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Synthesis of *C*-Glycosyl Type Iminosugar Building Blocks via a Multicomponent Reaction Strategy

DISSERTATION

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Technische Universität Graz

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Meinen Eltern und Geschwistern

Holzhacken ist deshalb so beliebt, weil man bei dieser Tätigkeit den Erfolg sofort sieht.

- Albert Einstein -

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ABSTRACT

The aim of this PhD thesis is the synthesis of various iminosugar building blocks with the help of the Staudinger/aza-Wittig reaction cascade and their evaluation as inhibitors of carbohydrate processing enzymes (CPE) or warheads for ligand directed protein profiling glycoprobes.

Iminosugars, excellent reversible inhibitors of CPEs, are an important tool to investigate, elucidate and manipulate their enzymatic functions and thus the synthesis of iminosugar based building blocks is a fundamental requirement for the diagnosis and treatment of many diseases, for example, diabetes, metastatic cancer and lysosomal storage diseases.

By employing different nucleophiles as imine traps in the Staudinger/aza-Wittig reaction sequence, various C-glycosyl type iminosugar building blocks have been synthesised and biologically evaluated in terms of their inhibition profile with a panel of glycoside hydrolases.

As these iminosugar derivatives are reversible, competitive inhibitors of glycoside hydrolases, they can be used as active site specific ligands for these enzymes. This allows for their employment as “warheads” in ligand directed protein profiling glycoprobes. Follow up chemistry on selected iminosugar derivatives gave access to these molecules.

KURZFASSUNG

Ziel dieser Arbeit ist die Synthese von verschiedenen Iminozuckerbausteinen mithilfe der Staudinger/aza-Wittig Reaktionskaskade und deren Evaluierung als Inhibitoren von kohlenhydratverarbeitenden Enzymen, oder als sogenannte „Warheads“ für „ligand directed protein profiling“ Glycoproben.

Iminozucker sind exzellente, reversible Inhibitoren von kohlenhydratverarbeitenden Enzymen. Sie werden als Werkzeuge für die Untersuchung, Aufklärung und Manipulation dieser Enzyme eingesetzt. Die Synthese dieser Iminozuckerbausteine ist eine fundamentale Voraussetzung für die Diagnose und Behandlung vieler Krankheiten, wie zum Beispiel Diabetes, metastatischem Krebs und lysosomaler Speicherkrankheiten.

Durch den Einsatz verschiedener Nukleophile in der Staudinger/aza-Wittig Reaktionssequenz, konnten C-glycosidisch derivatisierte Iminozuckerbausteine synthetisiert werden. Diese wurden als Inhibitoren von ausgewählten Glycosidasen evaluiert.

Iminozucker können außerdem als spezifische Liganden für das aktive Zentrum genannter Enzyme eingesetzt werden und so als „Warheads“ in „ligand directed protein profiling“ Glycoproben dienen.

Die Synthese dieser Glycoproben wurde durch Folgechemie an ausgewählten Iminozuckerderivaten realisiert.

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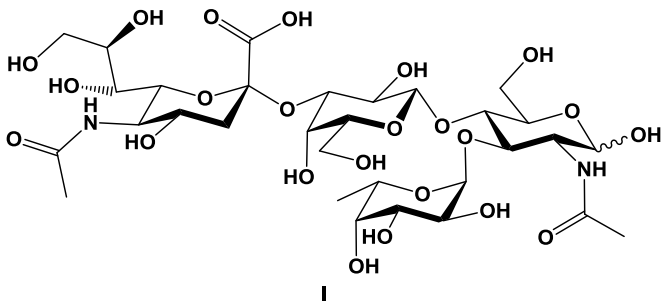
Abbreviations

General abbreviations			
$[a]_D^{20}$	specific optical rotation	TLC	thin layer chromatography
R_f	retardation factor	HRMS	high resolution mass spectrometry
Organic residues			
Cbz	carboxybenzyl	CN	nitrile
Et	ethyl	Me	methyl
Ph	phenyl	TBDMS	tert.butyl dimethylsilyl
Trit	triphenylmethyl	Bn	benzyl
Tos	p-toluenesulfonyl	Boc	tert.butyloxycarbonyl
Reagents and solvents			
Ac_2O	acetic anhydride	BnBr	benzylbromide
CH_2Cl_2	dichloromethane	DMAP	dimethylaminopyridine
CH	cyclohexane	EtOAc	ethyl acetate
DMF	dimethylformamide	Et_3N	triethylamine
H_2SO_4	sulfuric acid	HCl	hydrochloric acid
IR-120 H^+	acidic ion exchange residue	MeOH	methanol
Na_2CO_3	sodium carbonate	Na_2SO_3	sodium sulphite
Na_2SO_4	sodium sulfate	$NaBH_4$	sodium borohydride
NaH	sodium hydride	$NaHCO_3$	sodium hydrogencarbonate
NaN_3	sodium azide	NaOAc	sodium acetate
NaOH	sodium hydroxide	NaOMe	sodium methoxide
NH_4OH	ammoniumhydroxide	THF	tetrahydrofurane
TritCl	triphenylmethylchloride		

1. Introduction

1.1. Carbohydrates

Carbohydrates, also known as sugars or saccharides, form the most complex group of biopolymers. The deciphering of the “sugar code” or glycome is considered to be the most challenging field in cell biology due to the huge structural variety of natural occurring carbohydrate structures as well as their diverse biological functions. Monosaccharides exhibit multiple attachment sites, allowing them to build up linear and branched oligomeric and even polymeric structures. Carbohydrates can also be oxidized, reduced, dehydrogenated or substituted. An example, for a complex carbohydrate structure is Sialyl Lewis^x,(Figure 1 **I**).[1]



I

Figure 1: Structure of Sialyl Lewis^x (I).[1]

Additionally, carbohydrates can be linked to other biopolymers, such as proteins or lipids, forming glycoconjugates, like glycoproteins and glycolipids.[2]

The resulting diversity is responsible for the vital role of carbohydrates and their conjugates, as mediators as well as cell surface receptors and markers. Biologically important events such as cell-cell recognition, cell growth, cell development, cell-cell adhesion, inflammation, and metastasis, for example, are mediated by chemical signals transmitted either by sugars themselves or by their interactions with proteins.

The understanding of carbohydrate related processes on a molecular level is essential for the diagnosis and treatment of carbohydrate related diseases such as diabetes, metastatic cancer or lysosomal storage diseases, just to mention a few.[3]

However, advances in glycobiology are considerably slower than those of protein or nucleic acid biochemistry.

This is caused by several reasons: Firstly, for a long time, carbohydrates were considered to be important “only” for metabolic energy storage, or to function as inert structural polymers for plants, fungi, insects and crustaceans.
Secondly, as indicated before, the sheer complexity of carbohydrate structures and their conjugates, as well as the fact that only small quantities thereof can be isolated from natural systems, rendered it impossible to determine oligosaccharide sequences with the analytical instrumentation methods at hand.
To complicate things even more, homogenous and chemically well-defined glycoconjugates are very difficult to obtain by isolation, since their biosynthesis is neither template driven nor under direct transcriptional control. The assembly of oligosaccharides is performed in a step-wise fashion in the endoplasmatic reticulum (ER) and in the Golgi apparatus, which causes significant product microheterogeneity.[4]
Therefore, chemical synthesis is mandatory to get homogenous samples for the elucidation of carbohydrate related processes. This, however, requires strict regio- and stereochemical control in the glycosidic bond formation as well as sophisticated protecting group strategies. Additionally, the nature of these structures causes for very difficult and time demanding purification, isolation and analysis operations.

Nowadays, modern synthesis and analytical methods allow for the deduction of complex carbohydrate structures and the development of carbohydrate based drugs for diseases like cancer, diabetes, AIDS, influenza and bacterial infections.
A few selected examples of carbohydrate related drugs are given in Table 1.[5]

Table 1: Approved carbohydrate related based drugs.

Drug	Target	Company
Acarbose (**II**)	Diabetes (type I and II)	Bayer AG
Oseltamivir (**III**)	Influenza, antiviral	Hoffmann-La Roche
Celgosivir (**IV**)	HIV/AIDS	Hoechst Marion Roussel
Miglustat[6] (**V**)	Morbus Gaucher	Actelion
Miglitol[7] (**VI**)	Diabetes (type II)	Bayer AG

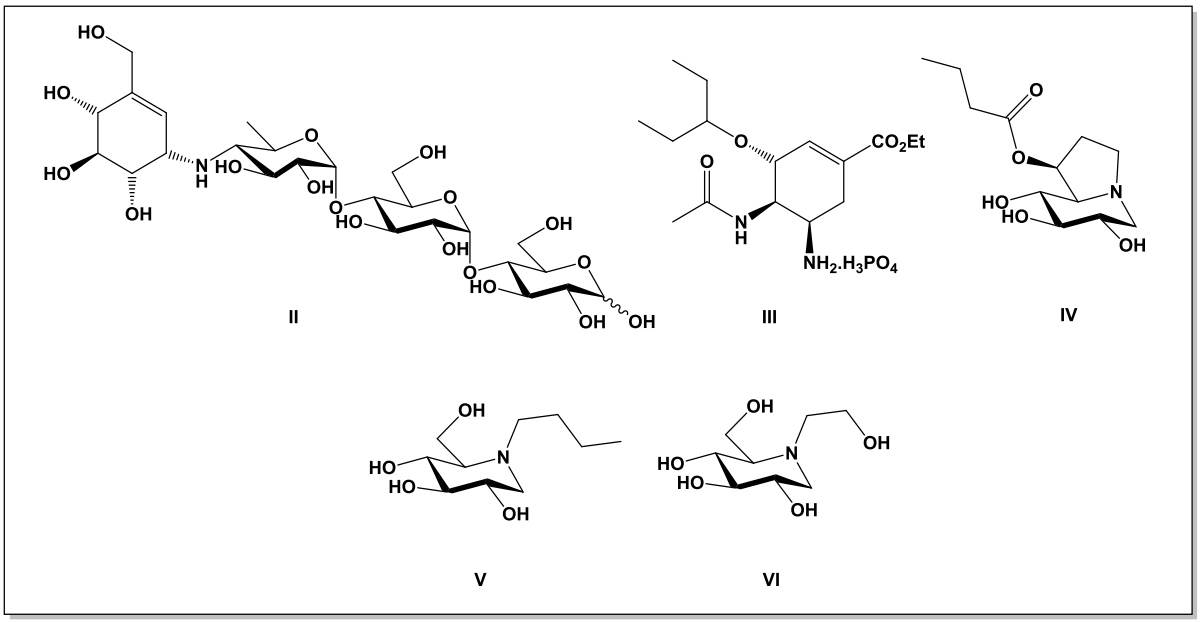


Figure 2: Examples of carbohydrate related based drugs.[5]

In order to be able to understand and treat carbohydrate related diseases it is important to understand, how monosaccharides are assembled, trimmed and shaped into bioactive glycoproteins or glycolipids by carbohydrate processing enzymes.

1.2. Carbohydrate processing enzymes (CPE)

The complexity and diversity of carbohydrates as well as glycoconjugates is controlled by CPEs or carbohydrate active enzymes.

Their main purpose is the assembly (glycosyl transferases) and the breakdown (glycoside hydrolases, polysaccharide lyases, carbohydrate esterases) of carbohydrate structures.

The huge variety of these enzymes is categorized in the Carbohydrate Active enZymes (CAZy) database.[8]

Two families are discussed for this purpose, namely glycoside hydrolases and glycosyl transferases, both of which involve cleavage of the glycoside bond linking a sugar's anomeric carbon with an oligo- or polysaccharide or a nucleoside diphosphate group.[9]

Although glycosyl transferases play a major role in post-translational modifications of proteins, namely the synthesis of *O*- and *N*-linked glycoproteins and glycolipids[10], herein the main focus will be laid on glycoside hydrolases.

1.2.1. Glycoside hydrolases

Glycoside hydrolases catalyze the cleavage of the glycosidic bond either at the non-reducing end of an oligo- or polysaccharide (*exo-glycosidases*) or within the polysaccharide chain (*endo-glycosidases*). These hydrolytic reactions can have two possible stereochemical outcomes. Inverting glycoside hydrolases, as the name suggests, invert the stereochemistry at the anomeric position of the molecule. In this process two carboxylic groups in the active site of the enzyme serve as general acid – base catalysts and are in a suitable distance to bind both the substrate and a water molecule (Figure 3, **i**). Afterwards, a single displacement reaction, involving an oxocarbenium ion-like transition state, occurs (Figure 3, **ii**), releasing the inverted, hydrolyzed substrate (Figure 3, **ii**, path **a**).[11]

The proposed reaction mechanism of retaining glycosidases suggests a double displacement mechanism, where first a carboxy function serves as acid-base catalyst to protonate the glycosidic oxygen, with concomitant bond cleavage, while the other carboxy group forms a covalent glycosyl-enzyme intermediate (Figure 3, **vi**). In the second step a water molecule is deprotonated by the carboxy function and attacks the anomeric center (Figure 3, **vii**), thus displacing the sugar with retention of the anomeric configuration (Figure 3, **viii**, path **b**). Both steps proceed via an oxocarbenium ion-like transition state (Figure 3, **v**, **vii**).[12]

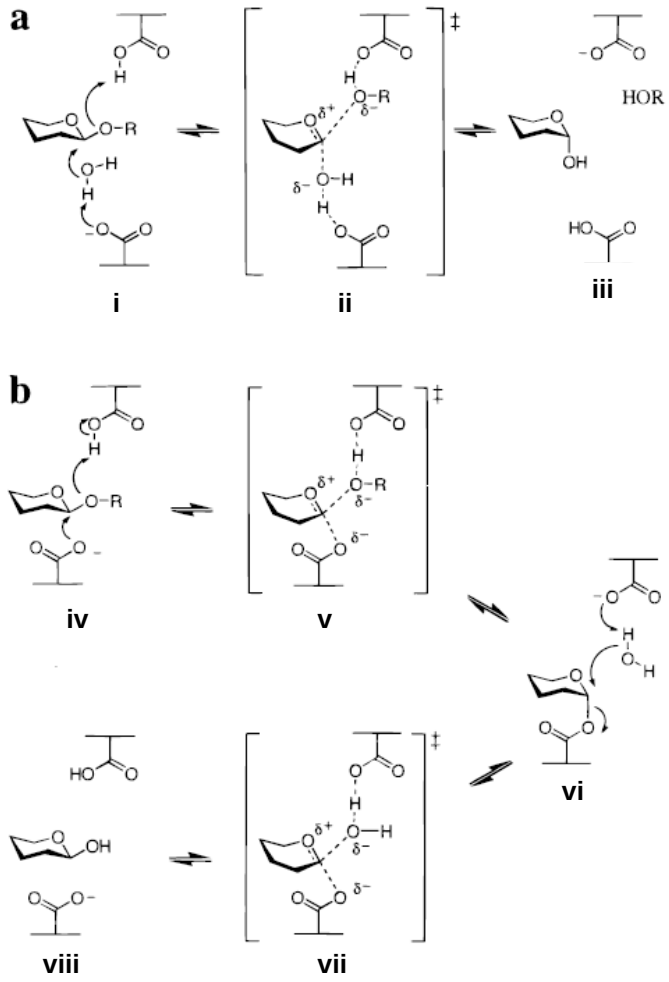


Figure 3: General mechanisms for inverting (a)[11] or retaining (b)[12] glycoside hydrolases.

Several biological processes are fundamentally influenced by the activity of glycoside hydrolases. Examples are the degradation of diet polysaccharides to obtain monosaccharides which can be metabolically absorbed, the catabolism of lysosomal glycoconjugates and glycoprotein processing as well as the conjugation of oligosaccharide units onto proteins or lipids.[13]

It is not surprising that, considering the vast biological impact of glycoside hydrolases, scientists have searched for valuable tools to investigate, elucidate and manipulate their physiological function with the help of biologically active compounds.

1.3. Inhibitors of glycoside hydrolases

Inhibitors in general decrease the enzyme activity by different mechanisms. In general they can be divided into covalent and non-covalent inhibitors. Both categories are valuable for different applications. Non-covalent inhibitors of glycoside hydrolases help with the elucidation of biological recognition processes[14] and are considered to have a high therapeutic value for the treatment of various diseases such as for example diabetes[15], obesity[16], HIV infections[17], tumors in general[18] and lysosomal storage diseases.[19]

Covalent inhibitors can be used for the identification of specific active site residues. Furthermore, they serve to study enzyme mechanisms and inactivate target enzymes. As activity based probes (ABP), covalent inhibitors can be applied for the discovery and characterization of enzymes.[20] The latter concept will be thoroughly discussed in chapter 1.6.

1.3.1. Non-covalent inhibitors of glycoside hydrolases

In general, non-covalent inhibitors of glycoside hydrolases are capable of mimicking the natural substrate or the transition state of the respective enzyme and are non-covalently bound in the active site, but are not enzymatically converted due to their chemical nature. As these interactions are non-covalent, the natural substrate can compete with the inhibitor for the active site, hence these molecules are often described as competitive, reversible inhibitors.

It is not surprising that most inhibitors closely resemble the respective carbohydrate substrates, the natural substrates of glycoside hydrolases. However, a few “non-carbohydrate” inhibitors are described in literature, e.g. chalcone (**VII**), a biosynthetic product of the shikimate pathway, which shows inhibitory properties against α-glucosidases (Figure 4).[21]

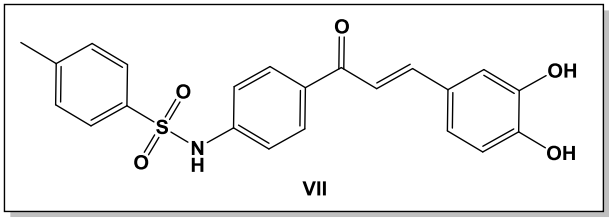


Figure 4: Chalcone, a non-carbohydrate inhibitor of glycoside hydrolases.[21]

Carbohydrate based inhibitors can be “generated” by manipulating functionalities of the monosaccharidic natural substrate of the enzyme.

For example by oxidation of the anomeric position, lactones, such as D-glucono-1,5-lactone (**VIII**) are generated (Figure 5).[22]

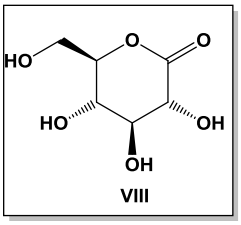


Figure 5: Example of a lactone.[22]

Another possibility is the introduction of a basic, trivalent nitrogen at the position of the ring oxygen, or at the anomeric position next to a methylene group at the former position of ring oxygen. These compounds are referred to as iminosugars or isoiminosugars, respectively. Paradigmatic examples of these compound classes are 5-amino-5-deoxy-D-glucono-1,5-lactam (**IX**)[22], deoxynojirimycin (**X**)[23] and isofagomine (**XI**)[24] (Figure 6).

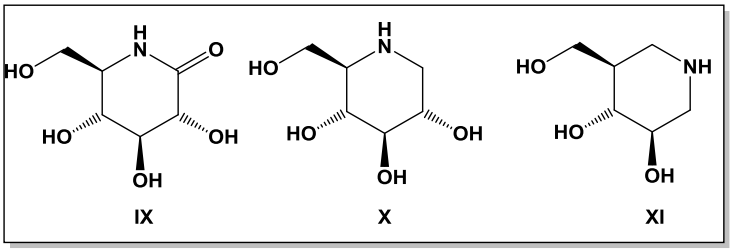


Figure 6: Example of iminosugars[22,23] and an isoiminosugar[24].

All these compounds have in common that they can mimic the conformation and, in case of imino- and isoiminosugars, also the charge of the oxocarbenium transition

state..[25] It is assumed that the oxocarbenium ion of a natural substrate adopts a half chair conformation in the transition state of the glycoside hydrolase reaction (**x**). The protonated 6-membered ring inhibitors mimic the charge development in the transition state (**ix**). A five membered iminosugar mimics both the half chair conformation in the transition state of the natural hydrolase reaction as well as the charge of the oxocarbenium ion (**xi**). The strong electrostatic interaction between the protonated ring nitrogen and the carboxylate residues in the active site causes for inhibition of the enzyme.[26]

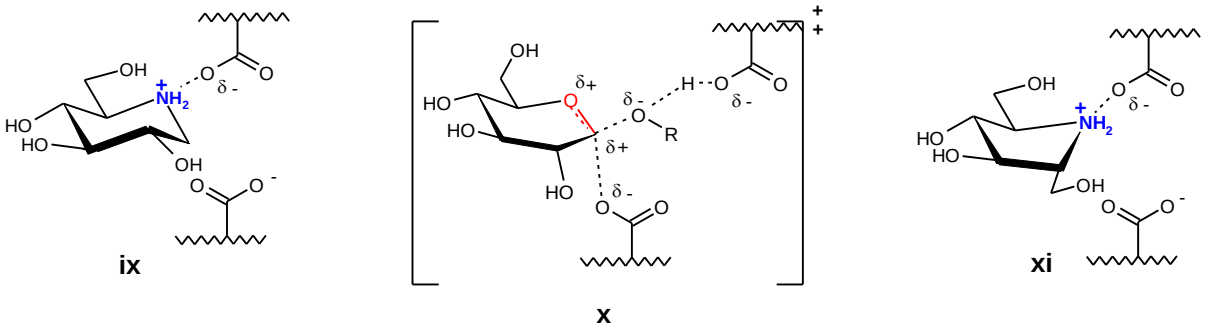


Figure 7: Transition state intermediates of the glycoside hydrolase reaction.[26]

Iminosugars are considered to be the most valuable research tool for the elucidation of glycoside hydrolase related processes as well as highly valuable drug candidates for various glycoside hydrolase related diseases such as mentioned above.

1.3.2. Iminosugars

In 1966 the first isolation[23] and synthesis[27] of deoxynojirimycin (DNJ, **X**) took place and in 1976 its biological activity was found. It was only in the 1990s, however, that the research interest and investigations in this field have increased dramatically.

Since then a big variety of structurally different polyhydroxy alkaloids have been isolated from natural sources and characterized. They can be subcategorized in five families, namely pyrrolidines, piperidines, pyrrolizidines, indolizidines and *nor*tropanes. Paradigmatic examples of those families are 2,5-dihydroxymethyl-3,4-dihydropyrrolidine (DMDP, **XII**), nojirimycin (**XIII**), 1-DNJ (**X**), australine (**XIV**), swainsionine (**XV**) and calysteine B_2 (**XVI**), respectively (Figure 8).[28]

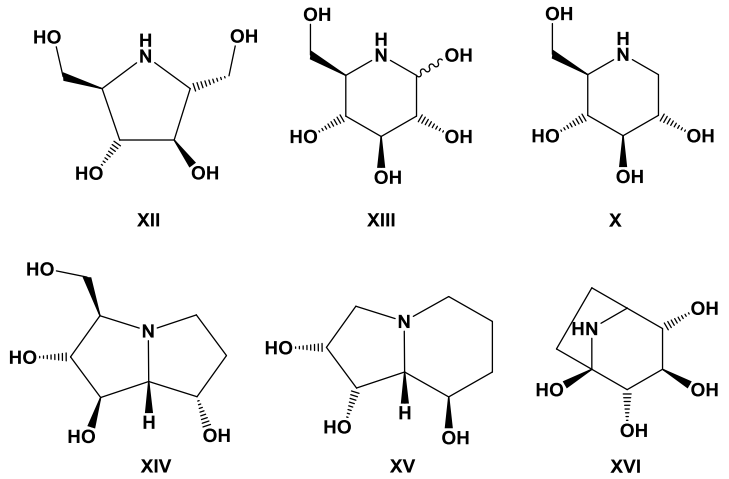


Figure 8: Paradigmatic examples of the iminosugar families.[28]

The fact that these substances are powerful inhibitors of glycoside hydrolases motivated scientists to improve their inhibition potency by synthesizing diverse derivatives of naturally occurring iminosugars.

It was hypothesized that additional interactions of the sugar with the enzymes result in better inhibition. This was indeed shown by derivatizing the ring nitrogen of these compounds. Many research groups have contributed to this field.[29]

Two examples of this compound class are *N*-butyl-DNJ (**V**, Miglustat), which has been approved for the treatment of Morbus Gaucher[6] and the *N*-(2-hydroxyethyl) derivative (**VI**, Miglitol) which is applied in the treatment of Type II Diabetes (Figure 9).[7]

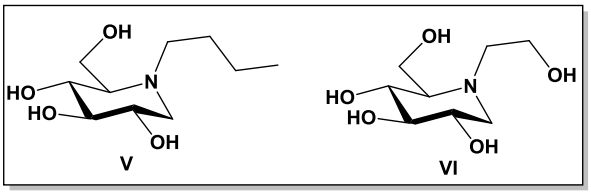


Figure 9: Structures of Miglustat[6] and Miglitol[7].

Although *N*-derivatized iminosugars are much more readily available, by reductive amination or alkylation of two parent compounds, various studies highlighted the advantage of iminosugar *C*-glycosides in terms of potency and selectivity, especially in the field of antiviral agents and glycoside hydrolases.[30]

This can be explained by a stronger influence on the conformation and the fact that, when cleverly designed, the substituent can have more favorable interactions with the putative lipophilic pocket of the human lysosomal β-glucocerebrosidase.[31,32]

An overview of how different chain lengths and substitution patterns influence the IC_{50} value of chosen compounds against human lysosomal β-glucocerebrosidase (GCase), the enzyme responsible for the Gaucher disease, are shown in Figure 10.[31] The unsubstituted 1,5-dideoxy-iminoxylitol **XVII** has an IC_{50} value of 2.3 µM. *N*-derivatisation with a nonyl side chain leads to an IC_{50} value of 1.5 µM of compound **XVIII**. By installing a heptyl- or dodecyl side chain at position C-1 of compound **XVII**, the obtained compounds **XIX** and **XX** reach highly improved IC_{50} values of 6.8 nM and 14 nM respectively. *N*-Nonyl derivatization of DNJ (**X**) improves its IC_{50} value from 240 µM to 660 nM in compound **XXI**. This value can be improved a hundredfold by implementing the nonyl side chain at position C-1 of DNJ (**X**) to give compound **XXII** with an IC_{50} value of 6.8 nM.

