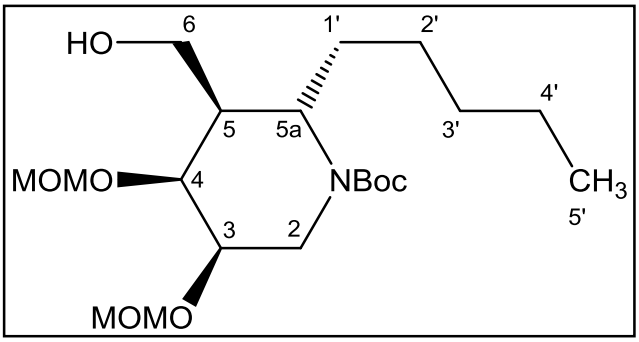
$[\alpha]_D^{20}$: -7.8 ° (c = 1.07, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.18, 5.06 (2s, 2H, H-11), 4.81 – 4.61 (m, 5H, H-5, 4x MOM-CH_2), 4.44 – 4.20 (m, 2H, H-1eq, H-3), 3.99 – 3.88 (m, 1H, H-2), 3.40 (2s, 6H, MOM-CH_3), 3.08 – 2.91 (m, 1H, H-1ax), 1.66 – 1.50 (m, 2H, H-6), 1.43 (s, 9H, Boc), 1.32 – 1.18 (m, 6H, H-7, H-8, H-9), 0.86 (t, *J*=6.7, 3H, H-10).

^{13}C NMR (76 MHz, $CDCl_3$): δ = 155.17 (Boc-CO), 141.70 (C-4), 110.34 (C-11), 95.05 (2C, MOM-CH_2), 79.86 (Boc-Cq), 73.45 (C-3), 72.08 (C-2), 59.39, 58.27 (C-5), 55.77, 55.56 (2C, MOM-CH_3), 42.45, 41.66 (C-1), 31.48, 26.11, 22.70 (3C, C-7, C-8, C-9), 30.98 (C-6), 28.52 (3C, Boc-CH_3), 14.09 (C-10).

(5a*S*)-*N*-tert-Butyloxycarbonyl-3,4-di-*O*-methoxymethyl-5a-*C*-pentyl-4-*epi*-isofagomine (115 b)

Under inert conditions (N_2 atmosphere), a 1 % solution (w/v) of **114 b** (41 mg, 0.11 mmol) in dry THF was cooled to 0 °C and treated with small portions of borane (total: 2.00 mL of a 1 M solution in THF, 2.00 mmol) over a period of 48 h and was allowed to warm up to ambient temperature. After full conversion, H_2O (5 mL), NaOH (3 M, 8 mL) and H_2O_2 (30 %, 8 mL) were added at 0 °C. The reaction mixture was diluted with Et_2O, extracted with brine and dried over Na_2SO_4. After purification by FCC, the desired compound **115 b** could be obtained as colourless syrup (18 mg, 44 µmol, 42 %).



$C_{20}H_{39}NO_7$

MW: 405.53 g/mol

TLC: CAM

R_f: 0.40 (C/EA 1:1 v/v)

FCC: (C/EA 5:1 → 1:1 v/v)

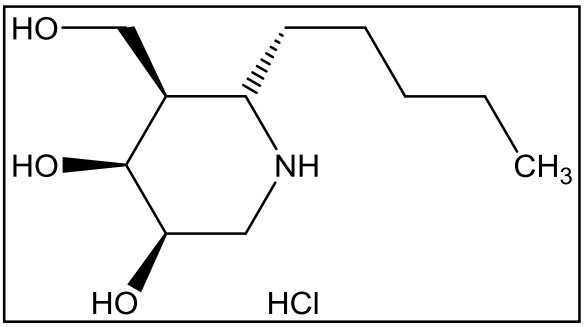
$[\alpha]_D^{20}$: -28.4 ° (c = 0.96, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.79 – 4.57 (m, 4H, MOM-CH_2), 4.51 – 4.34 (m, 1H, H-5a), 4.34 – 4.16 (m, 1H, H-2eq), 4.00 – 3.69 (m, 4H, H-3, H-4, H-6), 3.39 (2s, 6H, MOM-CH_3), 2.82 (d, $J_{2ax,2eq}$=14.2, 1H, H-2ax), 2.08 – 1.96 (m, 1H, H-5), 1.74 – 1.56 (m, 1H, H-1'a), 1.44 (s, 10H, H-1'b, Boc), 1.36 – 1.14 (m, 6H, H-2', H-3', H-4'), 0.94 – 0.81 (m, 3H, H-5').