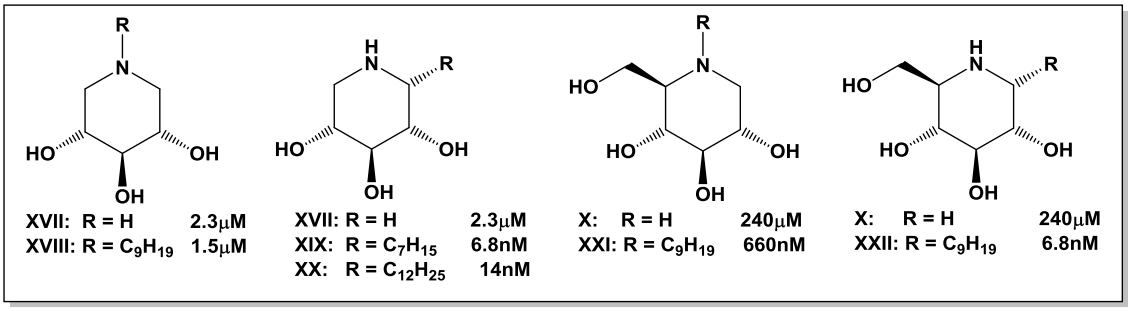


Figure 10: IC_{50} Values of chosen compounds.[31]

1.4. Lysosomal storage diseases (LSD)

Lysosomal storage diseases represent a group of genetically distinct, biochemically related, inherited diseases[33] and affect about 1 of 7000-8000 births.[34] In general, they appear because of genetic mutations, responsible for encoding lysosomal hydrolases, thus leading to malfunctions of these enzymes, affecting post-translational modifications, trafficking and activity. These enzymes are recognized and destroyed by a complex quality control mechanism in the endoplasmatic reticulum (ER) and therefore not available in the lysosomes. As a consequence, incompletely degraded metabolites of these enzymes are accumulated in various

organs such as liver and spleen, the nervous system or bones, depending on the disease. Basically any enzyme in a degradation cascade of macromolecules such as glycosphingolipids can be affected by these genetic mutations, leading to a vast number of different LSDs.

Figure 11 gives an overview of the lysosomal degradation cascade of glycosphingolipids and the related diseases.[35]

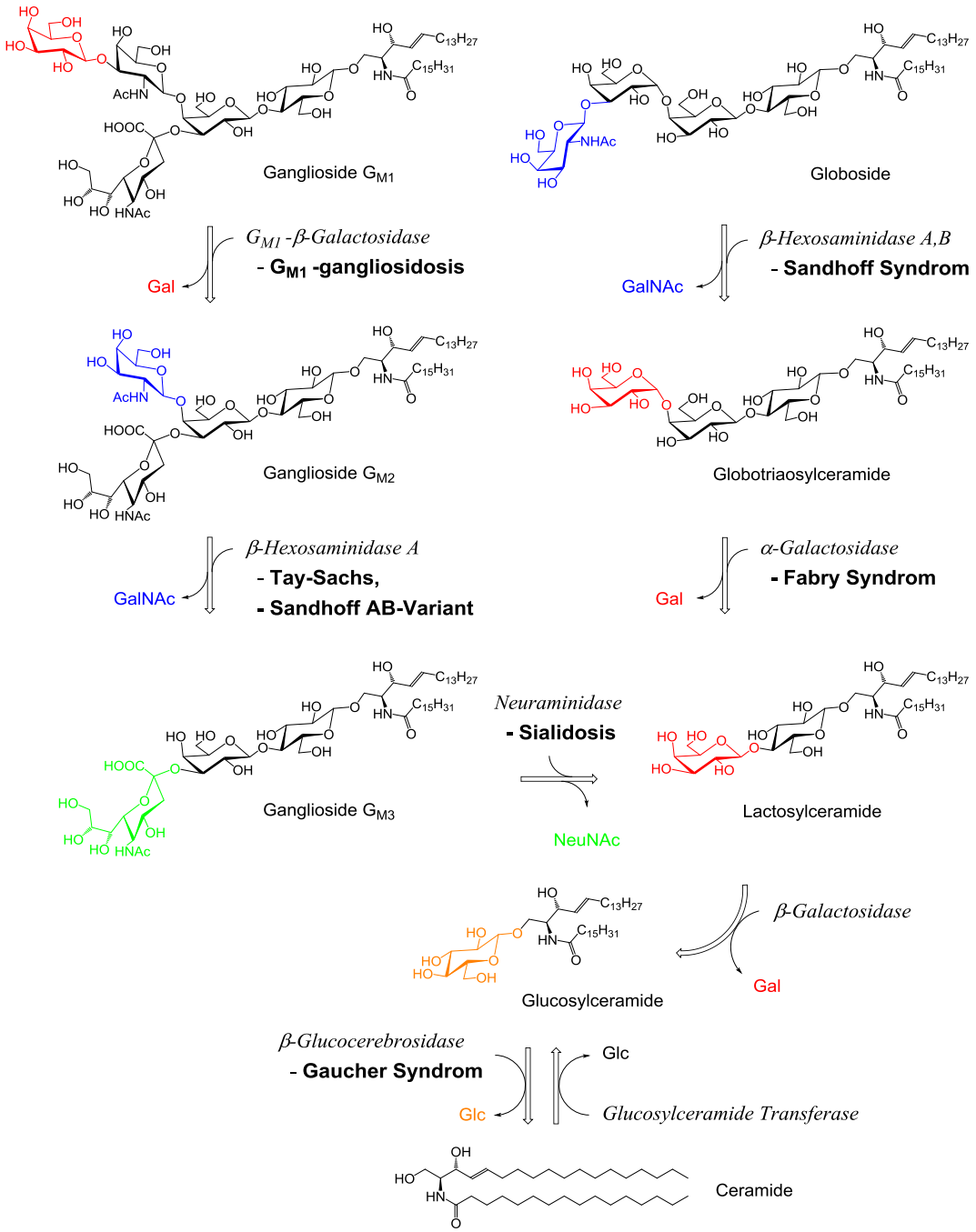


Figure 11: Lysosomal degradation cascade of glycosphingolipids and related diseases.[35]

Currently approved therapies, such as the enzyme replacement[36], the substrate reduction or the gene therapy[37] may reach, depending on the disorder, costs of up to 750000 US$ per case and year for the entire life of the patient.
It should be stressed that for many LSDs the therapies described above are not applicable.

A somewhat new and promising approach for the treatment of LSDs is the active site specific chaperone (ASSC) therapy, which will be quickly explained herein.
After the synthesis of the enzymes in the ribosomes they are transported into the endoplasmatic reticulum (ER) in a highly unfolded state. With the assistance of natural molecular chaperones, the protein is folded in its active conformation and transported to the Golgi apparatus for further maturation. The active enzymes are then transported into the lysosomes, where they process their natural substrates. If, however, the protein is folded improperly, for example due to an altered amino acid sequence, the endoplasmatic reticulum associated degradation (ERAD) pathway is induced. This complex quality control system degrades the improperly folded enzymes, even though partial or even full catalytic activity might be retained.
By application of a reversible, competitive inhibitor in sub-inhibitory concentrations, the proper folding of the mutant enzyme can be induced. The inhibitor binds to the catalytic domain of the enzyme, which induces the functional conformation and stabilizes the enzyme-inhibitor complex for further trafficking. In this context, these small molecule inhibitors are referred to as pharmacological chaperones (PCs), or active site specific chaperones (ASSCs).
This enzyme-inhibitor complex can be further maturated and transported into the lysosomes, where the inhibitor is replaced by the natural substrate, due to higher substrate concentrations and lower pH-values in the lysosomes, than in the ER. Thus the residual enzyme activity is increased. A schematic representation of the ASSC therapy with an iminoalditol as competitive, reversible inhibitor (pharmacological chaperone) is shown in Figure 12.[38]

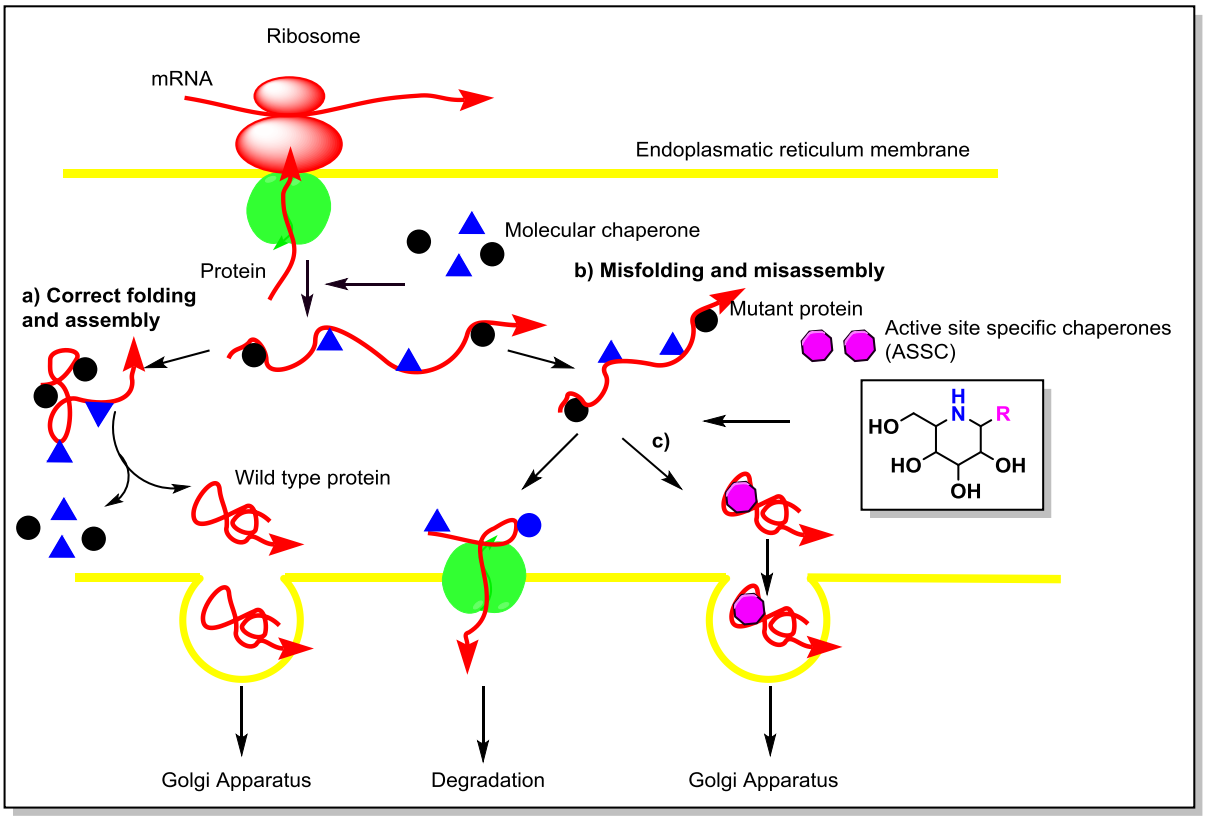


Figure 12: Schematic representation of the ASSC therapy. a) The unfolded protein is folded and assembled by natural molecular chaperones and transported to the Golgi apparatus. b) The misfolded protein is recognized by the quality control and degraded via the ERAD pathway. c) An active site specific chaperone promotes proper folding of the mutant protein and it is transported as enzyme-inhibitor complex to the Golgi apparatus. [38]

Although, in theory, any compound that interacts with the respective protein and induces its proper folding could have chaperone activity, focus is often laid on the active site of enzymes, because usually there is more structural information available. Basically three requirements have to be met for a molecule to be an effective chaperone.

Affinity to the biological active site

Generally it is assumed that compounds with a higher affinity to the active site bind more efficiently and therefore serve as better folding templates.

This was demonstrated by Fan *et.al.*[39] by comparing the inhibitory action and residual enzyme activity in lymphoblasts of α-galactose A (Gal A) in Fabry patients with several alkaloids, namely 1-deoxnojirimycin (**X**), 1-deoxygalactonojirimycin (DGJ, **XXIII**), 2-deoxy-DGJ (**XXIV**) α-*manno*-homonojirimycin (HNJ, **XXV**), α-*allo*-HNJ

(**XXVI**), α-*galacto*-HNJ (**XXVII**), *N*-methyl-DGJ (**XXVIII**), *N*-ethyl-DGJ (**XXIX**), *N*-propyl-DGJ (**XXX**), *N*-butyl-DGJ (**XXXI**), *N*-hydroxyethyl-DGJ (**XXXII**), β-1-*C*-butyl-DGJ (**XXXIII**) (Figure 13).

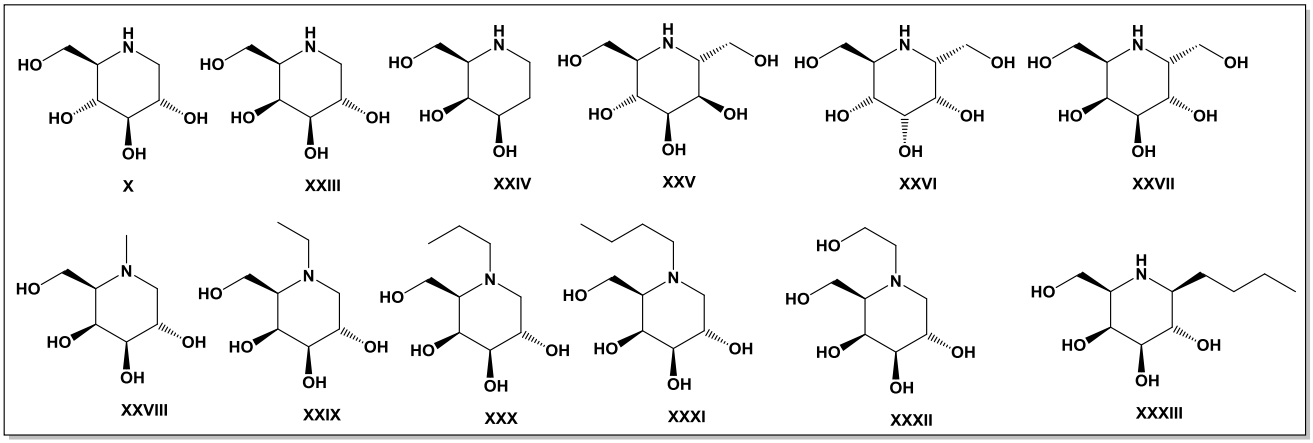


Figure 13: Structues of alkaloids X-XXXIII.

The results of this evaluation are shown in Figure 14.

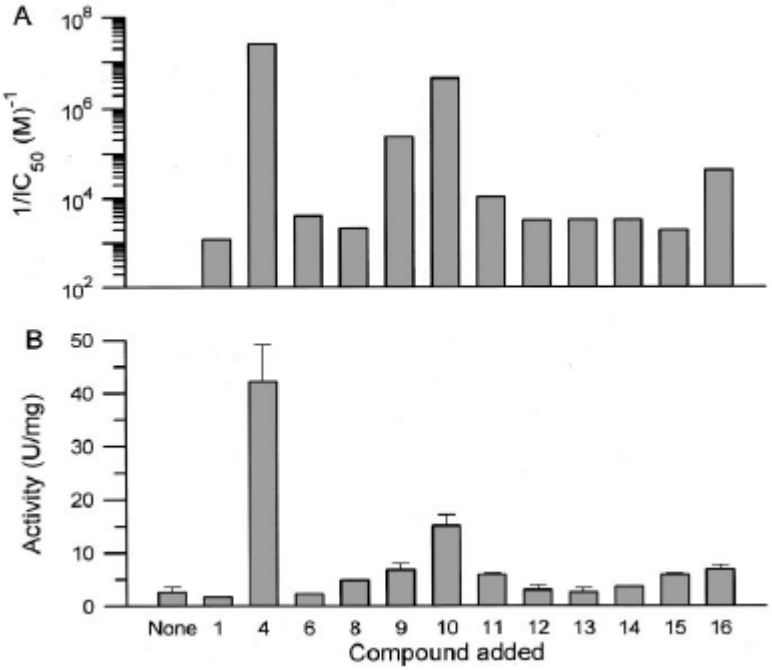


Figure 14: In vitro inhibition (A) and intracellular enhancement (B) of α-Gal A by inhibitors. Compound 1, 1-deoxynojirimycin (DNJ, X), 4, 1-deoxygalactonojirimycin (DGJ, XXIII), 6, 2-deoxy-DGJ (XXIV), 8, α-*manno*-HNJ (XXV), 9, α-*allo*-HNJ (XXVI), 10, α-*galacto*-HNJ (XXVII), 11, *N*-methyl-DGJ (XXVIII), 12, *N*-ethyl-DGJ (XXIX), 13, *N*-propyl-DGJ (XXX), 14, *N*-butyl-DGJ (XXXI), 15, *N*-hydroxyethyl-DGJ (XXXII), 16, β-1-*C*-butyl-DGJ (XXXIII).

These results indicate that the inhibitor potency is indeed important, but not the only relevant factor for ASSCs to be effective.

Lipophilicity

Figure 14 also shows that, although β-1-*C*-butyl-DGJ (**XXXIII**) is a less potent inhibitor than α-*allo*-HNJ (**XXVI**), the ASSC activity is approximately the same. It is considered that the increased lipophilicity resulting from the C-alkylation of the first compound leads to a better cell permeability and subcellular distribution, thus enhancing the ASSC efficiency.

Enzyme/Inhibitor dissociation

A smooth dissociation of the ASSC from the target enzyme in the lysosome is the third requirement for ASSCs to be effective. The biological function of the rescued enzyme is only maintained if the natural substrate can compete effectively with the inhibitor for the active site. A potent inhibitor, while very likely to be highly effective in rescuing enzymes, might be difficult to be removed competitively from the active site, which might not be favorable for a therapeutic agent.

Inspired by the development of highly potent inhibitors for the application as ASSCs, Zhu *et.al*.[40] designed and synthesized the most potent inhibitor for β-glucocerebrosidase (GCase), the enzyme responsible for Gaucher disease. It was rationalized that the active domain of the enzyme has two substrate binding sites, one for the glucosyl residue, the other one for the lipophilic ceramide moiety.
By mimicking not only the glucose and ceramide moiety, but also the transition state of the enzymatic cleavage, a highly potent inhibitor should be generated. The proposed interactions of an inhibitor with the domains of β-GCase are shown in Figure 15.

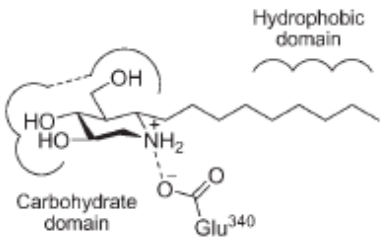


Figure 15: Proposed interaction domains between inhibitor and β-GCase.[40]

Starting from isofagomine (IFG, **XXXIV**), an isoiminosugar that closely resembles glucose bearing a nitrogen atom at the anomeric position, which already is a potent inhibitor of human β-GCase (K_i=56 nM), several hydrophobic side chains were introduced. Indeed, it could be shown that a C-5_a-nonyl side chain enhances the inhibitory potency of compound **XXXV** dramatically, reaching an IC_{50} value of 0.6 nM against human β-GCase. The evaluation of this compound as ASSC has not been conducted yet.

A similar result was obtained by Thonhofer *et.al*.[41] when applying the same strategy to design a potent inhibitor for human β-galactosidase (β-Gal), the enzyme responsible for G_{M1}-Gangliosidosis.
For this goal, several derivatives of 4-*epi*-IFG (**XXXVI**), a potent inhibitor of β-Gal with an IC_{50} value of 99.7 µM[42], were synthesized. Again it could be shown that the introduction of a C-5_a-butyl-dansyl side chain improves the IC_{50} value of compound **XXXVII** to 0.38 µM (Figure 16). The evaluation of compound **XXXVII** as a potential ASSC was tested on a R201C mutant enzyme employing patient's skin fibroblasts and revealed a maximum chaperone effect of 10-fold enhancement of residual β-Gal activity at a concentration of 2.5 µM of the inhibitor.

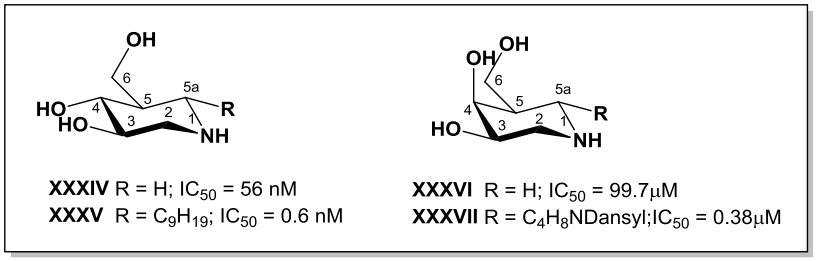


Figure 16: IFG and 4-*epi*-IFG derivatives.[40,41,42]

These findings clearly underline the postulated requirements for potent inhibitors and their potential to be effective ASSCs.

1.5. Protein modifications

The study of protein structure and function is of great interest for chemists, medical scientists and biologists.[43] In this respect, protein modification with molecular probes offers a powerful technique to elucidate their functions.

Although extensive studies have been performed on isolated proteins in the last decades, it is assumed that, in order to gain an overall and in-depth understanding of their structure, function and activity, the protein should be analyzed under the most natural conditions possible.[44] However, obtaining homogenously modified proteins within a biological sample is far from trivial. Several issues like protein selectivity and labeling site selectivity, among others, have to be addressed in the labeling process.[45]

There are basically two methodologies to introduce molecular probes to a target protein. The first possibility is to use a genetic modification system such as metabolic labeling, in which a protein is incorporated with an unnatural amino acid, which can be further modified with a molecular probe.[46] Another example for a genetic modification system makes use of a codon, which, instead of terminating the protein synthesis, encodes an unnatural amino acid.[47]

The other methodology is based on a chemical post-translational modification and in theory allows for a flexible, efficient and selective modification at any given time in the protein sequence.

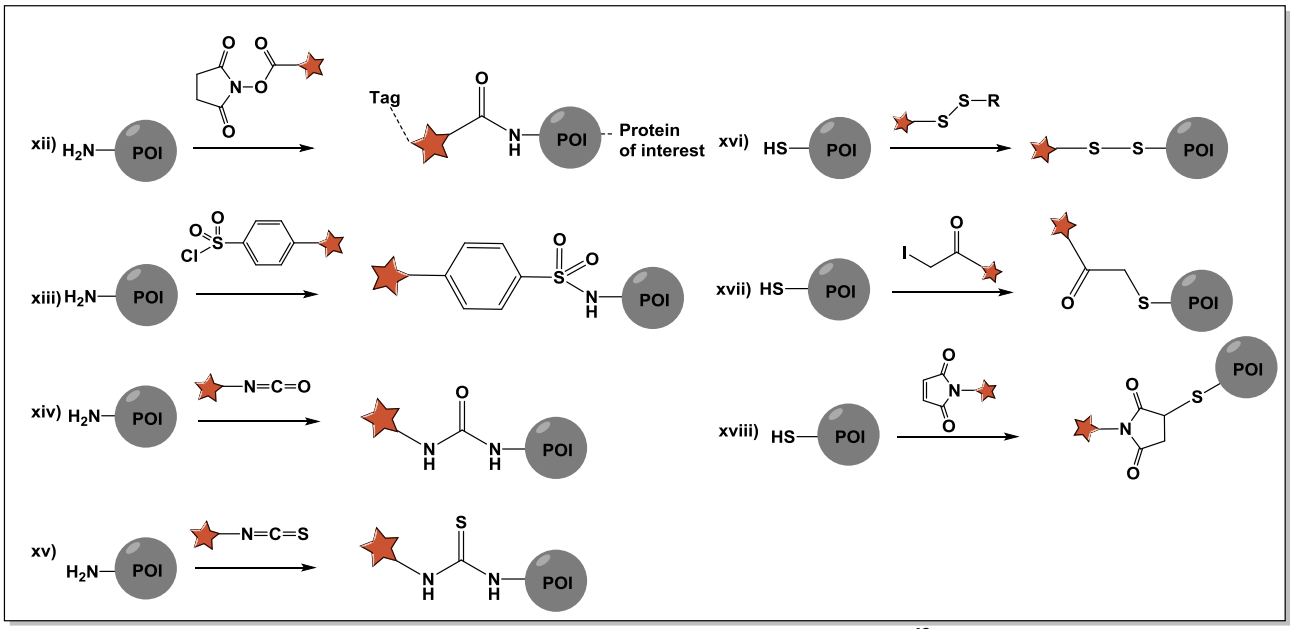
For the purpose of the latter, many different, orthogonal chemical and enzymatical protein modification methods have been developed.[43]

This field highly benefited from the preceding research in the field of proteomics, whose goal is to determine the amino acid components, structures and activities of proteins by means of selective amino acid modification.[48]

1.5.1. Labeling of natural amino acids on the protein surface

Classic approaches to conjugate chemical probes to the protein of interest (POI) make use of a simple second order reaction with functionalities at the side chains of amino acids residues present in the protein sequence. Of those, lysine residues are popular targets, since the terminal primary amino group can react selectively with many species such as activated esters (**xii**), sulfonyl chlorides (**xiii**), isocyanates (**xiv**), isothiocyanates (**xv**) or via reductive amination. Likewise, the N-terminus of proteins can be modified with these reagents.

The relatively rare amino acid cysteine is another popular "chemical target" for protein modification by means of disulfide transformation (**xvi**), alkylation with alkyl halides (**xvii**) or Michael additions (**xviii**), because it often allows for a single site modification (**Scheme 1**).[49]



Scheme 1: Classic protein modification reactions.[49]

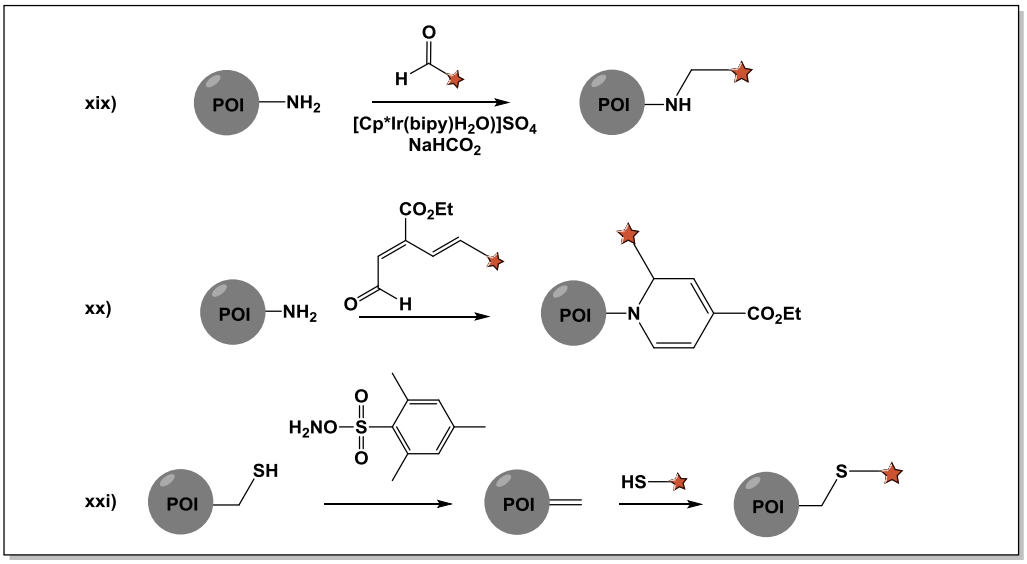
Since these classic reactions are often quite slow and require "suboptimal" reaction conditions for the cells, more sophisticated methods have been developed.

McFarland *et.al*.[50] developed a lysine-specific reductive alkylation reaction that proceeds at neutral pH conditions involving an iridium-catalyzed transfer hydrogenation (**xix**). Even though aliphatic aldehydes have a lower reactivity than aromatic aldehydes, the reaction conditions can be adjusted to meet satisfactory

conversions. Ketones, however, cannot be reductively alkylated with these reaction conditions.

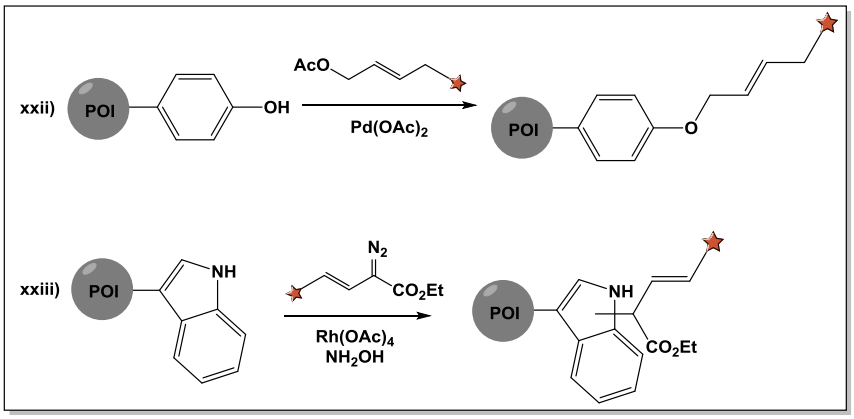
A novel lysine based labeling method was introduced by Fukase *et.al*.[51], making use of an extremely fast 6π-aza-electrocyclisation reaction (**xx**).

For cysteine modifications, a two-step method was developed by Davis *et.al*.[52]. Here, cysteine is first transformed to dehydroalanine via *O*-mesitylenesulfonylhydroxylamine and subsequently modified by a, for example, Michael addition (**xxi**) (Scheme 2).



Scheme 2: Advanced protein modification methods.[50,51,52]

Novel methods also focus on the modification of tyrosine and tryptophan residues, as they are relatively rare as components of proteins and often allow for a single site specific modification. For example, Francis *et.al*.[53] modified tyrosine residues via palladium π-allyl chemistry (**xxii**), likewise tryptophan was labeled with a diazo compound using a rhodium carbenoid (**xxiii**) (Scheme 3).[54]



Scheme 3: Tryptophan and tyrosine modifications.[53,54]

These methods do not allow only for a modification of proteins with chemical molecular probes such as fluorophores or biotin, but are also suitable for immobilization onto chromatography matrices, polymers and microarray chips.

It is easily comprehensible that these methods suffer from major drawbacks, especially because single site modification is very difficult to achieve unless the targeted amino acids appear only once in the protein and even then, dimerization or solubility problems can emerge. Furthermore, “suboptimal” or harsh chemical reaction conditions, metal complexes or irradiation with light are required often, which is likely to damage or destroy the samples. These problems are of course increased significantly in complex samples like living cells.

To overcome these problems, several techniques have been developed in order to achieve highly selective modification and bioorthogonality for further protein labeling.

1.5.2. Labeling with short amino acid sequences or enzyme tags

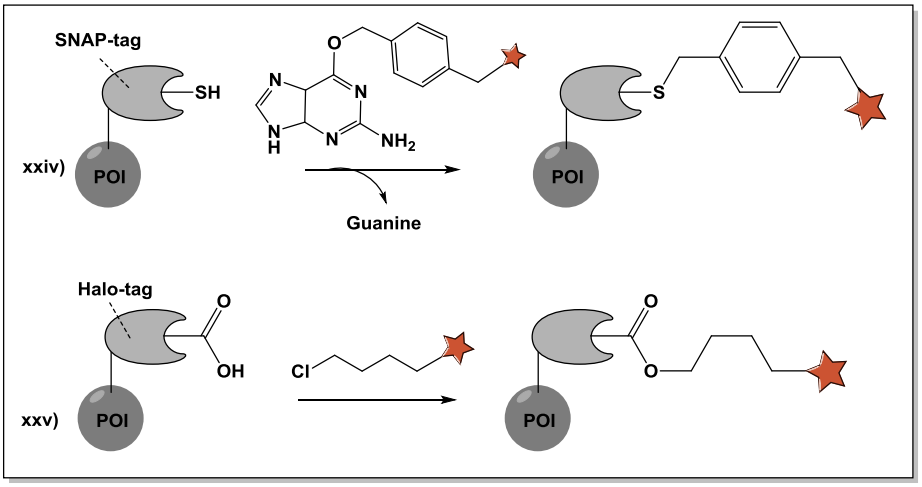
By use of genetic manipulation, a protein of interest (POI) can be equipped with a short amino acid sequence or even an enzyme tag, which after the labeling step can be selectively recognized by certain motifs of molecular probes, even in complex samples. Several techniques have been developed in this respect.

Enzyme tags

The first example of an enzyme tag is the SNAP-tag technology introduced by Johnsson *et.al*.[55] Here, a mutant of the human DNA repair protein O^6-alkylguanine

DNA alkyltransferase (hAGT) is genetically engineered onto the protein of interest and selectively reacts with O^6-benzylguanine derivatives (**xxiv**).

Another example makes use of a bacterial haloalkane dehalogenase, which removes halides from aliphatic hydrocarbons by a nucleophilic displacement mechanism (**xxv**) (Scheme 4).[56]



Scheme 4: Enzymatic modification technologies.[55,56]

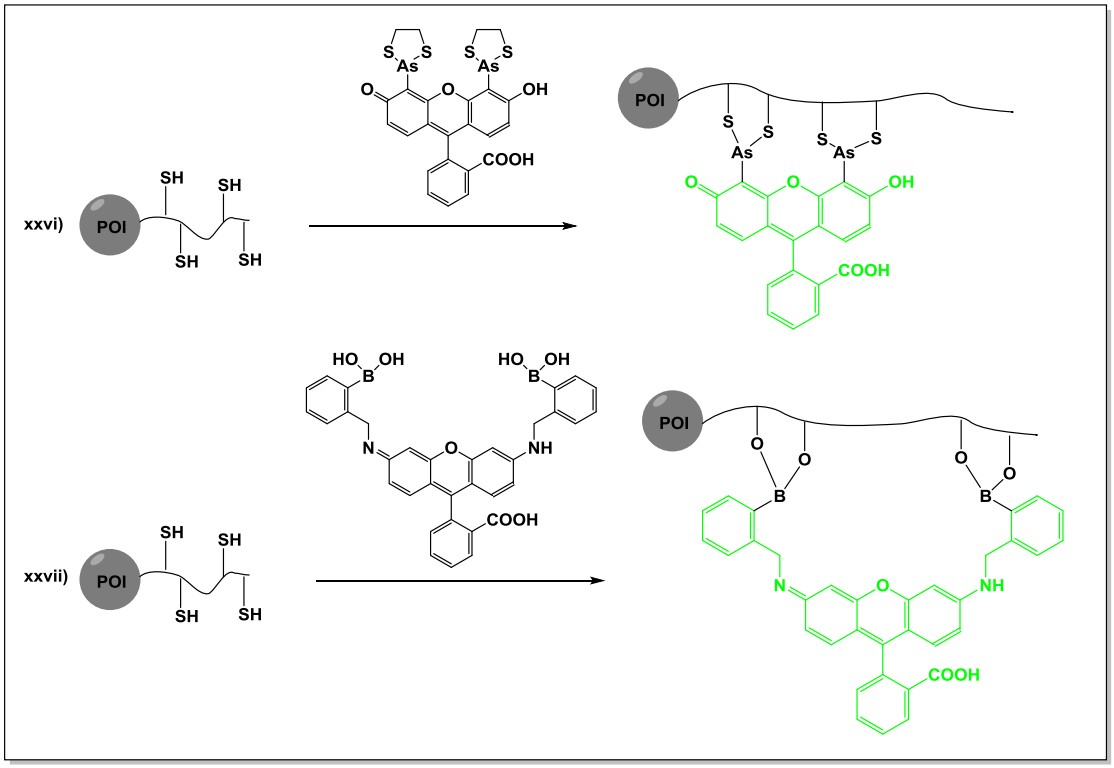
Although this method offers a very versatile biological research tool, the labeling of a protein with an enzyme may have an adverse impact on protein functions because of the large molecular weight of the enzyme units, which adds artificial molecular mass.

Short peptide tags

Similar genetic manipulations allow for the implementation of short peptide tags with certain amino acid motifs, which can be recognized by certain molecules, for example metal-complexes or enzymes corresponding to the tag.

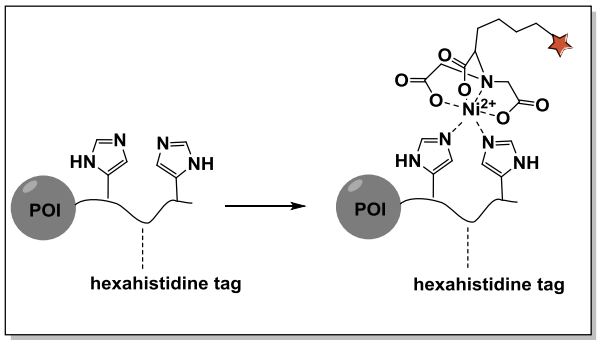
Tsien *et.al*.[57] introduced biarsenical-functionalized fluorescent dyes that specifically interact with a certain tetracystein motif on a peptide tag, engineered on the protein. Upon binding to the motif, fluorescence is dramatically enhanced, allowing for real-time imaging without excessive washing steps (**xxvi**).

A similar approach, which doesn't utilize the cytotoxic molecular arsenic was introduced by Schepartz *et.al*.[58]. Here a bisboronic acid rhodamine based dye binds to a tetraserine motif (**xxvii**) (Scheme 5).



Scheme 5: Short peptide tags.[57,58]

Hexahistidine is another applied peptide tag, commonly used as a purification tag for recombinant proteins. Its imidazole groups chelate nickel nitrilotriacetate (Ni-NTA) with high affinity.[59] Small molecular probes can be conjugated to Ni-NTA and used to image proteins containing hexahistidine peptides (Scheme 6).[60]



Scheme 6: Peptide tag metal complexes.[60]

The problem of the toxicity and the fluorescent quenching of nickel(II) ions was overcome by switching to zinc(II) complexes, a method introduced by Tsien *et.al.*[61] The complex consists of a fluorescin, derivatized with a pair of 2-pyridolsulfonamido functionalities to bind two Zn^{2+} ions. In this case, the vacant metal coordination shells of Zn^{2+} allow for binding of four imidazoles of the hexahistidine motif (Figure 17).

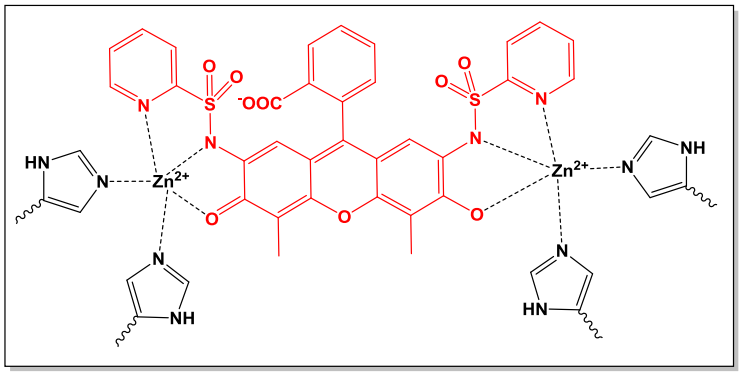
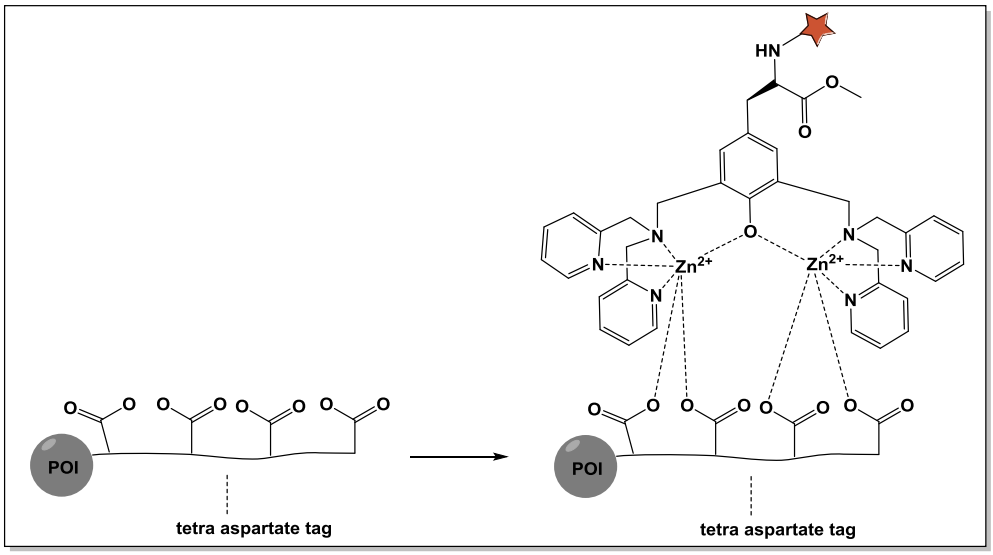


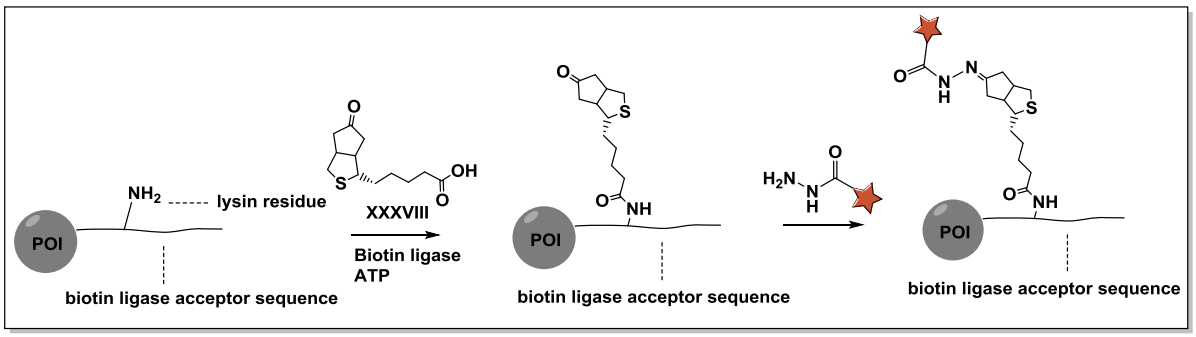
Figure 17: Hexahistidine - fluorescin Zn complex.[61]

A third example for exploiting peptide sequences was introduced by Hamachi *et.al*.[62] who used multinuclear zinc(II) complexes conjugated to fluorescin and cyanine dyes that selectively recognize tetraaspartate motifs in the amino acid sequence of the protein (Scheme 7).



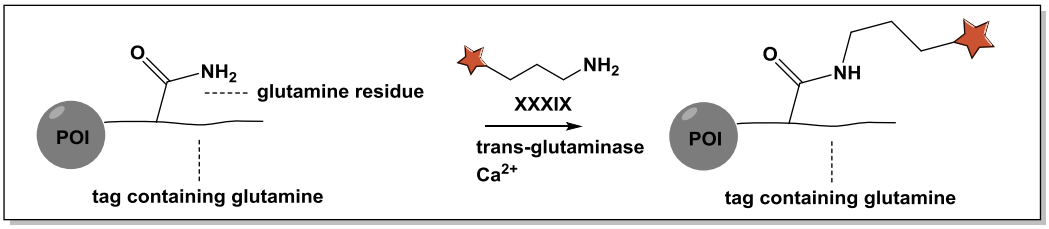
Scheme 7: Tetraaspartate - Zn complex.[62]

Certain enzymes allow for the introduction of bioorthonogal functional groups onto proteins of complex samples by means of recognizing short peptide sequences.
Ting *et.al*.[63] used biotin ligase from *E. coli*, which biotinylates a lysine residue within a 15-residue acceptor peptide and is not cleaved by mammalian biotin ligases. Furthermore, this ligase accepts ketobiotin **XXXVIII** as substrate, which can later be labeled with tagged hydrazide or aminooxy compounds to visualize the proteins.



Scheme 8: Protein modification utilizing biotin ligase.[63]

Another example is the modification of proteins equipped with a glutamine in their short peptide tag, using trans-glutaminases to catalyze the transaminase reaction with a tagged amine **XXXIX**.[64]



Scheme 9: Modification using trans-glutaminases.[64]

Even though specific protein labeling in crude conditions can be achieved with the methods described above, genetic manipulation is a necessary pre-requisite for the application of these strategies. An ideal scenario for precise and quantitative intracellular protein analysis however is only represented in endogenously expressed proteins without any manipulations.

This is achieved by preparing probes attached to molecules that are selectively recognized by the active site of an enzyme of interest, rather than artificially

implemented tags or enzymes, thus allowing to specifically label endogenously expressed enzymes in complex environments without any genetic manipulations or pre-treatment of the samples.

As these techniques are in excellent context with our interest in iminosugar based structures and their highly selective active site specific interactions with glycoside hydrolases, these strategies, namely the activity based protein profiling and the ligand directed protein profiling methodologies will be discussed in more detail in the next chapters.

1.6. Activity based protein profiling (ABPP)

As the name suggests ABPP relies on an activity based probe (ABP) which is only responsive to active enzymes but not their precursors or inhibited forms.[65] This allows ABPs to specifically modify an active enzyme. While the protein modifications described above can be performed on virtually all proteins, ABPs can only be applied on active enzymes (enzyme of interest, EOI). Activity based protein profiling allows for modification of these enzymes in complex samples like cell extracts, living cells or animal models.

Many different classes of enzymes can be targeted with these small molecule probes such as kinases,[66] phosphatases[67] and glycoside hydrolases.[68]

ABPs usually consist of three structural elements, namely a reactive group (warhead), a linker molecule to connect the warhead with the probe and to decrease steric hindrance between the warhead and the labeling tag, which is the third part of ABPs (Figure 18).[69]

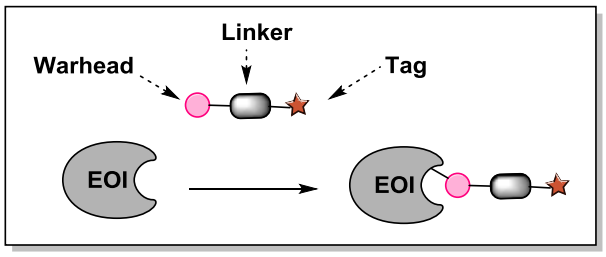


Figure 18: Schematic representation of an ABP.[69]

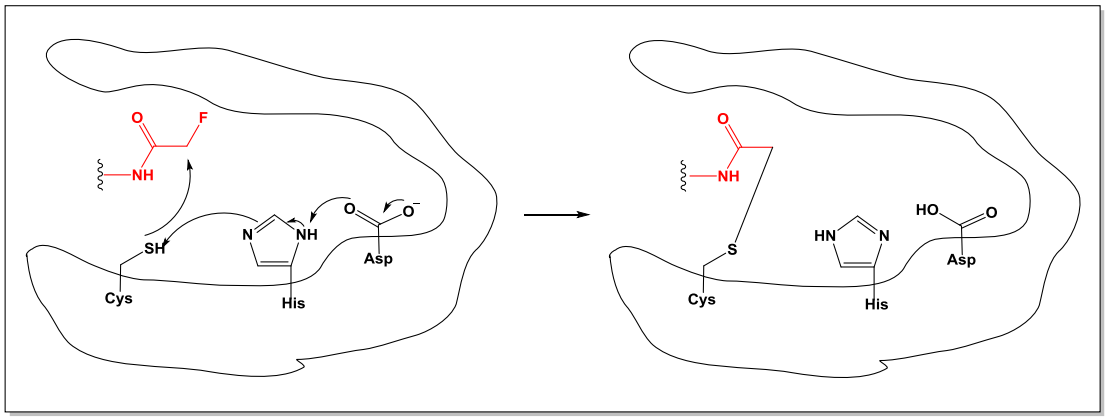
The preparation of such probes is guided by two designs. A directed approach targets specific classes of enzymes with highly reactive and specific warheads that react irreversibly with certain active site residues. This approach requires knowledge about the enzyme mechanism and structure, as well as some residual understanding of the enzyme's small molecule (inhibitor or substrate) binding preferences.
Non-directed approaches are used for less characterized enzymes. Mild electrophiles for example sulfonate esters, equipped with an array of binding groups address multiple enzyme classes simultaneously. [70]
In the next chapters, a closer look will be taken on the structural elements of ABPs.

1.6.1. The reactive group (Warhead)

It is safe to assume that the warhead is the most crucial part of an ABP. It is responsible to covalently link the probe to the enzyme of interest.
A proper balance between selectivity and reactivity is crucial for an effective targeting of the desired protein or protein family. Therefore, a warhead is often equipped with a recognition group that increases its affinity towards a target enzyme and places the warhead in close proximity to the relevant active site residues. Depending on how the covalent linkage of the probe to the enzyme is established, they can be subcategorized into mechanism- and affinity based warheads.[71]

Mechanism based warheads
In mechanism based ABPs, usually an electrophilic group is used as warhead, reacting with a nucleophilic residue in the active site.

For example, certain compounds equipped with fluoromethyl ketone can irreversibly inhibit the cysteine residue in the catalytic triad of the cytoplasmatic peptide *N*-glycanase (PNGase), an enzyme responsible for the deglycosylation of glycoproteins by cleavage of the *β*-aspartyl-glucosamine bond (Scheme 10).[72]



Scheme 10: Inhibition of the catalytic triad of PNGase with fluoromethylketones.[72]

Examples for warheads are methylketones/acetamides with a leaving group such as halides (**XL**), epoxides (**XLI**), aziridines (**XLII**) and Michael acceptors (**XLIII**) (Figure 19).[73]

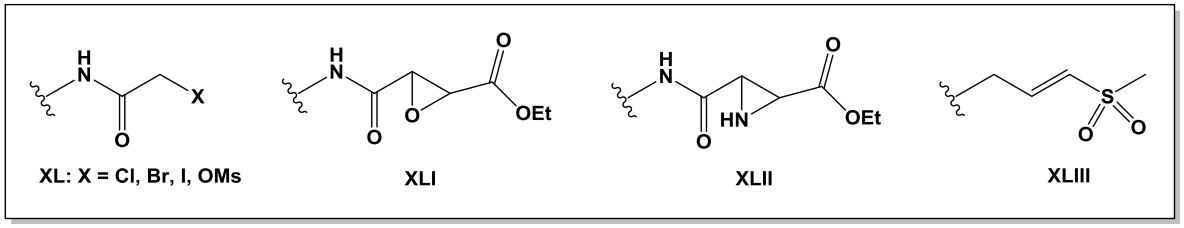


Figure 19: Examples of electrophilic warheads.[73]

Although outstanding in reactivity, the selectivity of such warheads is very poor since they can modify virtually all accessible cysteine residues in biological samples.
To overcome this problem, the warhead needs to be equipped with a recognition group to enhance its affinity to the enzyme of interest.

To underline the importance of the recognition element, Ito, *et.al*.[74] evaluated several recognition elements for PNGase which bear different warheads in terms of their inhibition potency. The IC_{50} values of selected recognition elements for PNGase with chloroacetamide as warhead is shown in Figure 20. Monosaccharides such as compound **XLIV** are not recognized by the active site of PNGase. The same is true for disaccharides without 2-*N*-acetyl substituents as in compound **XLV**. Only chloroacetamidyl chitobiose (**XLVI**) shows strong recognition with the PNGase (Figure 20).