^{13}C NMR (76 MHz, $CDCl_3$): δ = 156.24 (Boc-CO), 95.25, 94.48 (2C, MOM-CH_2), 79.87 (Boc-Cq), 72.37, 71.29 (2C, C-3, C-4), 61.67 (C-6), 55.78, 55.64 (2C, MOM-CH_3), 51.36 (C-5a), 43.85 (C-5), 41.52, 41.18 (C-2), 31.56, 26.15, 22.72 (3C, C-2', C-3', C-4'), 30.50 (C-1'), 28.50 (3C, Boc-CH_3), 14.12 (C-5').

(5a*S*)-5a-*C*-Pentyl-4-*epi*-isofagomine (105 b)

A 1 % solution (w/v) of **115 b** (18 mg, 44 µmol) in MeOH was treated with a few drops of H_2O and HCl (conc.) and was stirred for 24 h, at 60 °C. After full conversion, the solvents were removed under reduced pressure, and the crude product was treated with NH_3 (25 % aq.). After purification by FCC, the product was exposed to HCl (gaseous), to afford the desired hydrochloride of **105 b** (8.9 mg, 35 µmol, 80 %).



$C_{11}H_{24}ClNO_3$

MW: 253.77 g/mol

TLC: CAM

R_f: 0.30 ($CHCl_3$/MeOH/NH_3 3:1:0.01 v/v/v)

FCC: ($CHCl_3$/MeOH/NH_3 3:1:0.01 v/v/v)

$[\alpha]_D^{20}$: -19.4 ° (c = 0.89, MeOH)

^{1}H NMR (300 MHz, CD_3OD): δ = 4.19 – 4.10 (m, 1H, H-4), 3.93 – 3.81 (m, 1H, H-3), 3.71 (d, $J_{5,6}$=5.6, 2H, H-6), 3.40 – 3.23 (m, 1H, H-5a), 3.20 – 3.06 (m, 2H, H-2), 1.92 – 1.76 (m, 2H, H-5, H-1'a), 1.69 – 1.25 (m, 7H, H-1'b, H-2', H-3', H-4'), 0.93 (t, *J*=6.3, 3H, H-5').

^{13}C NMR (76 MHz, CD_3OD): δ = 68.94 (C-4), 67.22 (C-3), 61.49 (C-6), 53.81 (C-5a), 44.77 (C-5), 44.19 (C-2), 32.81, 25.23, 23.34 (3C, C-2', C-3', C-4'), 31.09 (C-1'), 14.27 (C-5').

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7 Appendix

7.1 Common abbreviations

Biochemical terms		**Miscellaneous abbreviations**	
CMT	chaperone-mediated therapy	**$[\alpha]_D^{20}$**	specific optical rotation
CoA	coenzyme A	**aq.**	aqueous solution
EC	enzyme commission number	**cat.**	catalytic
ER	endoplasmic reticulum	**conc.**	concentrated
ERAD	ER associated degradation	**Cq**	quaternary carbon atom
ERT	enzyme replacement therapy	**EWG**	electron withdrawing group
Gcase	glucocerebrosidase	**FCC**	flash column chromatography
GCS	glucosylceramide synthase	**HSQC**	heteronuclear single quantum coherence
GH	glycosyl hydrolase	**LG**	leaving group
GM1	G_{M1}-gangliosidosis	**M**	molar [mol/L]
GSL	glycosphingolipid	**MBH**	Morita-Baylis-Hillman
HSCT	hematopoietic stem cell transplantation	**MW**	molecular weight
IC_{50}	half maximal inhibitory concentration	**NMR**	nuclear magnetic resonance
K_i	enzyme inhibitor dissociation constant	**p.a.**	per annum
LSD	lysosomal storage disorder	**R_f**	retardation factor
mRNA	messenger ribonucleic acid	**sat.**	saturated solution
PC	pharmacological chaperone	**TLC**	thin layer chromatography
SL	sphingolipid	**T_m**	melting temperature
SRT	substrate reduction therapy	**v/v**	volume/volume
UDP	uridine diphosphate	**w/v**	weight/volume
Chemical substances and reagents		**Structural residues**	
9-BBN	9-borabicyclo[3.3.1]nonane	**Boc**	*tert*-butyloxycarbonyl (-$COOC(CH_3)_3$)
Boc_2O	di-*tert*-butyl dicarbonate	**Bn**	benzyl (-$CH_2C_6H_5$)
C	cyclohexane	**Et**	ethyl (-CH_2CH_3)
CAM	ceric sulfate, ammonium molybdate	**iPr**	isopropylidene (-$C(CH_3)_2$)
DABCO	1,4-diazabicyclo[2.2.2]octane	**Me**	methyl (-CH_3)
DEAD	diethyl azodicarboxylate	**MOM**	methoxymethyl (-CH_2OCH_3)
DGJ	1-deoxygalactonojirimycin	**Ph**	phenyl (-C_6H_5)
DMF	dimethylformamide	**TBS**	*tert*-butyldimethylsilyl (-$Si(CH_3)_2C(CH_3)_3$)