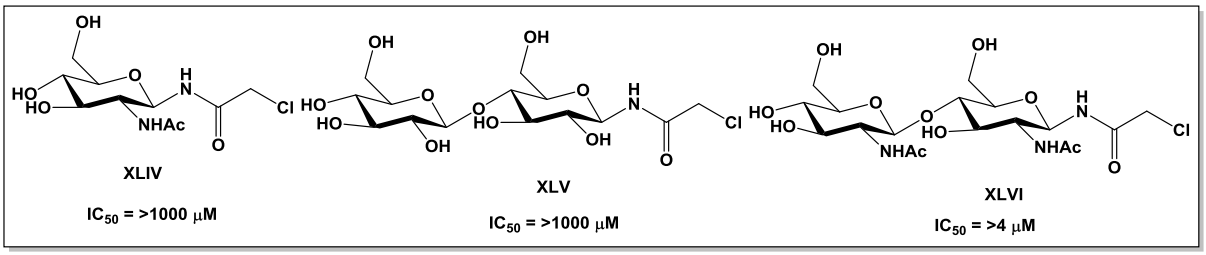
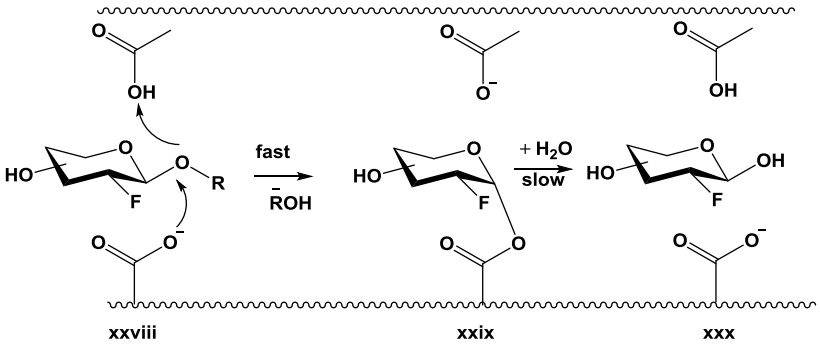


Figure 20: Recognition elements equipped with a warhead for PNGase.[74]

Mechanism based warheads that do not require a directing group for the selective recognition by the enzyme are developed using a warhead that is an inhibitor itself. Several research groups have contributed to the field of tagging retaining glycoside hydrolases, employing the strategy mentioned above.

Withers *et.al.*[75] introduced 2-deoxy-2-fluoroglycoside probes as inhibitors for retaining glycoside hydrolases. The destabilization of the oxocarbenium transition state during the glycosylation (**xxviii**) due to the 2-fluoride group, leads to a decreased rate of hydrolysis of the glycosyl-enzyme adduct (**xxix**), thus retaining the warhead in the active site with only slow hydrolysis to compound **xxx**. When carrying an activated anomeric leaving group the rate of glycosylation is enhanced and the glycosyl-enzyme adduct is accumulated, with lifetimes and stability that are sufficient for isolation and analysis (Scheme 11).



Scheme 11: Mechanism based inhibiton of glycosidases with fluorosugars.[75]

This approach inspired many research groups to develop ABPs based on fluoroglycosyl warheads, targeting both endo- as well as exo glycosidases. [76,77,78,79,80] Examples of these ABPs are given in chapter 1.6.4.

Another approach introduced by Legler *et.al.*[81] makes use of cyclitol epoxides, e.g. Conditurol B epoxide (CBE; **XLVII**) and exocyclic epoxides like compound **XLVIII** (Figure 21).

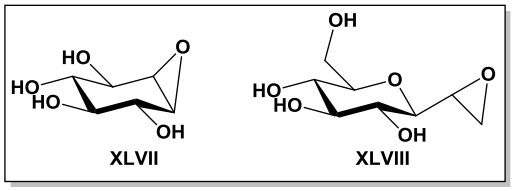
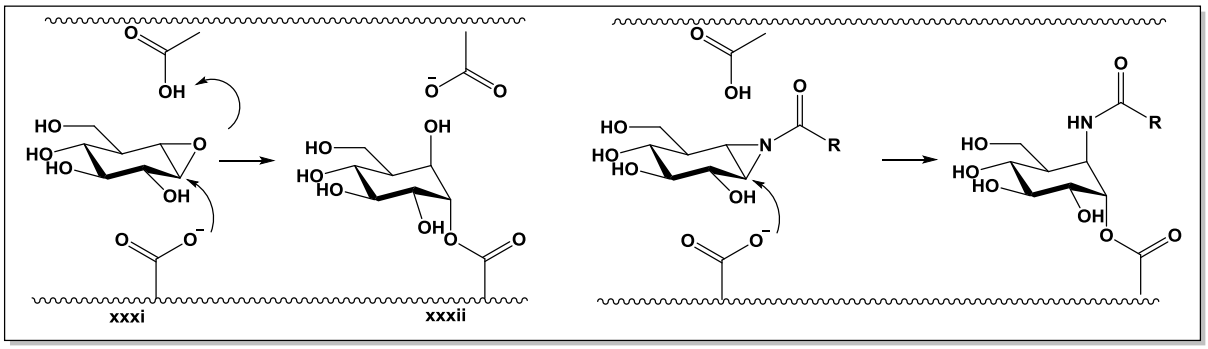


Figure 21: Examples of cyclitol epoxides and exocyclic epoxides.[81]

The inhibition properties of this compound class is based on the interaction of the substrates hydroxyl groups with the substrate-binding pocket (**xxxi**), ensuring specific binding of the inhibitor to the enzyme. *Trans*-diaxial opening of the epoxide by the nucleophilic carboxylic acid forms a stable ester bond (**xxxii**) .[82]
While compounds like CBE (**XLVII)** show excellent inhibition towards *exo*-glycoside hydrolases, their exocyclic epoxide counterparts, for example compound **XLVIII,** show excellent inhibition towards *endo*-glycoside hydrolases.[83]

Overkleeft *et.al*.[84] contributed to this field by designing "second-generation" cyclophellitol derivatives in which the epoxide is replaced by an aziridine moiety.
These were found to be much more potent inhibitors towards various retaining β-glucosidases such as the non-lysosomal enzyme β-glucosyl ceramidase (GBA2), the cytosolic broad-specificity β-glucosidase (GBA3) and the intestinal lactase/phlorizine hydrolase (LPH), all of which are either poorly inhibited by cyclophellitol derivatives or not at all. Another advantage of aziridine based cyclophellitol derivatives is that they do not require enzyme based protonation, allowing for an enzyme labeling at high pH conditions.
However, it should be stressed that cyclophellitols are able to specifically label the lysosomal enzyme β-glucocerebrosidase (GBA1) in living samples, while their corresponding aziridine derivatives also label GBA2, GBA3 and LPH (Scheme 12).



Scheme 12: Inhibition mechanism of CBE and aziridine based warheads.[82,84]

Affinity based warheads

Affinity based warheads do not react in a mechanism based fashion. Usually they consist of a moiety that is turned into a highly reactive species, either by irradiation with light in photoaffinity probes, or by enzymatic cleavage in, for example, quinone methide based probes.

In photoaffinity probes the warhead is a photoactivatable group that, upon irradiation with light of the appropriate wavelength, is converted into the highly reactive species that reacts with the enzyme. The selectivity is again established by a recognition element, in case of a photoprobe, a non-covalent inhibitor of the respective enzyme. Several photoactivatable groups connected to the non-covalent inhibitor DNJ (**X**) are described in literature, such as *p*-azidosalyicyl amide (**XLIX**)[85], benzophenone (**L**)[86] or aromatic azide groups (**LI**) (Figure 22).[87]

The second type of affinity based warheads was introduced in the early 90s with the family of quinone methide inhibitors.[88] The introduction of an *ortho*- or *para*-difluoromethylaryl group as the latent reactive group leads to an inhibitor **xxxiii**, which, when hydrolyzed in the active site, releases difluoromethyl phenolate (**xxxiv**). This compound eliminates fluorine and a reactive quinone methide intermediate (**xxxv**) is formed, which can be bound covalently by any nucleophile present in the active site of the glycosidase (**xxxvi**) (Figure 23).

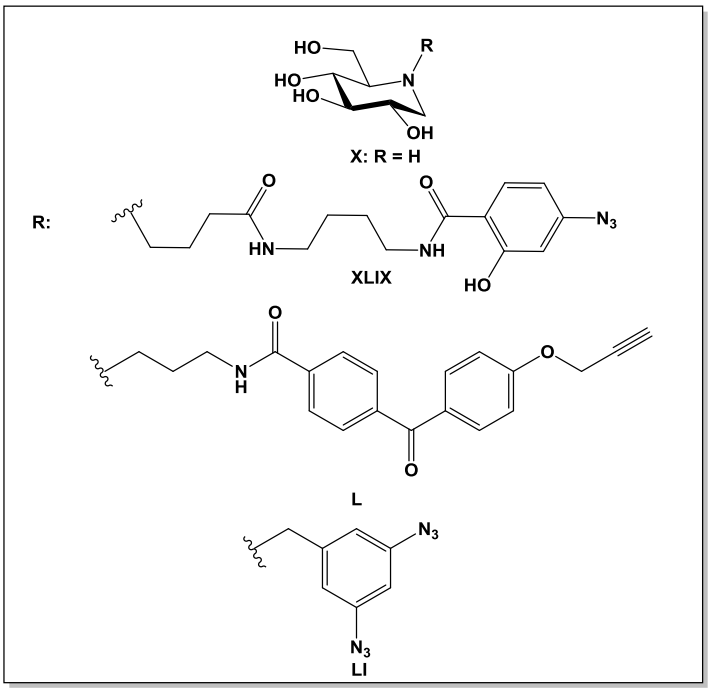


Figure 22: DNJ based photoaffinity labels.[85,86,87]

The mechanism of quinone methide inhibitors is shown in Figure 23

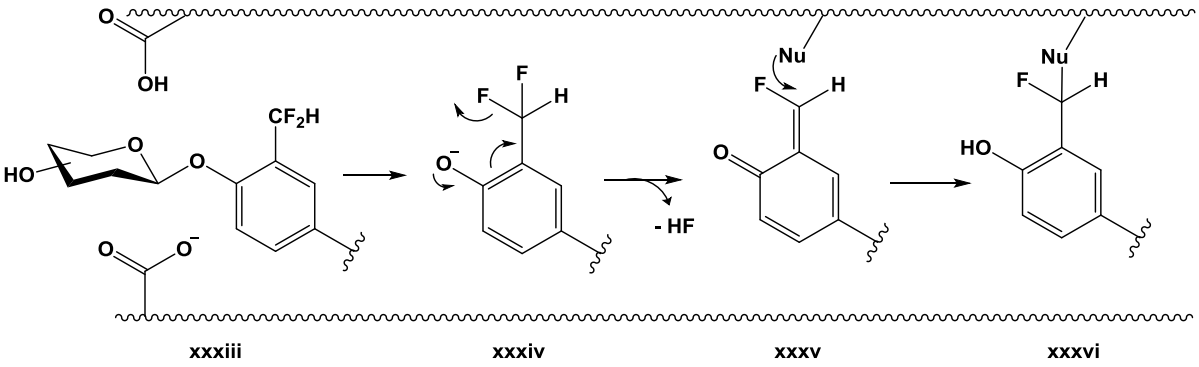


Figure 23: Mechanism of quinone methides.[88]

It is noteworthy to say that, even though photoaffinity and quinone methide based probes are the only method to label inverting glycoside hydrolases, they suffer from major drawbacks such as potential photodamage to living systems by irradiation with, for example UV–light. Once the glycosidic bond is cleaved, the reactive intermediate can diffuse out of the active site leading to cross-reactivity and labeling of the target enzyme unselectively at multiple sites. Additionally, the lifespan of the reactive intermediates can be very short in an aqueous environment, resulting in a low labeling efficiency.

In fact, even though several working groups contributed to this field, affinity based probes are essentially not considered suitable for the proposed *active-site specific* proteomics.[89,90,91,92]

1.6.2. The linker group

The linker group usually serves as special interface between the relatively large warhead and labeling tag moieties. The implementation of a simple CH_2-chain (**LII**) diminishes sterical hindrance, which could cause problems with the interaction of the warhead and the active site of the enzyme. It is, however, also possible to design special linkers for individual applications. Advantageous properties can be introduced by, for example, employing a polyethyleneglycol spacer (**LIII**), which increases the solubility in aqueous media. The linker can also be designed to increase hydrophilicity or lipophilicity for various applications. Furthermore, intelligent linker design may enhance the selectivity of the probe, for example, structural elements such as peptides may occupy binding pockets of specific proteases (**LIV**).[93]
Withers *et.al.*[79] incorporated a deuterium labeled linker moiety (**LV**), which allows for quantification by means of mass spectrometry after tryptolysis and enrichment of the labeled protein (Figure 24). [94]

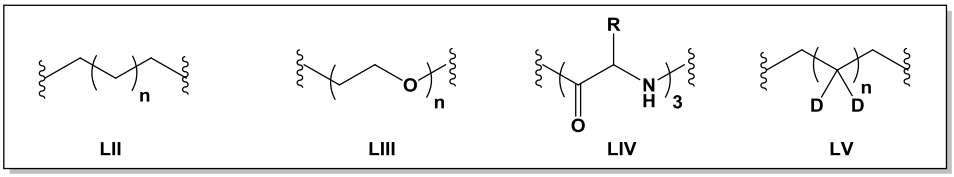


Figure 24: Linker molecules. [80,93]

1.6.3. The tag

A tag is employed in ABPs to allow for a rapid in-gel detection of labeled proteins or to study labeled enzymes in living cells by means of fluorescence imaging microscopy and fluorescence activated cell sorting. Commonly employed reporter groups are BODIPY (**LVI**), fluorescein (**LVII**) or a rhodamine moiety (**LVIII**) (Figure 25, Figure 27; path i) [69].

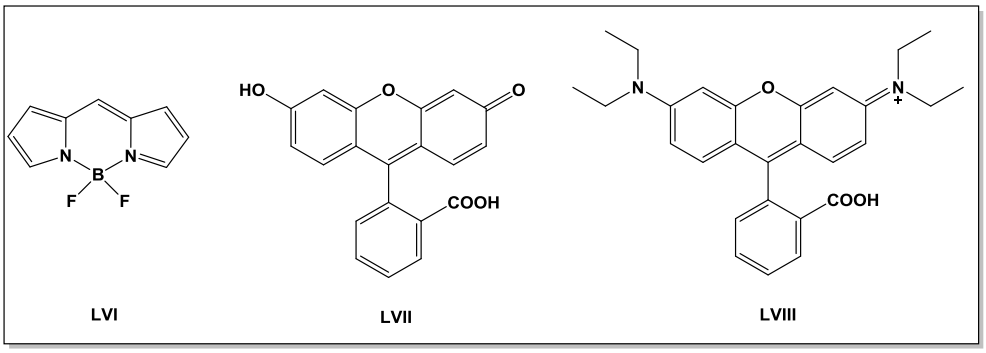


Figure 25: Commonly employed fluorophores as tags in ABPs.[69]

Alternatively, biotin (Figure 26, **LIX**) can be used as an affinity tag to enrich labeled proteins via a streptavidin pulldown and identify them by mass spectrometry (Figure 27; path ii)[69,95]

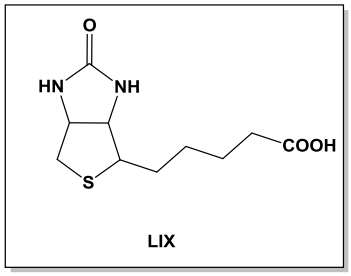


Figure 26: Structure of racemic biotin.[69]

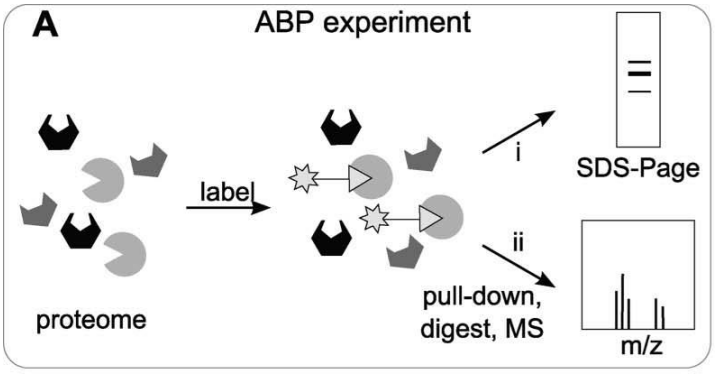


Figure 27: ABP experiment.[69]

These tags are sterically demanding and may obstruct binding to the target protein. Also, detrimental effects on the cell permeability may occur.

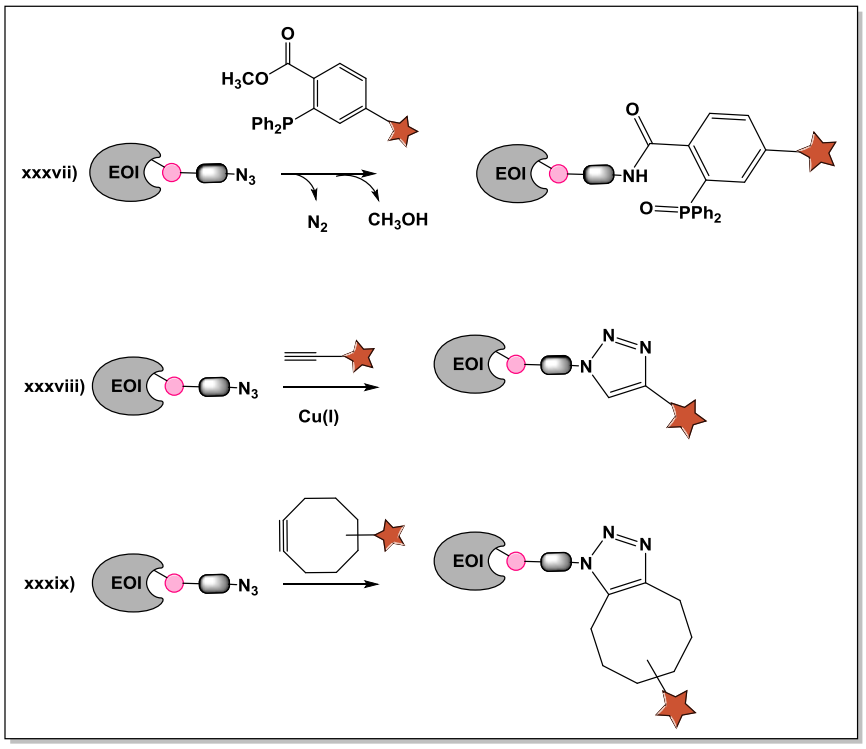
These drawbacks can be overcome by a two-step labeling strategy where, instead of the tag, a bioorthogonal ligation handle is implemented which can be conjugated to the tag of choice after recognition.

Several bioorthogonal handles have been evaluated for this purpose.

Azides and alkynes

In ABPs this handle is suitably an azide group, since it is small, possesses orthogonal reactivity to most of the biological functional groups and is not naturally present in biological systems.[96]

Azides can be readily conjugated to molecules containing a tag via various reactions, such as the Staudinger-Bertozzi ligation (**xxxvii**)[97], a copper catalyzed "click" reaction (**xxxviii**)[98] or a strain promoted "click" reaction (**xxxix**)[99] with alkynes (Scheme 13).

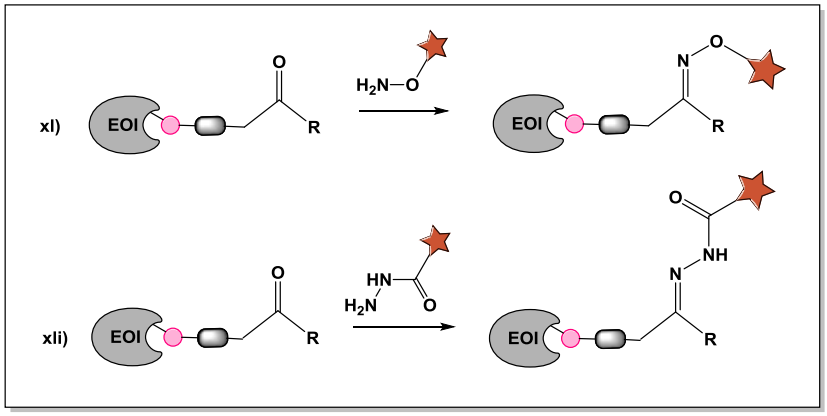


Scheme 13: Bioorthogonal conjugation reactions with azides.[97,98,99]

All reactions mentioned in Scheme 13 do also work vice versa, by conjugating an alkyne to the enzyme of interest and performing a bioorthogonal reaction with a tagged molecule containing an azide function.

Ketones/Aldehydes

Likewise, ketones and aldehydes are not present on cell surfaces and can therefore serve as unique chemical reporters for bioorthogonal conjugation reactions. By integrating these functional groups in a cell surface, they can be treated with a tagged molecule containing an amino oxy (**xl**) or a hydrazine function (**xli**) to form stable oxime or hydrazone linkages, respectively (Scheme 14). [100]

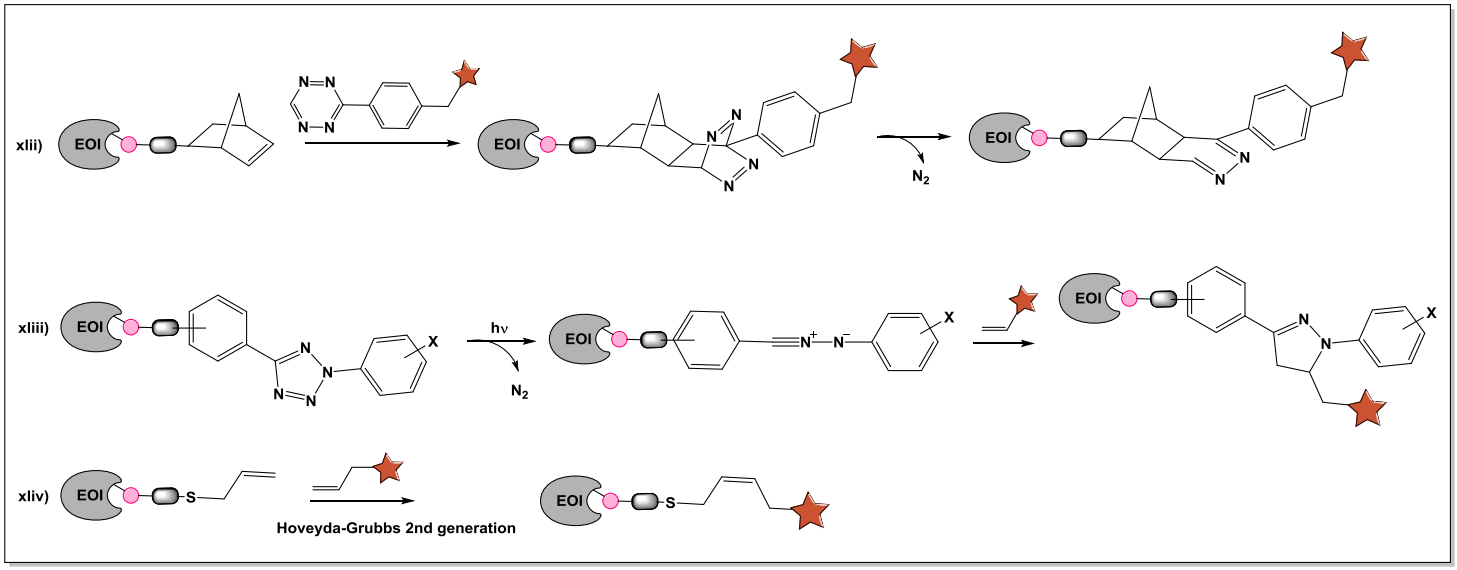


Scheme 14: Bioorthogonal reactions with carbonyl functions.[100]

Alkenes

Alkenes can be used as bioorthogonal ligation handles allowing for cycloadditions of 1,3-dipoles and dienes. Hilderbrand *et.al.*[101] introduced an inverse demand Diels-Alder reaction of norbornenes and tetrazines as ring strain promoted cycloaddition reaction (**xlii**). By irradiation of substituted diaryl tetrazoles, a nitrile-imine dipole is formed, which readily reacts with alkenes in a 1,3-dipolar cycloaddition reaction (**xliii**).[102]

By modifying proteins with allyl sulfide groups, Davis *et.al*.[103] developed a cross methatesis reaction in water, employing a Hoveyda-Grubbs second generation catalyst, suitable for bioorthogonal conjugation of tagged molecules to alkenes in living systems (**xliv**) (Scheme 15).



Scheme 15: Bioorthogonal reactions with alkenes.[101,102,103]

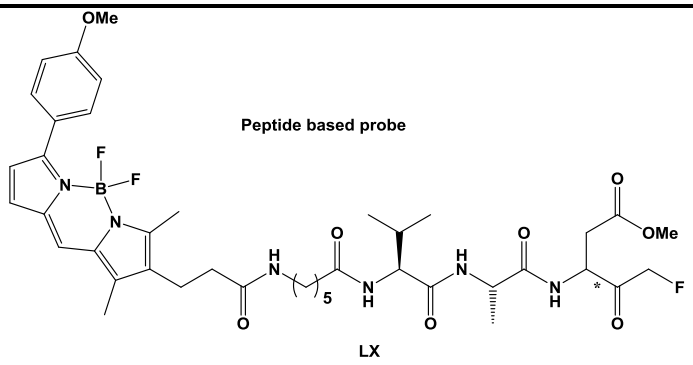
Again, all reactions mentioned in Scheme 15 can also be performed vice versa by inverted placement of the reactive groups.

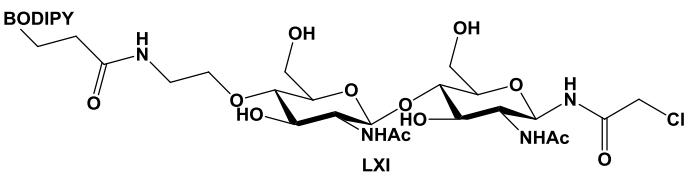
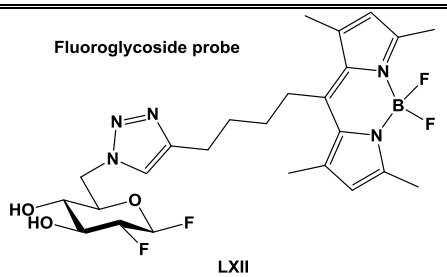
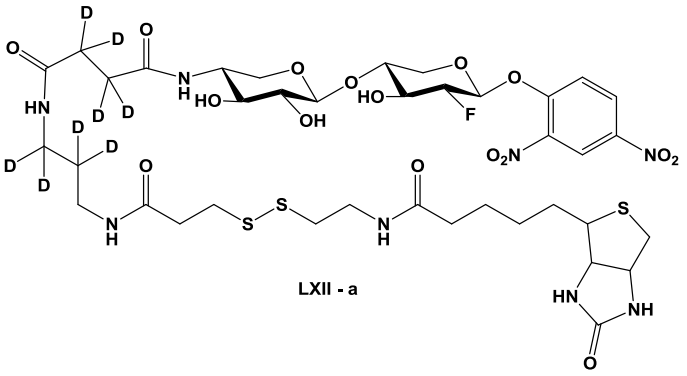
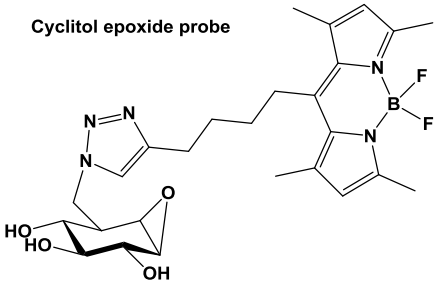
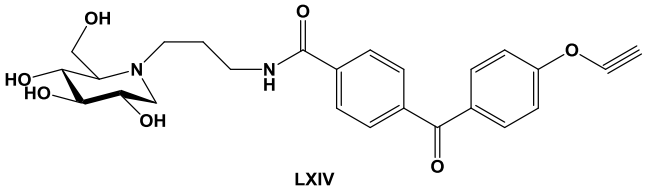
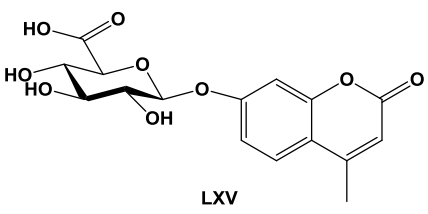
1.6.4. Overview and application

By combining the concepts mentioned in chapter 1.6, a big variety of activity and affinity based probes for several enzyme classes have been synthesized and evaluated.

Table 2 gives an overview of selected ABPs.[69]

Table 2: Selected assortment of probes.

Enzyme	Probes	Advantages	Disadvantages
PNGase	 Peptide based probe LX	-potent	-less selective than carbohydrate based probes

PNGase	**Carbohydrate based probe**  LXI	-more selective than peptide probes	-synthetically challenging
Glycosidase	**Fluoroglycoside probe**  LXII  LXII - a	-very selective -mechanism based	-covalent adduct might be unstable -only for retaining glycoside hydrolases
	Cyclitol epoxide probe  LXIII	-selective -mechanism based -very potent -highly stable adduct	-less selective than fluoroglycoside probes -only for retaining glycoside hydrolases
	Photoaffinity probe  LXIV	-for retaining and inverting glycoside hydrolases	-labeling is affinity based (may result in multiple labeling sites)
	Quinone methide probe  LXV	-for retaining and inverting glycoside hydrolases	-non-specific labeling (diffuses out of the active site)

The peptide based probe **LX** is equipped with a highly potent fluoromethyl ketone warhead and a peptide based recognition element for PNGase, which is less selective than a carbohydrate based probe. A BODIPY tag for identification purposes is installed via a hexyl linker to the recognition element.

A more selective ABP for PNGase is realized with a chitobiose recognition element conjugated to a chloromethyl ketone warhead and a BODIPY tag as recognition element (**LXI**). The synthetical challenge of this probe might be a disadvantage.

Retaining glycosyl hydrolases can be transiently linked very selectively to 2-deoxy-2-fluoroglycosides as warhead, in a mechanism based fashion. An azide ligation handle at position C-6 can be bioorthogonally conjugated to a BODIPY tag via azide/alkyne click reactions to give ABP **LXII**. **LXII-a,** respectively is equipped with a better leaving group at the anomeric position and, more importantly, features an isotope labeled linker moiety for quantification purposes. The covalent glycosyl-enzyme adduct of both compounds **LXII** and **LXII–a** might be unstable towards hydrolysis in the active site of the enzyme.

By using a cyclitol epoxide warhead, a less selective but more potent recognition element is employed, which forms a highly stable adduct in the active site. Again, installing an azide as ligation handle at position C-6 can lead to a BODIPY modified ABP via click chemistry (**LXIII**).

Affinity based warheads offer the only possibility to label inverting glycoside hydrolases. By conjugating a benzophenone as photoactivatable moiety to DNJ **X** as recognition element and an alkyne as biorthogonal ligation handle, the photoaffinity based probe **LXIV** is realized. Since irradiation with light is a necessity for the activation of the probe, photodamage can occur in living systems and multiple labeling sites may occur due to the affinity based labeling.

The quinone methide probe **LXV** can also label inverting glycoside hydrolases, however, non-specific labeling may occur since the reactive intermediate can diffuse out of the active site.

This selected assortment (Table 2) shows that by well-thought-out probe design, labeling of basically any enzyme in living systems can be realized. The combination of warheads, recognition elements, linker groups and labeling tags allows for a great number of activity and affinity based probes, designed for the required purpose.

However, it should be stressed that mechanism based warheads alter the active site and therefore inactivate the target enzyme, which limits the value of activity based probes for some applications such as real-time monitoring of protein activity rather than their abundance in cells.

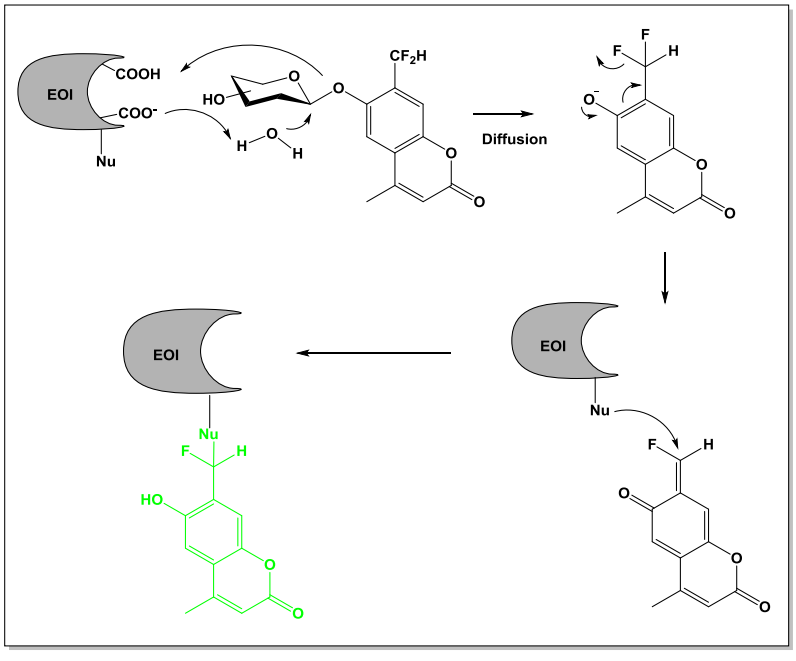
For these applications, restoration or retaining of the original function of the enzyme is essential.

These requirements can be met by developing probes that specifically label proteins or enzymes outside the active site. Many strategies have been developed for this purpose and will be discussed in more detail in the next chapter.

1.7. Ligand directed protein profiling (LDPP)

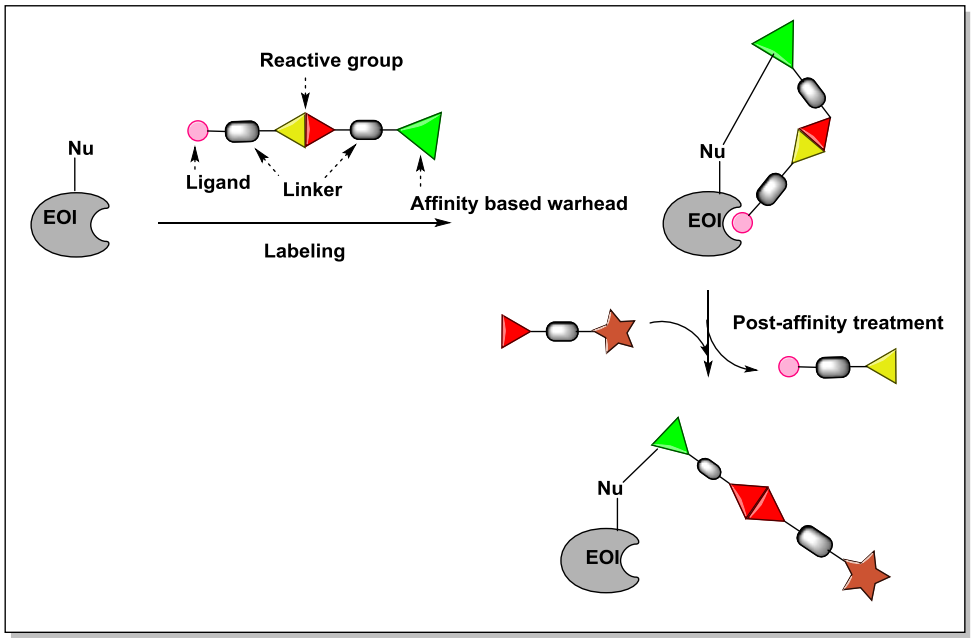
Ligand directed probes resemble activity based probes, especially those equipped with affinity based warheads in many aspects.

In fact, Withers *et.al.*[104] elegantly took advantage of the mentioned drawbacks of quinone methide based affinity probes by developing coumarin glycosides that, upon enzymatic cleavage, release a fluorescent coumarin based quinone methide, which diffuses out of the active site and readily reacts with a proximal nucleophilic amino acid in the same subcellular compartment as the target glycoside hydrolase (Scheme 16).



Scheme 16: Coumarin based quinone methide labeling.[104]

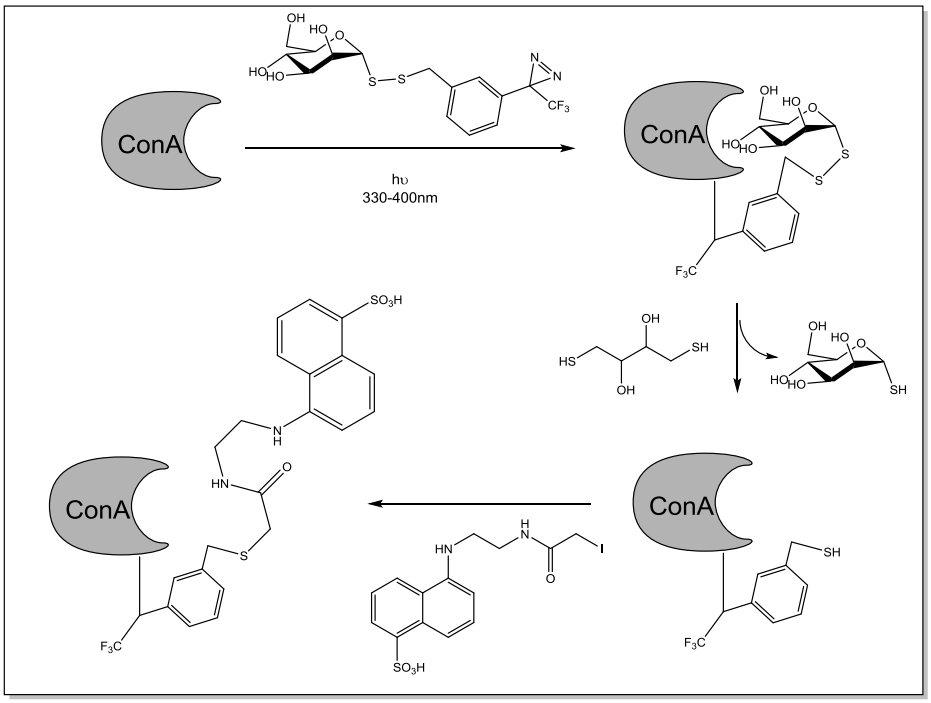
Initial approaches to label the enzyme outside the active site also rely on photoaffinity or epoxide based warheads. For this purpose, a ligand with high affinity to the enzyme of interest, e.g. a reversible inhibitor of glycoside hydrolases, is equipped with a reactive group as cleavage site and a photoaffinity- or epoxide based reaction site, which can be attacked by a nucleophile present at the enzyme. Subsequent chemical cleavage of the reactive site and concomitant release of the inhibitor from the active site yields a tagged enzyme, which can be further equipped with a fluorescent or affinity label of choice (Scheme 17).



Scheme 17: Post affinity labeling method.[105]

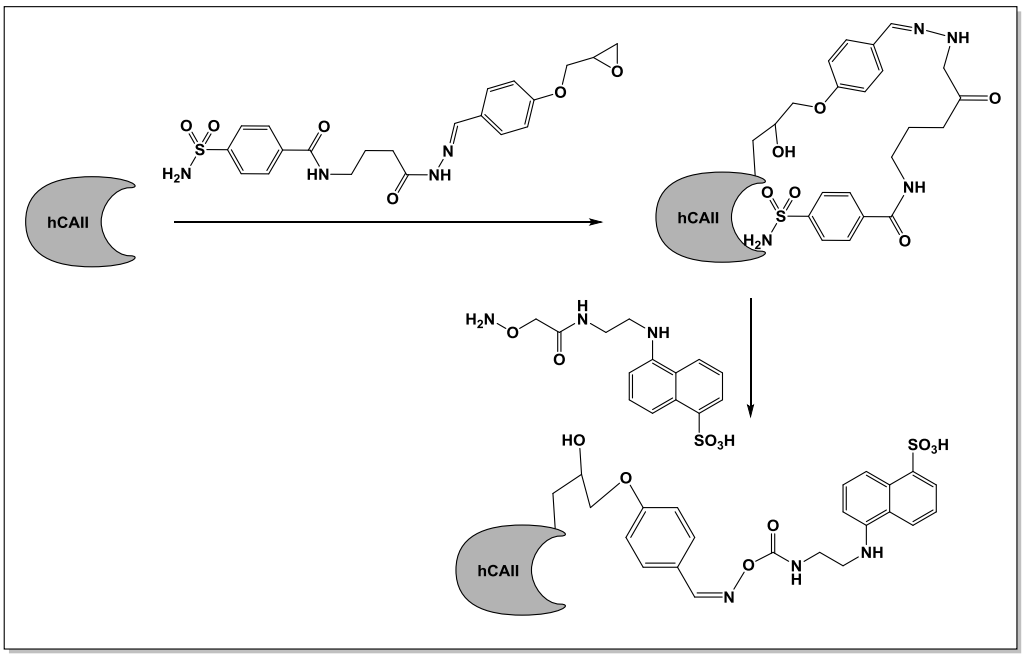
Hamachi *et.al*.[105] introduced a post photoaffinity labeling method, utilizing mannose as ligand, containing a disulfide bridge as cleavage site and a 3-(triflouormethyl)-3-phenyldiazirine unit as a photoactivatable group to selectively label the saccharide binding protein concanavalin A (ConA).

Upon irradiation of the molecule with light of an appropriate wavelength the molecule is linked to ConA outside its active site. Treatment with dithiothreitol cleaves the disulfide bridge, and the fluorescent iodoacetylated dansyl group can be attached (Scheme 18).



Scheme 18: Post photoaffinity labeling method.[105]

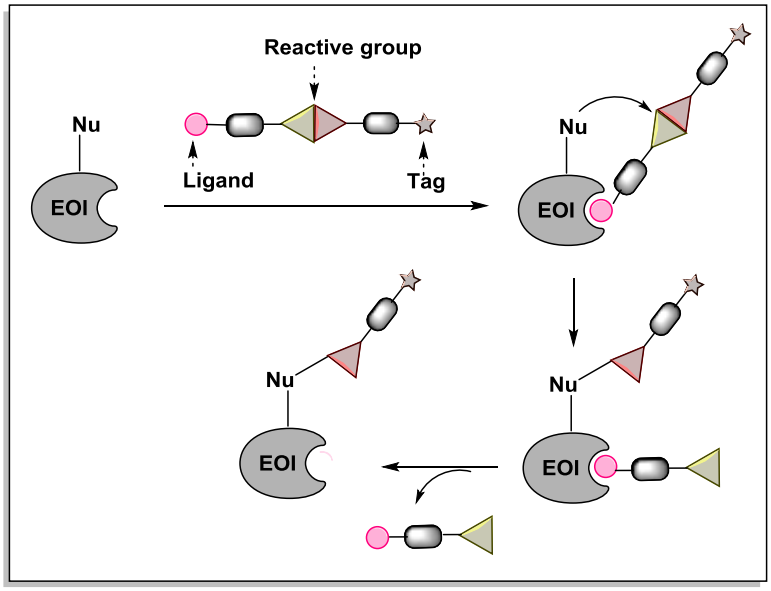
A similar strategy was shown by the same group for labeling human Carbonic Anhydrase II (hCAII). Here the reversible inhibitor benzenesulfonamide was equipped with a linker molecule and a hydrazone unit as reactive site. An epoxide is used to covalently link the molecule to hCAII. The hydrazone moiety can be readily exchanged with aminooxy reagents in a transamination reaction (Scheme 19).[106]



Scheme 19: Post affinity labeling method.[106]

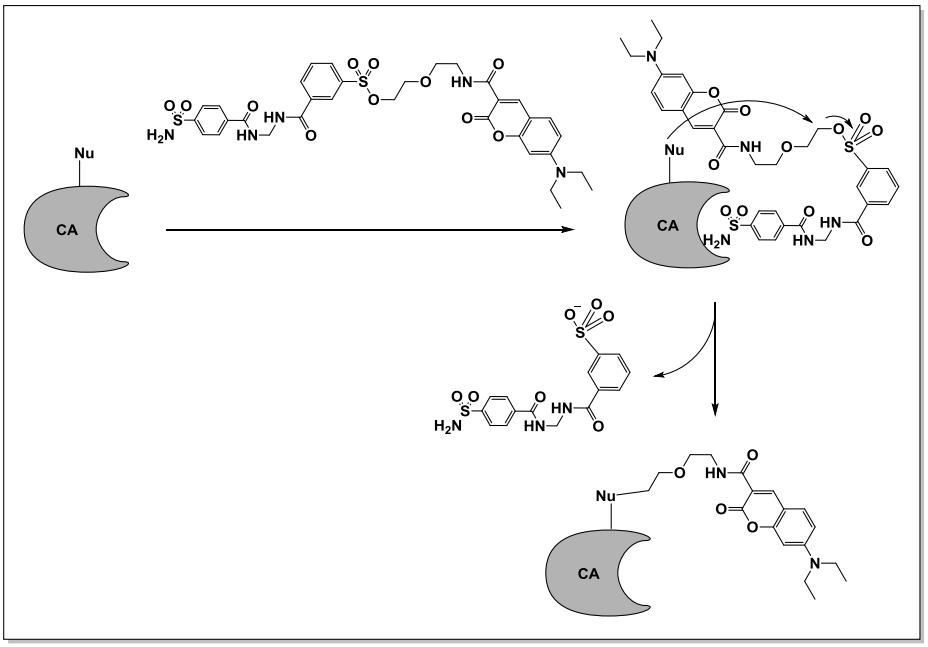
Although very useful for selectively labeling enzymes with a fluorescent or affinity tag, the post affinity labeling technique is not ideal in complex samples, because the bioorthogonality of these reactions is low.

To overcome this problem, Hamachi *et.al*. [107] developed ligand directed probes that contain an affinity based warhead as ligand, a reactive group which is cleaved by a nucleophile present at the enzyme, and a tag molecule. Thus the ligand can leave from the active site and the natural activity of the enzyme should be retained (Scheme 20).



Scheme 20: Ligand directed labeling method.[107]

First approaches employed phenylsulfonate as reactive group. This ligand directed tosyl chemistry (LDT) strategy is similar as the post-affinity labeling approach. Carbonic Anhydrase (CA) was labeled with a molecule, containing a benzenesulfonamide as specific ligand, connected to a phenylsulfonate moiety which presents a coumarin group as fluorescent tag. The ligand binds to the target protein with high affinity and brings the reactive group in close proximity to an amino acid located at the enzyme. After cleavage of the reactive tosyl group, the ligand is released from the active site and the fluorescent coumarin molecule is covalently attached on the enzyme (Scheme 21).



Scheme 21: LDT approach.[107]

Several working groups evaluated different reactive groups for this methodology. Fenical *et.al*.[108], for example, employed a 2-hydroxybenzoyl moiety (**LXVI**), while Hamachi *et.al*.[44] utilized acyl imidazole (**LXVII,** Figure 28).

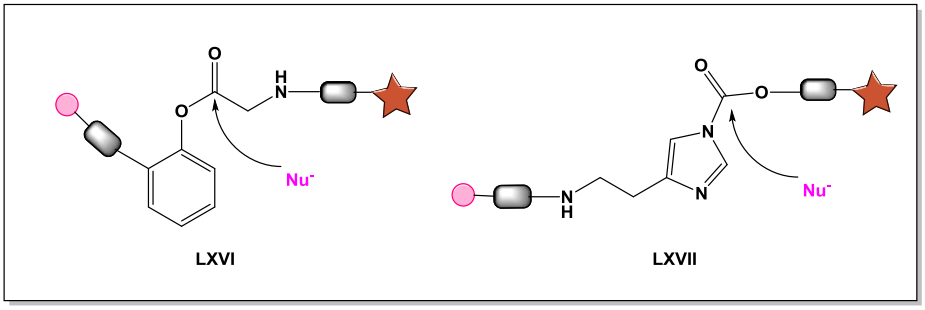


Figure 28: 2-Hydroxybenzyl and acyl imidazole based reactive groups.[108,44]

LDPP offers a powerful approach towards specific labeling of the enzyme outside its active site and should allow for applications such as real time monitoring of enzyme activities rather than abundances in living systems.

Our interest to synthesize glycoprobes for the investigation of carbohydrate processing enzymes in living cells quickly led to the realization that even though activity based glycoprobes are quite popular, ligand directed protein profiling glycoprobes are rarely found. This might be due to the huge variety of CPEs which renders a general probe design impossible. Additionally, intramolecular side

reactions might occur between the carbohydrate based recognition feature and the reactive group of the probe.

However, our aim was to synthesise a ligand directed protein profiling glycoprobe through well thought-off synthetic design and strategies.

The synthesis of powerful affinity based warheads is arguably the most crucial part and a necessary prerequisite for this challenge.

We figured that iminosugar C-glycosides would make excellent affinity based warheads for ligand directed protein profiling, as they are strong, competitive, reversible inhibitors of glycoside hydrolases.

Synthetic strategies towards these compounds will be discussed in the next chapter.

1.8. Synthetic strategies of iminosugar C-glycosides

As mentioned in chapter 1.3.2, *C*-derivatised iminosugars, such as compound **XIX**, **XX** and **XXII** can surpass their *N*-derivatised counterparts in many aspects. In general, they are more potent and show increased selectivity towards the target enzymes.[31]

A general and efficient synthetic route towards iminosugar *C*-glycosides and the generation of structural diversity is a necessity for the discovery of biologically relevant compounds. The establishment of high stereochemical control, an efficient build-up of the piperidine or pyrrolidine scaffold, as well as a judiciously selection of protecting groups due to the high density of functional groups in the molecule are only some of the challenges to be faced.

In a retrosynthetic analysis of the piperidine scaffold, two main synthetic strategies are feasible, namely the bond disconnection between C5-N or C1-N (Figure 29, path I), or by making use of an electrophilic iminosugar donor, disconnecting C1-CH_2-R (Figure 29, path II).

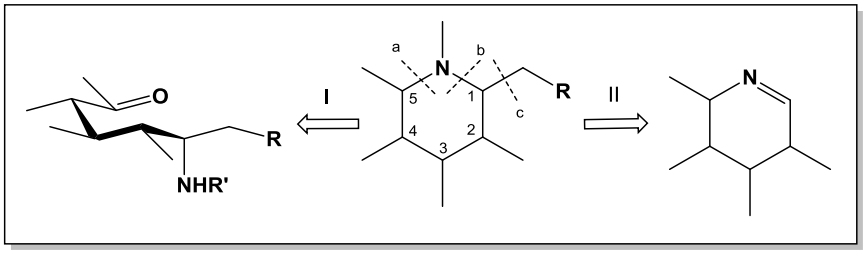


Figure 29: Retrosynthetic analysis for the piperidine ring.

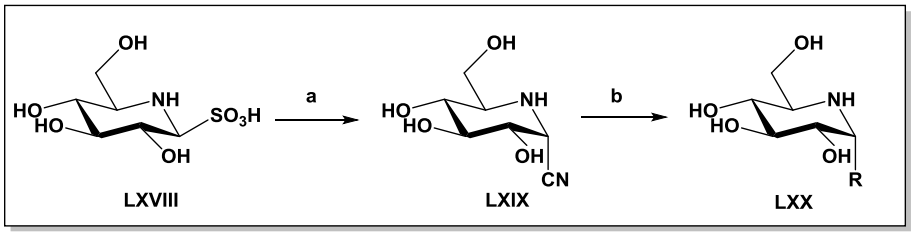
Although a few de novo syntheses are reported in literature[109], most of the synthetic approaches make use of carbohydrates as starting materials because of their availability, their presentation of stereocenter and their close structural relationship with iminosugars.

The iminosugar synthesis following path I will not be discussed here. Excellent reviews, e.g. from Martin *et.al*.[109] are available to cover this topic.

1.8.1. Electrophilic iminosugar donors

Electrophilic iminosugar donors almost always involve imine, iminium or *N*-acyliminium ions as reaction intermediates.
Selected examples for several strategies will be presented in the following.