Chemical substances and reagents (*continued*)	
DMSO	dimethyl sulfoxide
DNJ	1-deoxynojirimycin
EA	ethyl acetate
IFG	isofagomine
LAH	lithium aluminium hydride
NANA	*N*-acetylneuraminic acid
NOEV	*N*-octyl-4-*epi*-β-valienamine
pTSA	*p*-toluenesulfonic acid
THF	tetrahydrofuran
VAN	vanillin, sulfuric acid

7.2 Curriculum vitae

René Lebl, born in Salzburg on October 04th 1990

Since Mar. 2014	Master studies: Technical Chemistry at Graz University of Technology. Thesis: *A pre-commercial de novo synthesis of 5a-C-pentyl-4-epi-isofagomine – a powerful pharmacological chaperone for GM1-gangliosidosis.* Supervision: Prof. Arnold Stütz
Jun. – Aug. 2016	Summer school: REU research program at Syracuse University, NY, USA. Working on: *Peptide-based Iridium (III) complexes as catalysts for asymmetric one-pot hydrogenations.* Supervision: Prof. Ivan Korendovych
Feb. – Jun. 2015	Exchange student: chemistry graduate program at *POSTECH,* Pohang University of Science and Technology, South Korea. Supervision: Prof. Young Ho Rhee
Oct. 2010 – Apr. 2014	Bachelor of Science in chemistry: completed at Graz University of Technology. Thesis: *6-Azido-6-deoxy-D-fructose as substrate for the Staudinger-aza-Wittig-cyanide multicomponent reaction.* Supervision: Prof. Tanja Wrodnigg
Sept. 2001 – Jun. 2009	Secondary school Matura: completed Bundesgymnasium Seekirchen.

7.3 List of publications

Posters

Lebl, R., Hojnik, C., Schalli, M., Thonhofer, M., Stütz, A., & Wrodnigg, T. (**2013**). *6-Azido-6-deoxy-D-fructose as substrate for the Staudinger-Aza-Wittig-Cyanide multicomponent reaction*. Poster session presented at 15th Austrian Chemistry Days, Graz, Austria.

Presentations

Hojnik, C., Gloe, T., Lebl, R., Lindhorst, T., Schalli, M., Stütz, A., Torvisco Gomez, A., Thonhofer, M., Weber, P., Wrodnigg, T., Zoidl, M. (**2016**). *The Amadori rearrangement as conjugation method: scope and limitations*. 20th Austrian Carbohydrate Workshop, Vienna, Austria.

Lebl, R., Hojnik, C., Schalli, M., Stütz, A., Thonhofer, M., Torvisco Gomez, A., Weber, P., Wrodnigg, T., Zoidl, M. (**2016**). *The Staudinger/aza-Wittig nucleophile reaction cascade: Scope and limitations*. 20th Austrian Carbohydrate Workshop, Vienna, Austria.

Schalli, M., Fischer, R., Hojnik, C., Lebl, R., Thonhofer, M., Stütz, A., Weber, P., Wrodnigg, T., Zoidl, M. (**2016**). *Synthesis of carbacyclic inhibitors for ß-galactosidases*. 20th Austrian Carbohydrate Workshop, Vienna, Austria.

Thonhofer, M., Fischer, R., Gonzales-Santana, A., Hojnik, C., Lebl, R., Papst, B., Schalli, M., Stütz, A., Tschernutter, M., Weber, P., Windischhofer, W., Withers, S. G., Wrodnigg, T., Zoidl, M. (**2016**). *Synthesis and biological evaluation of C-5a extended derivatives of 4-epi-isofagomine*. 20th Austrian Carbohydrate Workshop, Vienna, Austria.

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Lebl, R., Stütz, A., Thonhofer, M., Wrodnigg, T. (**2013**). *6-Azido-6-desoxy-D-fructose als Substrat für die SAW-CN-Reaktion.* Oral presentation at the 17th Austrian Carbohydrate Workshop, Graz, Austria.