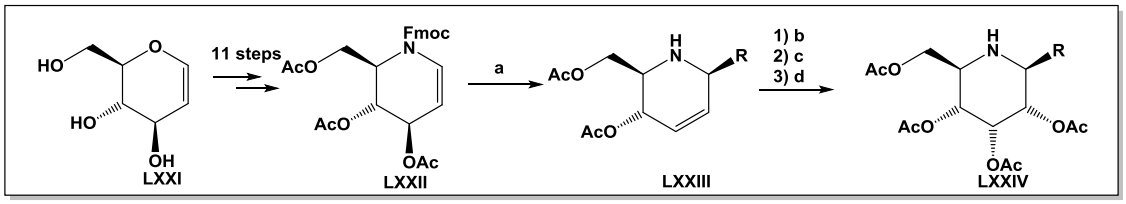
Nucleophilic substitution
Although widely used in the synthesis of classical C-glycosides, Lewis acid catalyzed reactions of carbon nucleophiles with activated iminosugar donors is not obvious, because of the relative instability of the intermediate.
Nevertheless, Böshagen *et.al*.[110] reported a synthesis of C-glycosides based on sulfonate/cyanide (**LXVIII** to **LXIX**) and cyanide/alkyl group (**LXIX** to **LXX**) exchange (Scheme 22).



Scheme 22: Sulfonate/cyanide and cyanide/alkyl group exchange approach. a) $Ba(OH)_2$, H_2O, then NaCN, 7.5 M HCl b) $(TMS)_2NH$, imidazole, then RMgX, THF.[110]

Iminoglucals

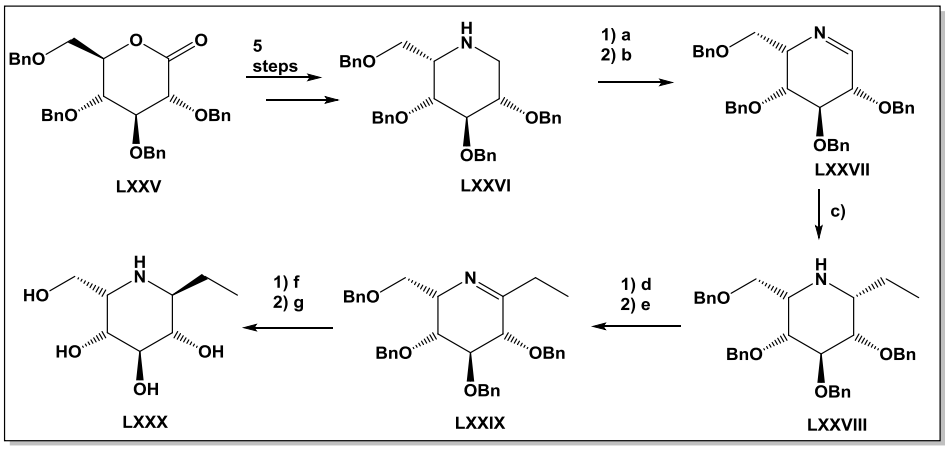
Similar to classic glycal chemistry, iminoglucals such as compound **LXXII** can undergo Lewis-acid-mediated carbon carbon bond formation reactions by allylic displacement of the C-3 acetate group to give compound **LXXIV** (Scheme 23).[111]



Scheme 23: Synthesis with Iminoglucals. a) Nu, Lewis Acid, CH_2Cl_2 b) OsO_4, NMO, acetone/H_2O c) Ac_2O, pyridine d) piperidine, CH_2Cl_2.[111]

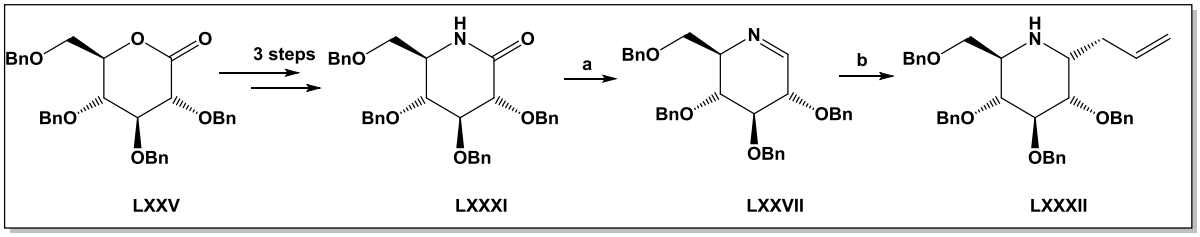
Addition to the endocyclic C=N bond

Davis *et.al*.[112] showed that the treatment of a protected 1-deoxy-L-iodonojirimycin (**LXXVI**), available from the protected lactone **LXXV**, with *N*-chlorosuccinimide (NCS), gives a cyclic chloramine nitrogen function. This allows for regioselective elimination utilizing 1,8-diazabicyclo[5.4.0]undic-7-ene (DBU) to give compound **LXXVII**. Addition of ethylmagnesium bromide yielded 1-*epi*-adenophorine **LXXVIII** with high diastereoselectivity. To synthesise adenophorine **LXXX**, compound **LXXVIII** was treated with NCS and lithium tetramethylpiperidine (LiTMP) to give elimination product **LXXIX**. Addition of a small nucleophilic hydride provided by lithium aluminium hydride (LAH) and subsequent hydrogenolytic deprotection utilizing $PdCl_2$ as catalyst gave adenophorine **LXXX** with high diastereoselectivity (Scheme 24).



Scheme 24: Approach via chlorination-elimination. a) NCS, CH_2Cl_2 b) DBU, Et_2O c) EtMgBr, Et_2O/dioxane d) NCS, CH_2Cl_2 e) LiTMP, Et_2O f) LAH, THF g) H_2, $PdCl_2$, EtOH[112]

Another approach towards endocyclic C=N bonds was shown by Szcześniak *et.al.*[113], who started from the protected lactone **LXXV** to synthesise lactame **LXXXI** in three steps. Further treatment with $Cp_2Zr(H)Cl$ gave the endocyclic imine intermediate **LXXVII**. Addition of allyltributylstannane and $Yb(OTf)_3$ afforded homoallylic compound **LXXXII** with excellent diastereoselectivity.



Scheme 25: Zirconium Yttrium approach. a) $Cp_2Zr(H)Cl$, THF b) allyltributylstannane, THF, $Yb(OTf)_3$.

A third possibility to create endocyclic imine intermediates is provided by the Staudinger/aza-Wittig (SAW) reaction sequence, which reasonably became the cental reaction of this PhD thesis, because it offers a quick and efficient access to *C*-glycosyl iminsugar compounds. Therefore, it will be discussed in more detail in the next chapter.

1.9. The Staudinger/aza-Wittig (SAW) reaction sequence

The SAW reaction sequence allows for synthesis of imine intermediates from azidodeoxy sugars. When treating compound **xlv** with a phosphine reagent, the terminal nitrogen is attacked by the phosphine, leading to a 4-membered ring transition state (**xlvi**). This gives, under the release of molecular nitrogen, a phosphinimine species **xlvii**. The phosphinimine nitrogen can attack the aldehyde in an intramolecular fashion thus forming another 4-membered ring transition state (**xlviii**). Phosphine oxide is released, thereby generating the iminium ion species **xlix**.[114]

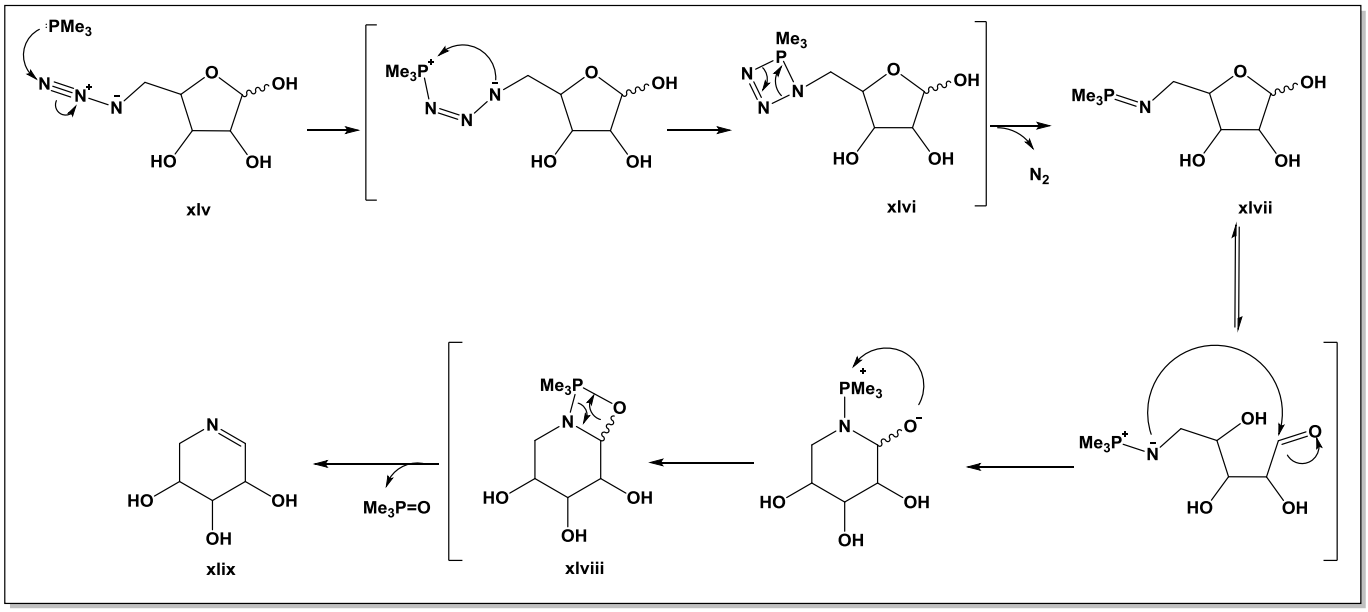
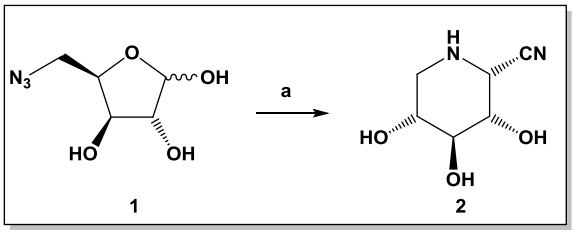


Figure 30: Reaction mechanism of the SAW reaction sequence.[114]

When applying 5-azido-5-deoxy-α,β-D-xylose (**1**) as substrate in the SAW reaction sequence, it could be demonstrated, that the formed iminium ion can be trapped quantitatively by cyanide as nucleophile in a diastereoselective manner, yielding the β-configurated product **2** (Scheme 26).[115]



Scheme 26: SAW - Cyanide reaction sequence. a) PMe_3, MeOH then NaCN.

Overkleeft *et.al.*[116] combined the Staudinger/aza-Wittig sequence with the Ugi-reaction by allowing hexylidene protected 5-azido-5-deoxy-α,β-D-ribose (**LXXXIII**) to undergo the SAW reaction and adding an acid as well as an isocyanide reagent to give compound **LXXXIV** as single diastereomer.

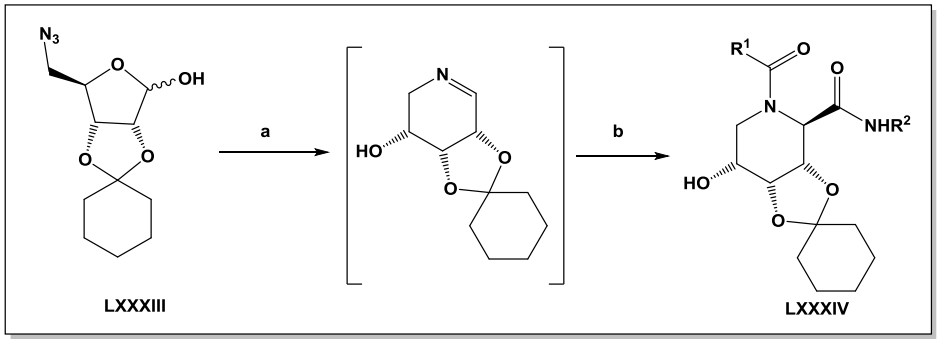


Figure 31: SAW-Ugi reaction cascade. a) PMe_3, MeOH b) R^1COOH, MeOH, then R^2-NC.[116]

The stereochemical outcome of the reactions mentioned earlier is influenced by the favored *H* conformation of the unsaturated piperidine systems as well as stereoelectronic effects and steric interactions.[117]

When the reaction is driven by conformational control, a useful tool for the prediction of the stereochemical outcome is the Woerpel model for iminium ion intermediates and will be explained with the example of a *xylo* configurated compound.[118]

The cyclic iminium ion intermediate is in equilibrium of two possible conformations wherein all substituents are found in an axial position (**I**) or in an all equatorial position (**Ii**). Both, an attack from the bottom side on the all axial- (**I**) or an attack from the top site on the all equatorial conformation (**Ii**), would give a highly twisted and therefore unfavored transition state (**Iii** or **Iiii** respectively), both of which will unlikely form the products **Iv** or **Ivii**, respectively. An attack from the top site on the all axial conformation (**I**) leads to a transition state, where the substituents at the sugar molecule would enter a 1,3-diaxial position (**Iiv**), which is fairly unfavorable to give

product **lv**. An attack from the bottom site at the all equatorial conformation (**li**) however leads to a favored transition state (**lvi**), which is most likely entering product formation (**lvii**) (Figure 32).

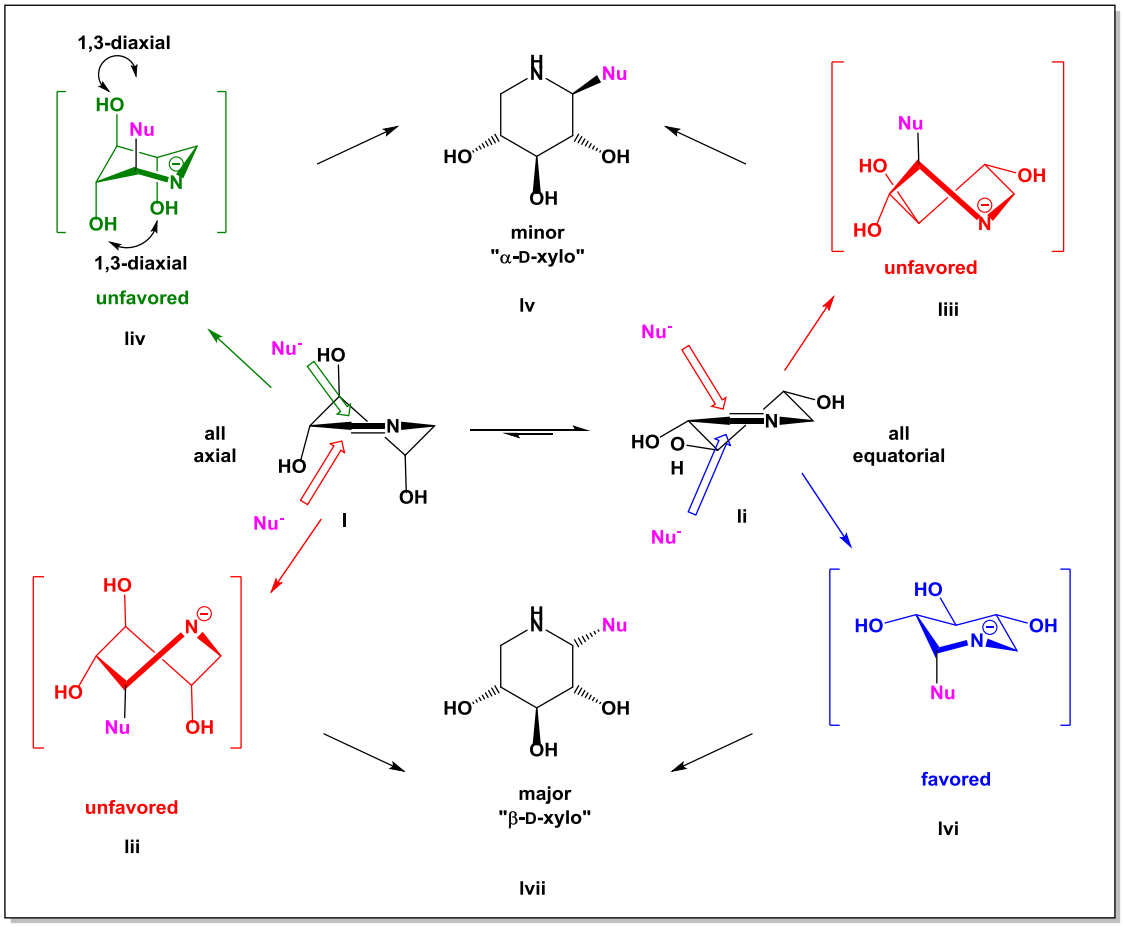


Figure 32: Woerpel model for iminium ion intermediates.[118]

2. Aims of the thesis

Iminosugars and especially their 1-C-derivatised analogues (**I**) have been shown to exhibit excellent inhibition properties towards glycoside hydrolases. Countless synthetic strategies towards these molecules are described in literature (Figure 33).

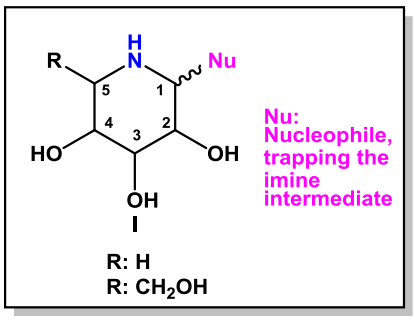
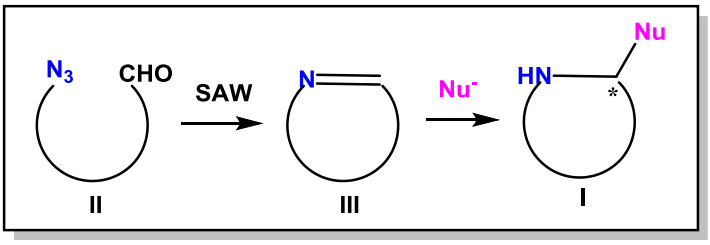


Figure 33: 1-C-derivatised iminosugar building block.

In this thesis the intramolecular Staudinger-aza/Wittig reaction is employed to provide an electrophilic iminosugar donor, which can be trapped by various nucleophiles, thus allowing for a rapid and efficient synthesis of the proposed C-1 modified iminosugar based glycoside hydrolase inhibitors (Scheme 27).



Scheme 27: SAW - nucleophile reaction cascade.

The decoration of the molecules will be guided by the respective applications, either as glycoprobes for carbohydrate processing enzymes, as inhibitors, or as affinity based warheads for ligand directed protein profiling probes.

Scope and limitations of different nucleophiles in the Staudinger-aza/Wittig nucleophile reaction cascade will be investigated. C-1 derivatised, unprotected iminosugars will be biologically evaluated towards their inhibitory properties with

selected glycoside hydrolases. The biological evaluation will be performed in the laboratories of Prof. Steven Withers, University of British Columbia, Vancouver, Canada.

Furthermore, a convenient and reliable synthesis for a ligand directed protein profiling probe will be established, with iminosugar moieties as affinity based warheads (Figure 34).

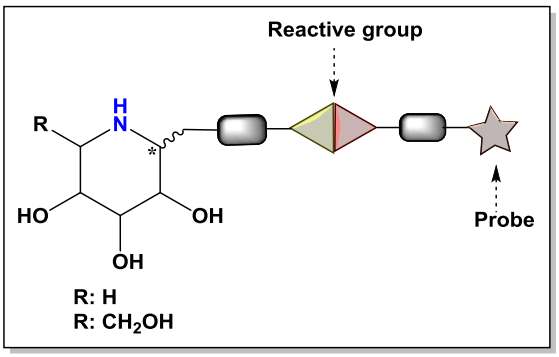
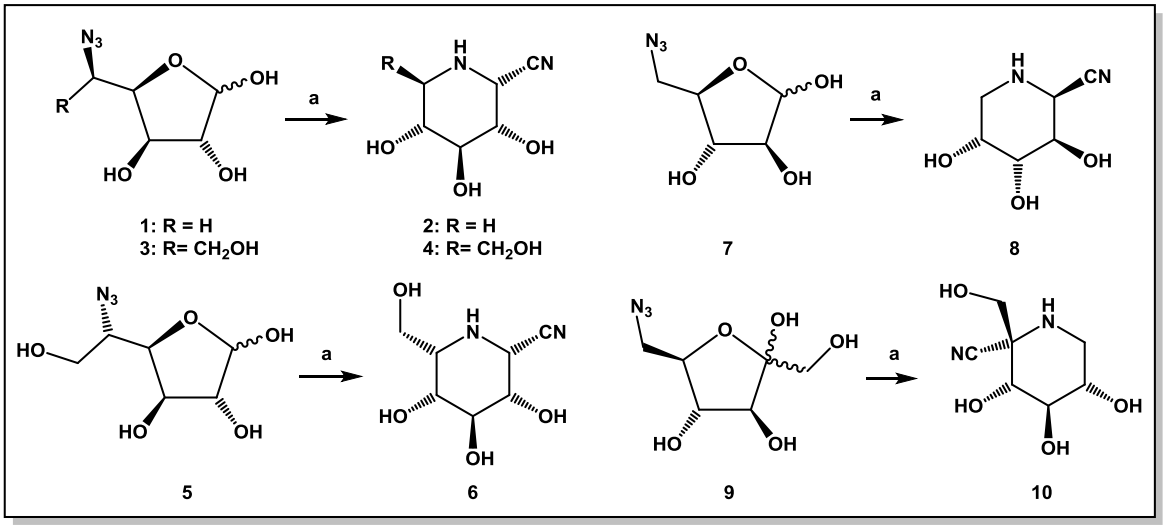


Figure 34: LDPP probe with an iminosugar as warhead.

3. Results and discussion

3.1. Preliminary studies

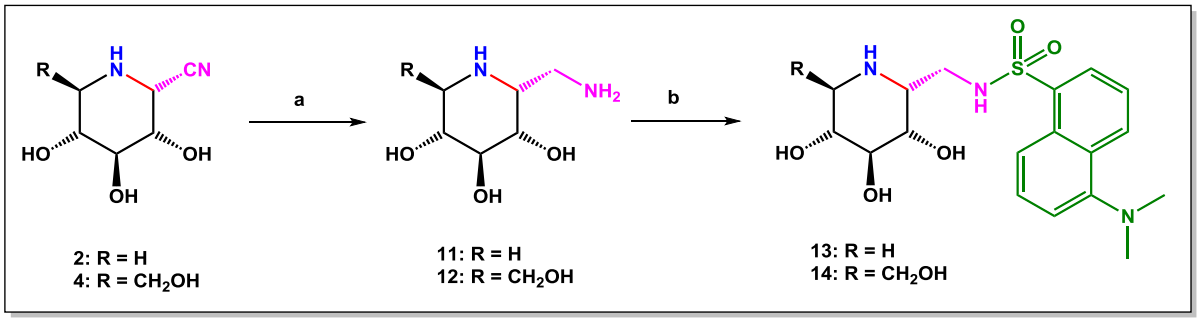
As described in an earlier work,[115,119] the Staudinger/aza-Wittig reaction sequence is a versatile approach towards 1-*C*-cyano-iminosugar building blocks. The reaction has been optimized and evaluated with various unprotected 5-azido-5-deoxy sugar substrates, namely 5-azido-5-deoxy-α,β-D-xylose (**1**), 5-azido-5-deoxy-α,β-D-glucose (**3**), 5-azido5-deoxy-α,β-L-idose (**5**), 5-azido-5-deoxy-α,β-D-arabinose (**7**) and 6-azido-6-deoxy-α,β-D-fructose (**9**). Sodium nitrile was used as nucleophile to give the corresponding 1-*C*-cyano iminosugar products **2**,**4**,**6**,**8** and **10** respectively in a stereoselective reaction, with the introduced nitrile being in *cis*-orientation to the adjacent alcohol group (Scheme 28).[119]



Scheme 28: Preliminary SAW/CN-reactions. a) PMe_3, MeOH, then NaCN.[119]

The obtained products have the potential to serve as diversely functionalisable building blocks. The reduction of the nitrile group of compounds **2** and **4** gives access to the primary amine functions (**11** and **12**, respectively). These can be further alkylated, acylated or sulfonylated in a chemo- and regioselective fashion. Sulfonylation with dansylchloride, for example, gives compounds **13** and **14**, respectively which proved to be highly potent inhibitors of human lysosomal *β*-glucocerebrosidase. The result of the biological evaluation will be presented in

chapter 4 (Scheme 29). These promising results cause motivation to further investigate the scope of the Staudinger/aza-Wittig reaction sequence as a concise synthetic strategy towards a large number of diverse compounds.[115]



Scheme 29: Follow up chemistry. a) H_2, MeOH/H_2O, $Pd(OH)_2$/C, H^+ b) dansylchloride, MeOH, Et_3N.[115]

In theory, any molecule that offers nucleophilicity can be attached to the iminium ion intermediate of the SAW reaction sequence.

3.2. Synthesis of starting materials

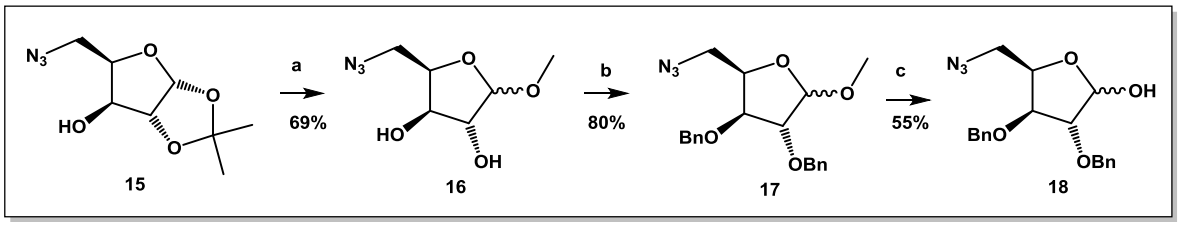
The SAW reaction and cyanide as nucleophile has been tested solely on unprotected sugar compounds. When employing different nucleophiles, however, this strategy might not be very constructive.

To evaluate different nucleophiles as agents for the SAW reaction, a protecting group strategy has to be developed to provide protected 5-azido-5-deoxy sugars as starting materials.

3.2.1. 5-Azido-5-deoxy-2,3-di-*O*-benzyl-α,β-D-xylofuranose (18)

Starting from compound **15**,[119] reacetalisation occurs in methanol with acetylchloride to give the α,β-mixture of the methyl glycoside **16**. After benzylation of the unprotected hydroxy groups the protected intermediate **17** is formed. The methyl glycoside can be cleaved under acidic conditions employing concentrated HCl and

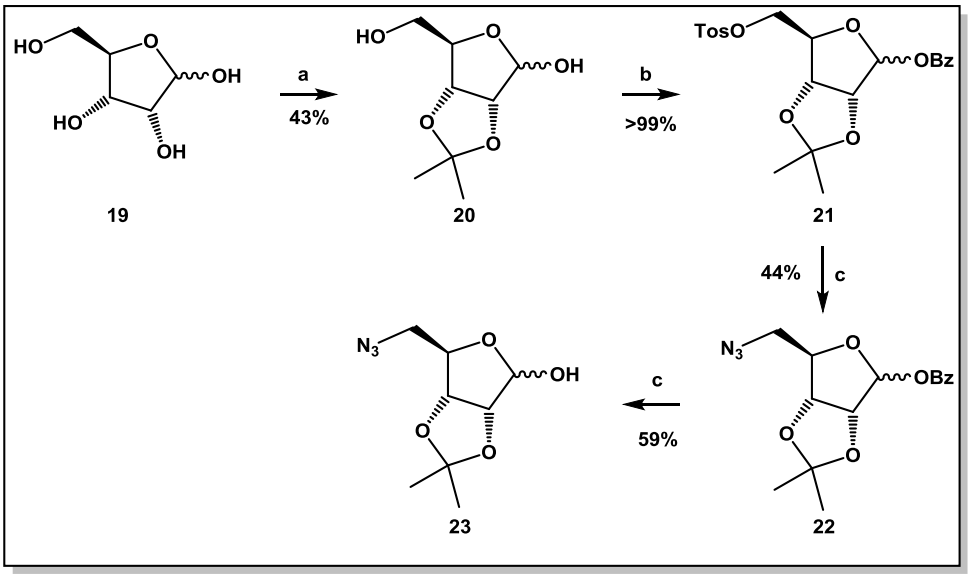
elevated temperatures to give the protected 5-azido-5-deoxy-α,β-D-xylose starting material **18** (Scheme 30).



Scheme 30: Synthesis of 5-azido-5-deoxy-2,3-di-*O*-benzyl-α,β-D-xylofuranose. a) AcCl, MeOH, b) NaH, DMF, then BnBr c) HCl, CH_3CN/H_2O, reflux.

3.2.2. 5-Azido-5-deoxy-2,3-*O*-isopropylidene-α,β-D-ribofuranose (23)

Starting from α,β-D-ribofuranose (**19**), the alcohol groups at positions C-2 and C-3 can be protected with an isopropylidene protecting group, using acetone as solvent under acidic conditions to give compound **20**. Tosylation of position O-5 and subsequent benzoylation of position O-1 gives an α,β-mixture of compound **21**. The tosyl group can be substituted with an azido group using sodium azide as reagent in DMF. The obtained compound **22** can be deprotected under Zemplén conditions to give compound **23** (Scheme 31).

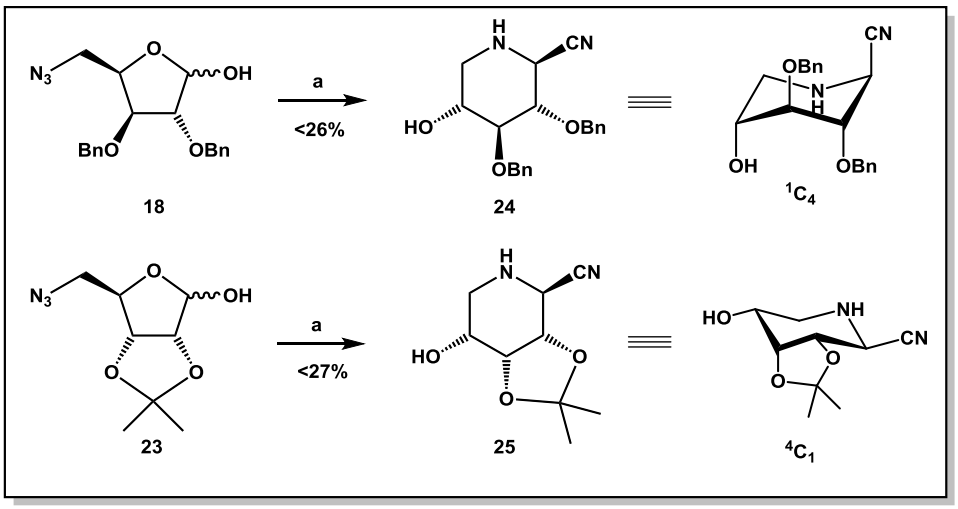


Scheme 31: Synthesis of 5-azido-5-deoxy-2,3-*O*-isopropylidene-α,β-D-ribofuranose. a) acetone, H_2SO_4 b) TosCl, pyridine, then BzCl c) NaN_3, DMF d) NaOMe 1M, MeOH.

3.3. Sodium cyanide as nucleophile

To evaluate the suitability of the SAW reaction sequence with protected 5-azidodeoxy aldoses, nitrile is employed as nucleophile. When applying 5-azidodeoxy-2,3-di-*O*-benzyl-α,β-D-xylofuranose (**18**) as substrate, compound **24** can be isolated with a yield less than 26%.

When applying 5-azidodeoxy-2,3-*O*-isopropylidene-α,β-D-ribofuranose (**23**) to these reaction conditions, however, less than 27% of compound **25** can be isolated.



Scheme 32: SAW-nitrile reaction sequence. a) PMe_3, MeOH then NaCN.

Although the yields of these reactions are poor and pure products cannot be isolated, the results prove that protected sugar compounds are indeed suitable for the SAW-nucleophile reaction sequence.

Compound **24** shows a lack of big coupling constants in the ^{1}H NMR, thus suggesting a $^1C_4$ chair conformation and a *trans*-orientation of the nitrile group to the adjacent *O*-benzyl group.

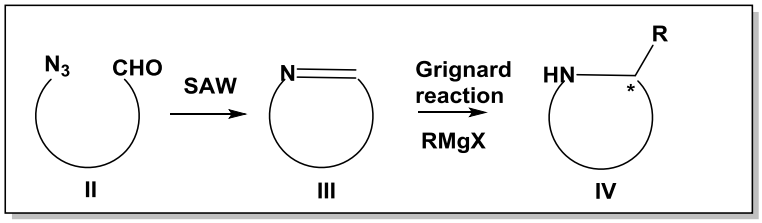
Compound **25**, however, shows a coupling constant of J_{H1-H2} 8.8 Hz, which is consistent with a $^4C_1$ chair conformation and again a *trans*-orientation of the nitrile group to the adjacent isopropylidene protecting group.

These results are somehow surprising as the Woerpel model proposes an attack from the bottom site of the transition state. It is assumed that the sterical hindrance of

the protecting groups causes steric- rather than conformational control of these reactions.

3.4. Grignard reagents as nucleophiles

Grignard reagents are well known nucleophiles in organic synthesis and should be able to trap the cyclic iminium ion intermediate provided by the SAW reaction sequence (Scheme 33).



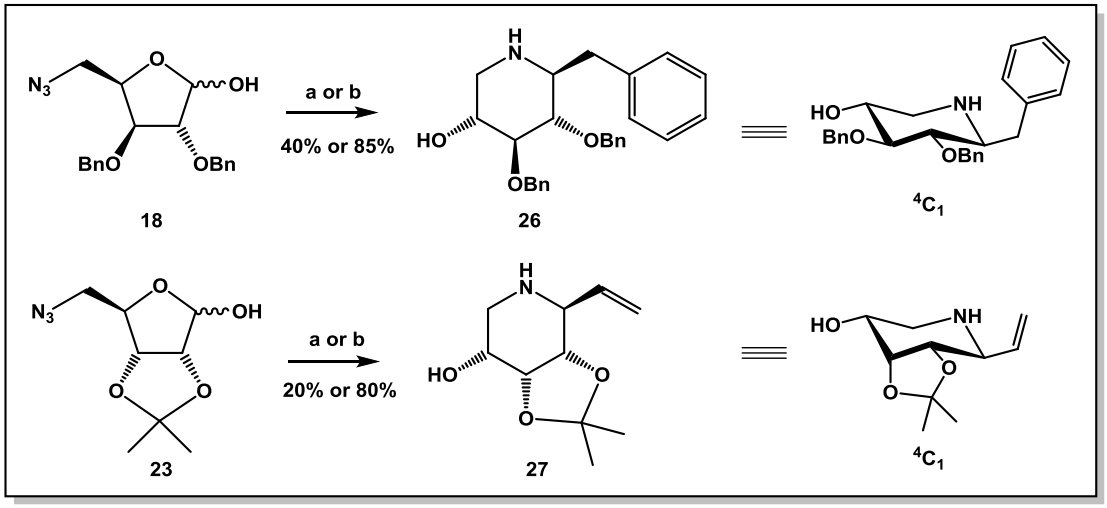
Scheme 33: SAW-Grignard reaction sequence.

It is presumed that both, the SAW sequence as well as the Grignard reaction, would be compatible with the choice of THF as solvent. Indeed, compound **18** is smoothly converted into the corresponding cyclic iminium ion intermediate when treated with PMe_3 in THF. After addition of commercially available benzylmagnesium bromide, 1-*C*-benzyl-2,3-di-*O*-benzyl-iminoxylitol (**26**) is formed in a yield of 40%.

A similar result is obtained when applying the same reaction conditions to 5-azidodeoxy-2,3-*O*-isopropylidene-D-ribofuranose (**23**) as substrate with commercially available vinylmagnesium bromide as nucleophile. The corresponding isopropylidene-protected 1-*C*-vinyl iminoribitol (**27**) is obtained in 20% yield.

To improve the somehow disappointing yields of this reaction sequence, a solvent exchange method, introduced by Overkleeft *et.al*.[116] is evaluated.

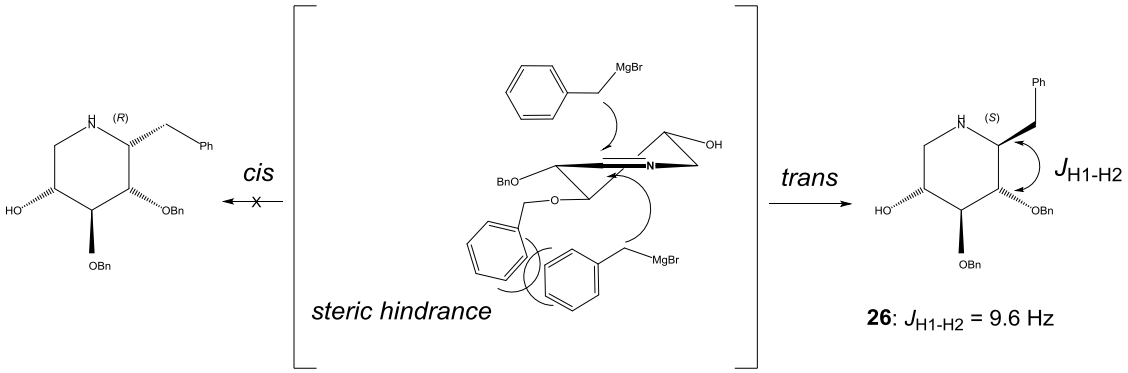
Therefore, the SAW transformation is conducted in MeOH employing PMe_3 as phosphine reagent. After complete consumption of the starting material the solvent is removed under reduced pressure and the crude iminium ion intermediate is dissolved in THF. After addition of the corresponding Grignard reagents and consumption of the iminium ion intermediate, the yields of the reactions can be increased to 85% in the case of compound **26**, and 80% in the case of compound **27** (Scheme 34).



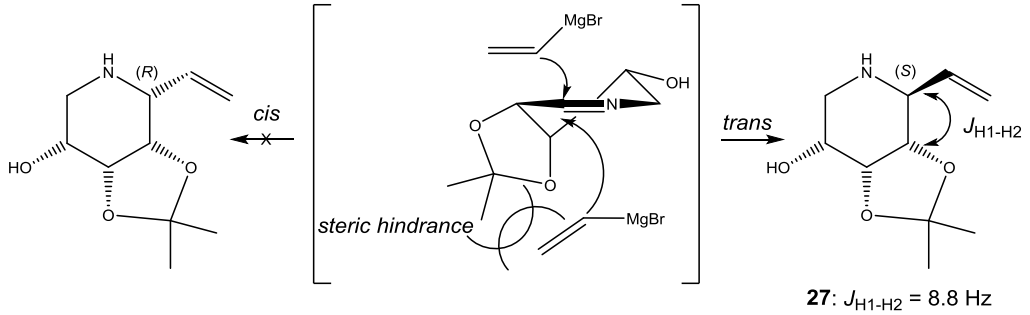
Scheme 34: SAW-Grignard reactions. a) PMe_3, THF, then RMgX b) PMe_3, MeOH, then THF, RMgX.

NMR spectroscopy indicates an (*S*)-configuration at the newly formed stereocenter in both compounds with a coupling constant of J_{H1-H2} = 9.6 Hz for compound **26** and J_{H1-H2} = 8.8 Hz for compound **27** respectively. These results are consistent with a *trans*-orientation of the 1-*C*-alkyl substituent to the adjacent position C-2 in the $^4C_1$ chair conformation.

Again, these results are somehow surprising, as the Woerpel model suggests a *cis* approach of nucleophiles. However, a closer look indicates steric hindrance of the bottom side caused by the protecting groups (Scheme 35, Scheme 36).

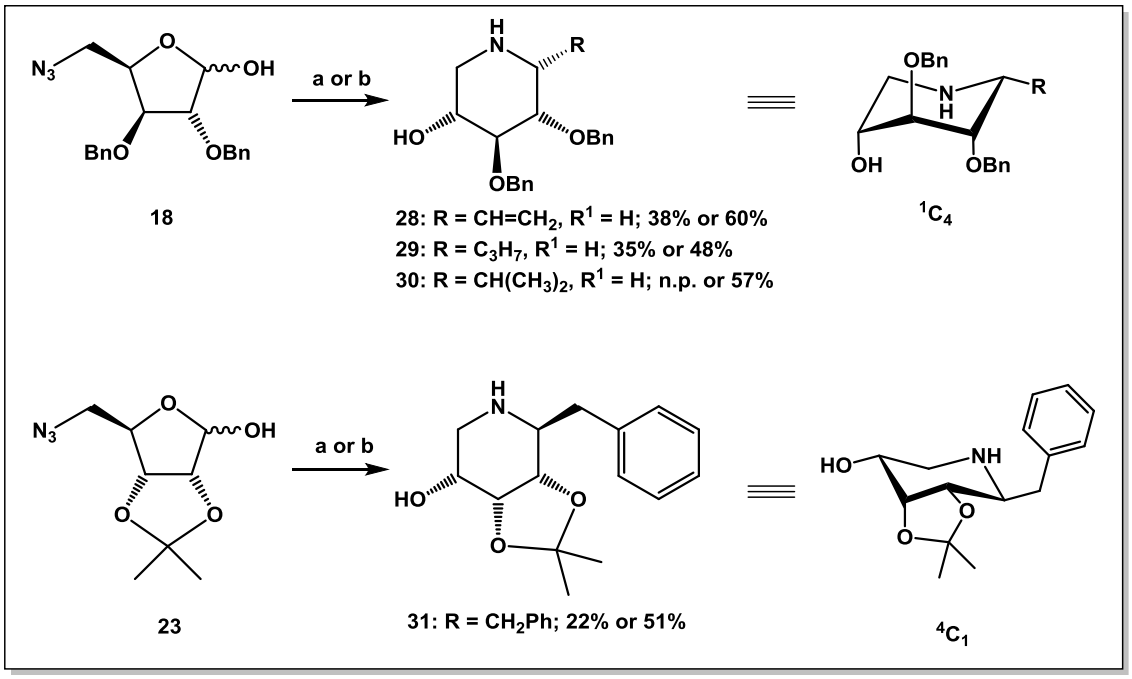


Scheme 35: Woerpel's model for the steric control of the benzyl protecting group.



Scheme 36: Woerpel's model for the steric control of the isopropylidene protecting group.

Alternative commercially available nucleophiles such as propylmagnesium bromide and isopropylmagnesium bromide are also evaluated and conduced to both solvent strategies. Again, far better yields are achieved with the solvent exchange method (Scheme 37).



Scheme 37: SAW-Grignard reactions overview. a) PMe_3, THF, then RMgX b) PMe_3, MeOH, then THF, RMgX.

Small H-H coupling constants in the NMR spectra of compounds **28**, **29** and **30** indicate a $^1C_4$ chair conformation of the piperidine ring. Small nucleophiles are not sterically hindered by the *O*-benzyl protecting groups and attack from the bottom site following the Woerpel model, thus, the introduced residue is in *cis*-orientation to the

adjacent position C-2. X-Ray analysis of compound **26** and compound **29** determine these findings (Figure 35).

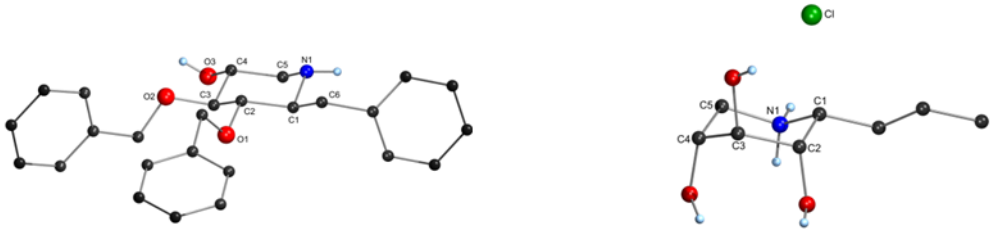


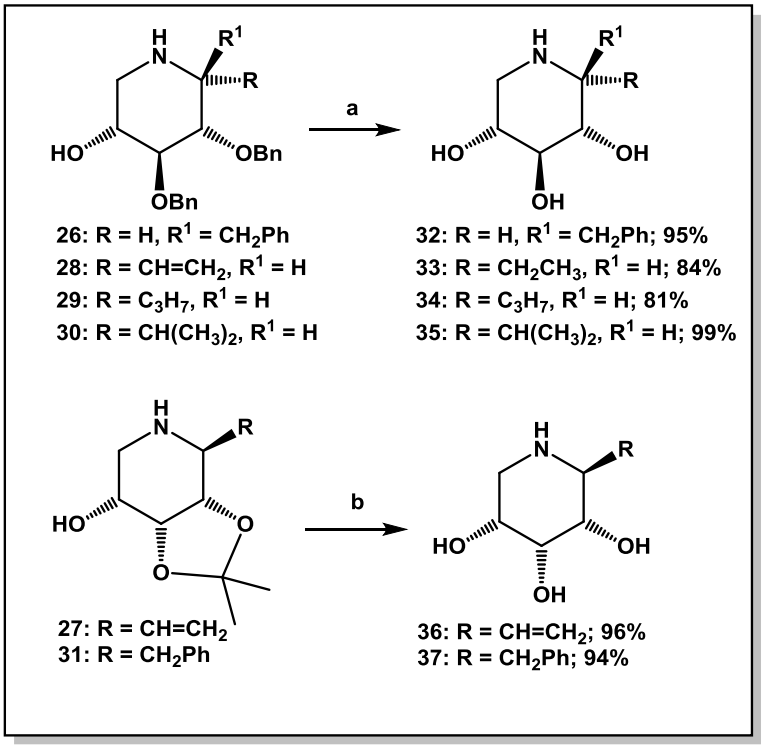
Figure 35: X-Ray structure of compound 26 and compound 29

A summary of the obtained yields, configurations and conformations is given in Table 3.

Table 3: SAW-Grignard reactions.

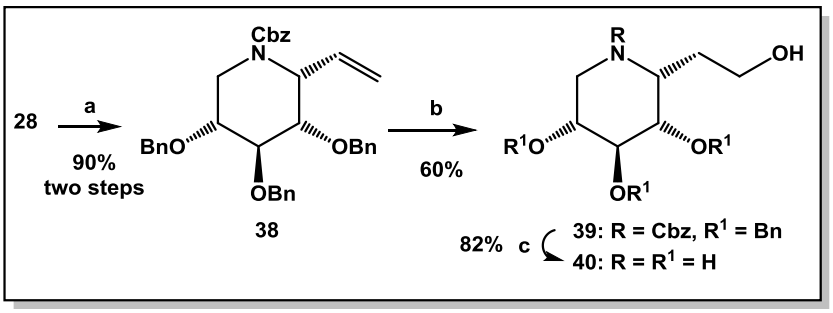
Sugar substrate	Grignard reagent	product	yield %	configuration	conformation
18	BnMgBr	**26**	a: 40 b: 85	(*S*), 1,2-*trans*	$^4C_1$
18	vinylMgBr	**28**	a: 38 b: 60	(*R)*, 1,2-*cis*	$^1C_4$
18	C_3H_7MgBr	**29**	a: 35 b: 48	(*R)*, 1,2-*cis*	$^1C_4$
18	$(CH_3)_2CHMgBr$	**30**	a: n.p. b: 57	(*R)*, 1,2-*cis*	$^1C_4$
23	vinylMgBr	**27**	a: 20 b: 80	(*S*), 1,2-*trans*	$^4C_1$
23	BnMgBr	**31**	a: 22 b: 51	(*S*), 1,2-*trans*	$^4C_1$

Debenzylation of compounds **26, 28, 29** and **30** by hydrogenolysis under catalytic conditions or acidic removal of the isopropylidene protecting group of compound **27** and **31** give the unprotected 1-*C*-alkyl iminosugar compounds **32-37** ready for biological evaluation (Scheme 38).



Scheme 38: Deprotection of the compounds. a) H_2, $Pd(OH)_2$/C, MeOH/H_2O 1/1 b) HCl, H_2O.

To increase the diversity of these products, the vinyl moiety can be exploited for follow up chemistry. Starting from compound **28** protection of the secondary amine with benzylchlorformiate and benzylation of the remaining hydroxy group with benzyl bromide gives compound **38.** The vinyl group can undergo hydroboration to give compound **39**. Global deprotection under hydrogenolytic conditions utilizing $Pd(OH)_2$ on activated charcoal gives compound **40** (Scheme 39).



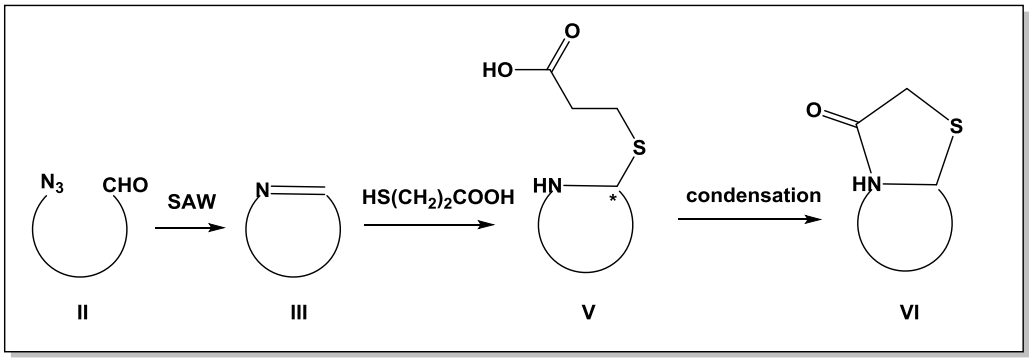
Scheme 39: Follow up chemistry on the vinyl moiety. a) CbzCl, pyridine, then BnBr, NaH, DMF b) $NaBH_4$, THF, then DMS c) $Pd(OH)_2$/C, MeOH/H_2O 1/1.

The biological evaluation of the unprotected compounds obtained, will be presented in chapter 4.

3.5. Amino- and mercaptoacids as nucleophiles

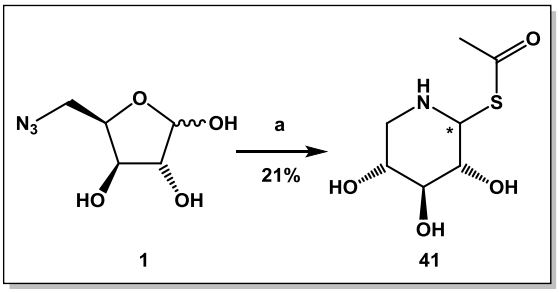
Li *et.al.*[120] introduced mercaptoacids as suitable nucleophiles for the SAW reaction sequence.

They propose an attack of the sulfur as nucleophile at the cyclic iminium ion intermediate, which is further condensated to a five-membered lactam to give a fused bicyclic system (Scheme 40).



Scheme 40: Proposed SAW-mercaptoacid reaction.

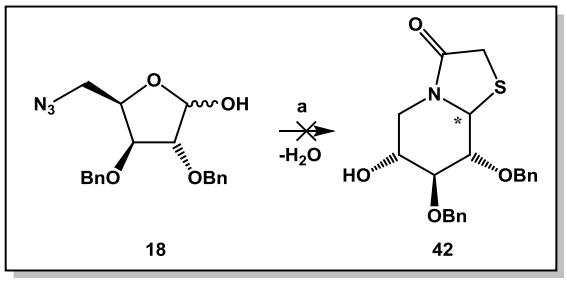
To evaluate sulfur as a suitable nucleophile, 5-azido-5-deoxy-α,β-D-xylofuranose (**1**) is treated with PMe_3 in MeOH. After consumption of the starting material, thioacetic acid is added to the reaction mixture leading to product **41** in poor yields. The reaction suggests that sulfur is a possible nucleophile for the synthesis of a variety of 1-C-sulfurylated iminosugar building blocks (Scheme 41).



Scheme 41: SAW thioacetic acid reaction sequence. a) PMe_3, MeOH, then thioacetic acid.

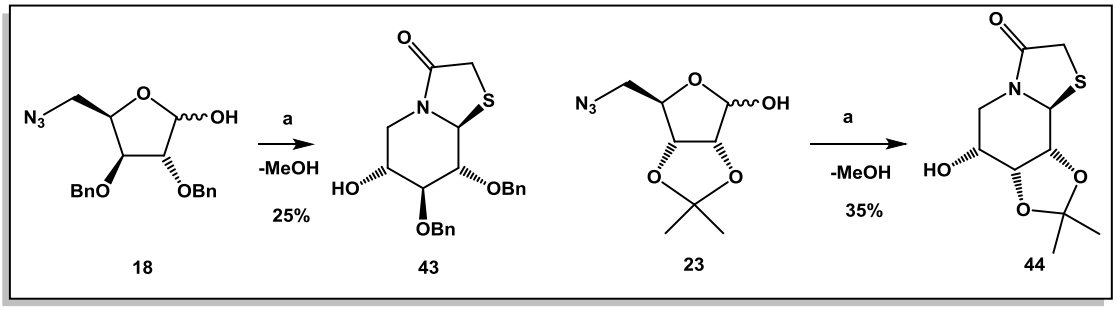
Finding the condensation step hard to believe, 5-azidodeoxy-2,3-di-*O*-benzyl-α,β-D-xylofuranose (**28**) is brought to reaction with PMe_3 in MeOH and, after consumption of the starting material mercaptoacetic acid is added to the reaction mixture.

However, no conversion to the desired product **42** is observed, suggesting that water is no suitable leaving group for the proposed condensation (Scheme 42).



Scheme 42: SAW-mercaptoacetic acid reaction. a) PMe_3, MeOH, then mercaptoacetic acid.

The reaction is repeated with mercaptoacetic acid methyl ester to provide a better leaving group and indeed, the desired product **43** can be isolated.
The reaction is likewise performed with 5-azido-5-deoxy-2,3-*O*-isopropylidene-α,β-D-ribofuranose (**23**) as substrate and product **44** is obtained (Scheme 43).

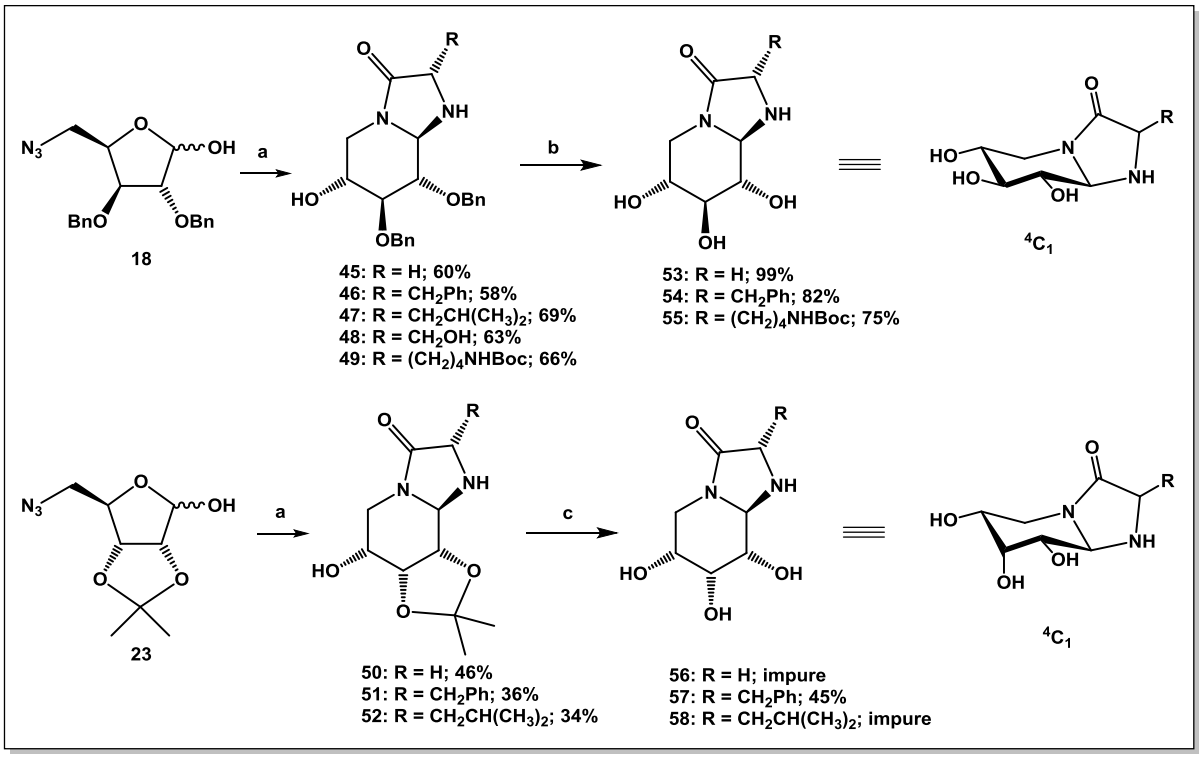


Scheme 43: SAW-mercaptoacetic acid reaction. a) PMe_3, MeOH, then mercaptoacetic acid methyl ester.

Inspired by these results various commercially available amino acid methyl ester hydrochlorides are evaluated as suitable agents in the SAW amino acid reaction cascade.
The starting materials compound **18** and compound **23** are converted into the cyclic iminium ion intermediate employing PMe_3 in MeOH. After the consumption of the starting material, the amino acid methyl ester hydrochlorides, namely L-glycin, L-phenylalanine, L-leucine, L-serine and L-lysine, treated with basic Merk III ion exchange residue to liberate the respective free amine, is added to the reaction mixture. The SAW amino acid reaction sequence with compound **18** gives the desired products **45-49** in satisfying yields.[121]

Compound **23** is subjected to the SAW reaction with L-glycine, L-phenylalanine and L-leucine and gives compounds **50**, **51** and **52** in good yields.

Deprotection by hydrogenolysis under catalytic conditions of compound **45**, **46** and **49** gives the unprotected bicyclic iminosugar products **53**, **54** and **55**. Acidic removal of the isopropylidene protecting group of compound **50**-**52** gives the unprotected bicyclic iminosugar products **56**-**58**. The yield of compound **56** and **58** is found to be higher than 100%. Purification, employing concd. NH_4OH solution forms NH_4Cl salt, which cannot be removed by means of silica gel chromatography (Scheme 44).



Scheme 44: SAW-amino acid reaction sequence. a) PMe_3, MeOH then amino acid methyl ester b) H_2, $Pd(OH)_2/C$, MeOH/H_2O 1/1 c) HCl, H_2O

All obtained products show a coupling constant $J_{H1\text{-}H2}$ of approx. 8 Hz, indicating a *trans*-orientation of the substituent at C-1 to the adjacent *O* group in the $^4C_1$ chair conformation.

A crystal structure of compound **48** determines these findings (Figure 36).[122]

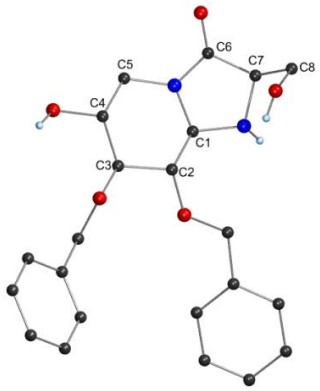


Figure 36: Crystal structure of compound 48.

3.6. Ligand directed acyl imidazole probes

As described in chapter 2, the target LDPP probe molecule is composed of three parts. Our approach utilizes iminoxylitol derivatives as ligand (warhead), easily accessible via the SAW-Grignard reaction sequence or reductive amination of compound **1**, an acyl imidazole derived reactive site, as well as the fluorescent dansyl tag (Figure 37). Spacer molecules between the warhead and the reactive site, as well as the dansyl tag and the reactive site reduce steric hindrance between the sterical demanding components.

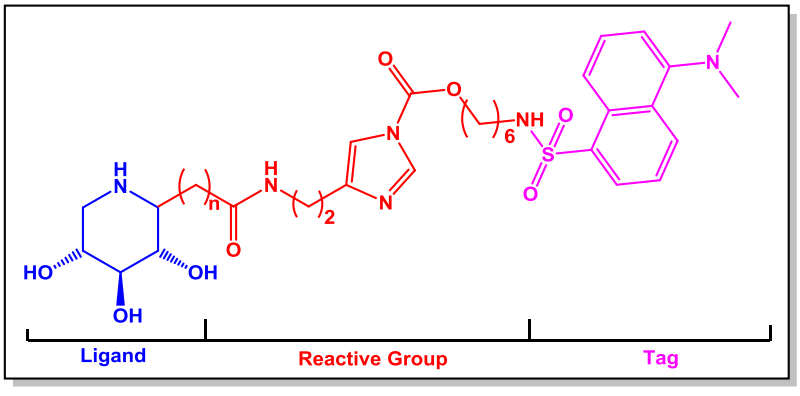


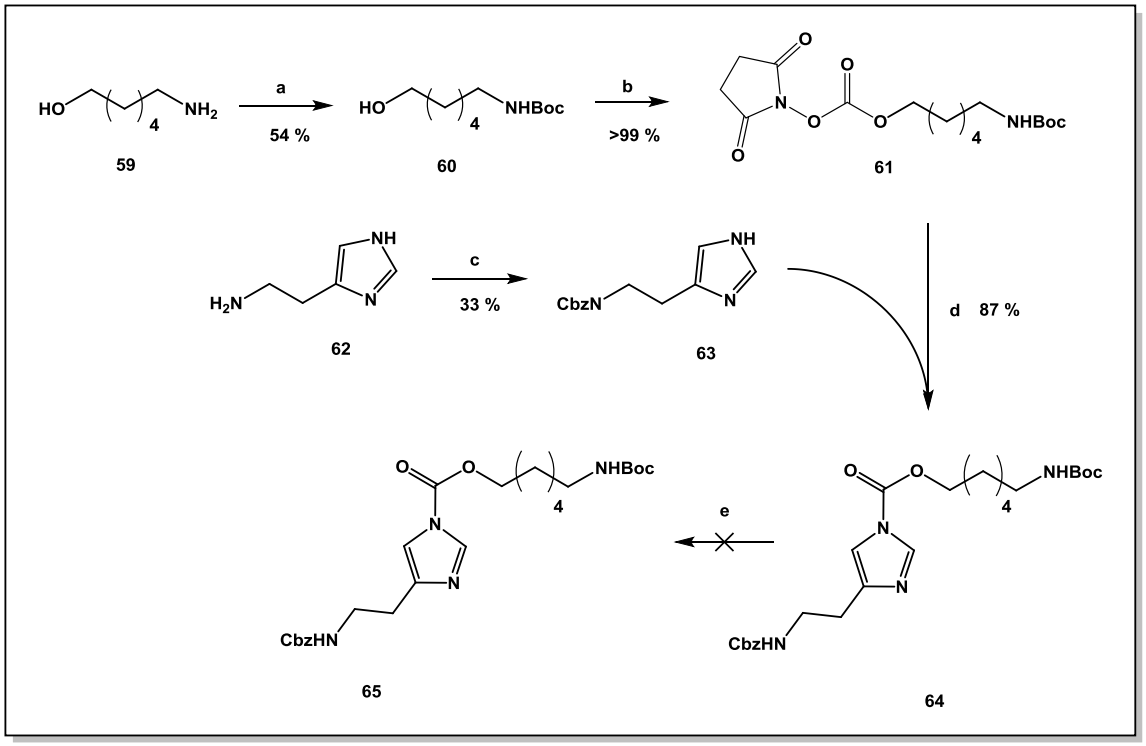
Figure 37: Target acyl imidazole probe molecule.

Initially, our focus is the synthesis of a protected acyl imidazole which, after deprotection, will be coupled to the warhead or the tag.

3.6.1. Protected acyl imidazole moiety

The protected acyl imidazole moiety **64** is successfully synthesized by protecting the amine function of 6-aminohexanol **59** utilizing *tert*-butyloxycarbonyl anhydride (Boc_2O) under basic conditions in CH_2Cl_2. The hydroxy group is further activated with *N,N'*-disuccimidyl carbonate, yielding the protected, activated spacer moiety **61**. The terminal amine of histamine (**62**) is protected, using benzylchloroformiate (CbzCl) under basic conditions in MeOH to give compound **63**. Molecules **61** and **63** are coupled in DMF using diisopropyl ethylamine (DIEA) as base to give the protected acyl imidazole **64** in good yields.

For later coupling of the dansyl tag to the reactive group, compound **64** is subjected to Boc-deprotection under acidic conditions employing trifluoro acetic acid (TFA). These reaction conditions however, only lead to the decomposition of the molecule (Scheme 45).



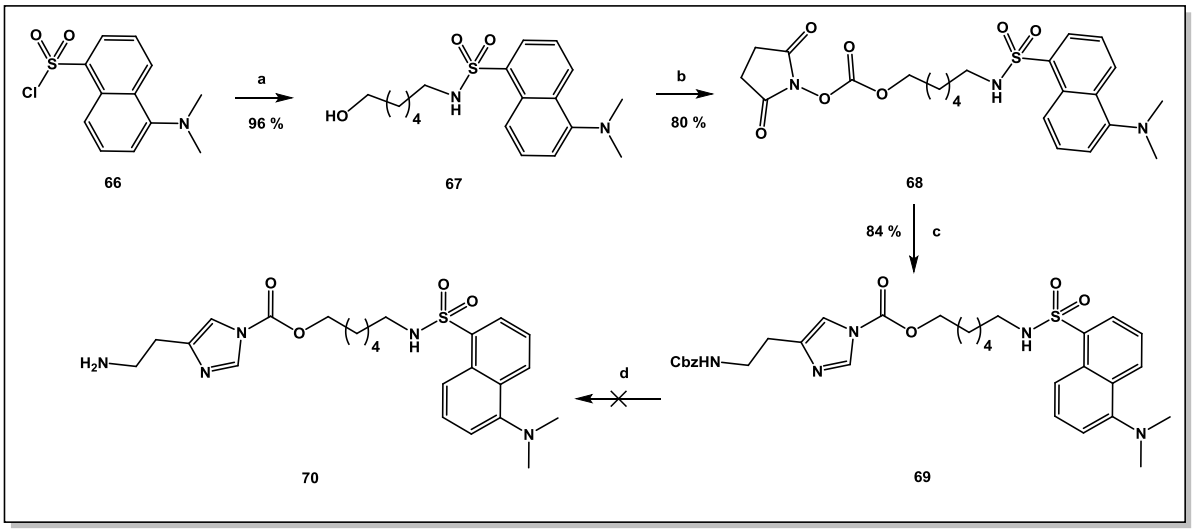
Scheme 45: Synthesis of the protected histamine moiety. a) Boc_2O, CH_2Cl_2, Et_3N b) *N,N'*-disuccimidyl carbonate, CH_3CN, Et_3N c) CbzCl, MeOH, Et_3N d) 63, DMF, pyridine, then 61 e) TFA.

Since the reactive acyl imidazole group does not seem to be stable under acidic conditions, the dansyl moiety will be installed prior to activation and coupling of the spacer molecule to the histamine moiety.

3.6.2. Dansylated histamine moiety

Dansylchloride (**66**) is equipped with a 6-aminohexanol spacer molecule under basic conditions in methanol. Further activation of compound **67** with *N,N'*-disuccimidyl carbonate gives the succimidyl activated fluorescent moiety **68**. Molecules **63** and **68** are coupled in DMF and (DIEA) as base to give the protected acyl imidazole presenting the fluorescent tag **69** in good yields.

The deprotection of the primary amine under hydrogenolytic conditions utilizing $Pd(OH)_2$ on activated charcoal as catalyst, however, leads to decomposition of compound **69**. No deprotected compound **70** is obtained (Scheme 46).



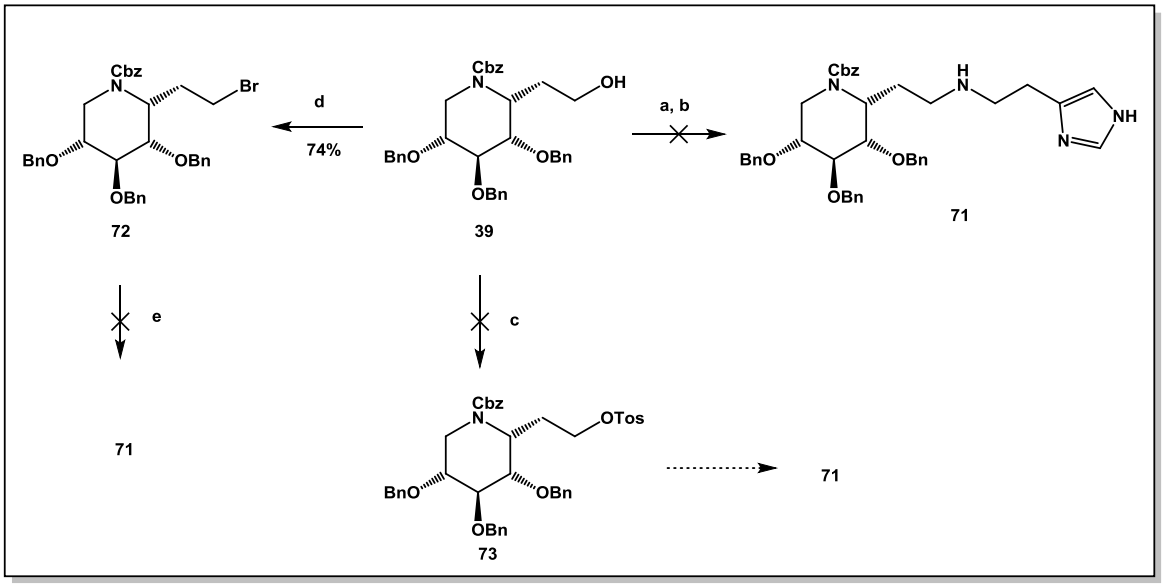
Scheme 46: Synthesis of the histamine moiety. a) 1-Aminohexanol, MeOH, Et_3N b) *N,N'*-disuccimidyl carbonate, CH_3CN, Et_3N c) Compound 63, DMF, Et_3N d) H_2, MeOH, $Pd(OH)_2$/C.

Unfortunately, the acyl imidazole component appears to be unstable under hydrogenolytic or acidic deprotection conditions. As a result it is obvious that the carbamate coupling has to be last step in the probe synthesis.

3.6.3. Modification of SAW-Grignard reaction iminosugar compounds

The SAW-Grignard follow-up chemistry offers compound **39** containing a primary hydroxy function, which can be converted into an aldehyde, tosylate or bromide ready for the reaction with the primary amine of histamine.

Oxidation with Dess-Martin periodinane (DMP) of compound **39** proceeds smoothly on TLC while reductive amination with sodium cyanoborohydride ($NaCNBH_3$) does not give the desired product **71** surprisingly. Tosylation of compound **39** shows no conversion to compound **73**. Bromination with triphenylphosphine dibromide gives a smooth conversion to compound **72**. The nucleophilic attack of the histamine moiety to obtain compound **71** however, did not give the desired product (Scheme 47).



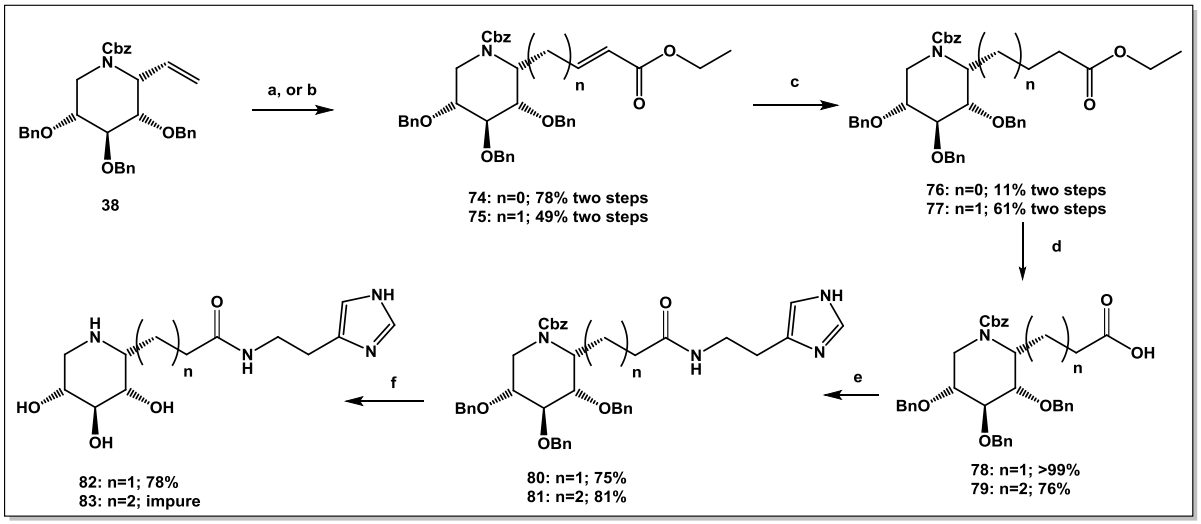
Scheme 47: Functionalities after SAW-Grignard. a) DMP, CH_2Cl_2 b) Histamine dihydrochloride, MeOH, 3A MS then $NaCNBH_3$ c) TosCl, pyridine d) PPh_3Br_2, CH_2Cl_2, pyridine e) histamine dihydrochloride, DMF, K_2CO_3.

Although these results are somehow disappointing, a secondary amine in the spacer part is not considered ideal anyway, because it would also be able to serve as a nucleophile opening the reactive group of the probe.

Therefore, an amide function should be the better choice as attachment site of the histamine moiety.

A retrosynthetic analysis reveals that the amide can be formed between the primary amine of histamine and an acid functionality of the spacer molecule employing amide coupling conditions. This acid is readily available via saponification of an ethyl ester, provided by a Horner Wadsworth Emmons (HWE) reagent, which can be readily attached to an aldehyde of the sugar molecule.

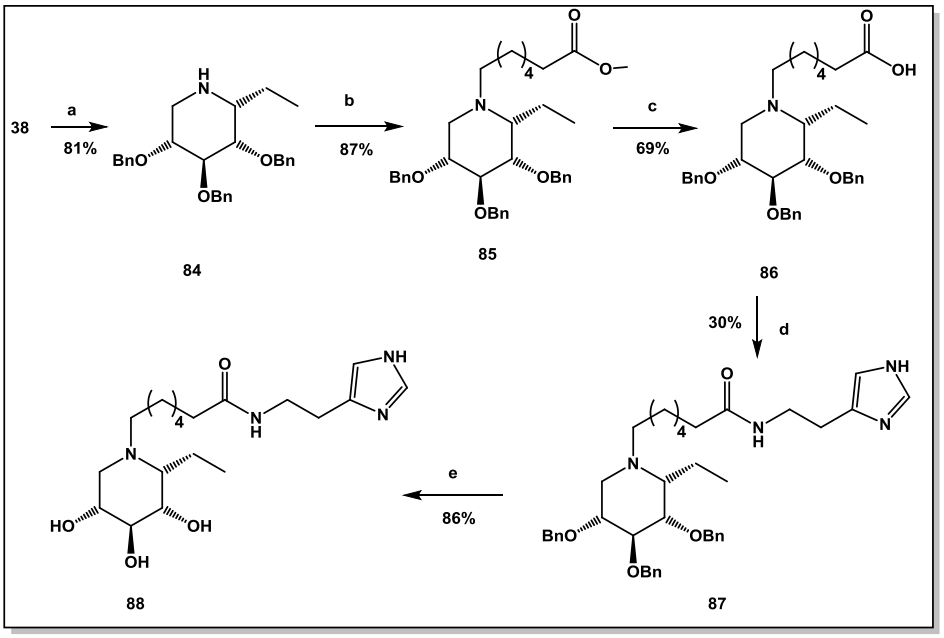
This aldehyde is formed via oxidation of the primary alcohol from compound **39** to give compound **75**, or via ozonolysis of the vinyl function of compound **38** to give compound **74**. Hydrogenolytic reduction in MeOH with $Pd/BaSO_4$ of the double bond, formed in the HWE reaction sequence, results in the loss of the Cbz protecting group, the benzyl protecting groups, however, remain on the molecules under these reaction conditions. The Cbz protecting group is reattached at the ring nitrogen using benzylchlorformiate and Et_3N as base to give the fully protected ester compounds **77** and **76**, respectively. Saponification of the ethyl ester gives the free acid moieties **79** and **78**, respectively, which can be converted into the corresponding histamine amide with 1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium-hexafluorophosphate (COMU) coupling conditions in DMF and DIEA as base to give compounds **81** and **80**, respectively. Hydrogenolytic deprotection of the sugars proceeds smoothly with $Pd(OH)_2$ on activated charcoal as catalyst yielding the unprotected warhead molecules **83** and **82** (Scheme 48).



Scheme 48: Synthesis of warheads. a) O_3, CH_2Cl_2/MeOH; triethylphosphonoacetate, THF, then KOtBu (gives compound 74); b) $NaBH_4$, THF, then DMS; DMP, CH_2Cl_2; HWE reagent, THF, then KOtBu (gives compound 75); c) H_2, $BaSO_4$, MeOH; CbzCl, MeOH, Et_3N d) NaOH 3M, dioxane/H_2O e) COMU, DMF, DIEA, then histamine dihydrochloride f) H_2, $Pd(OH)_2$/C, MeOH/H_2O.

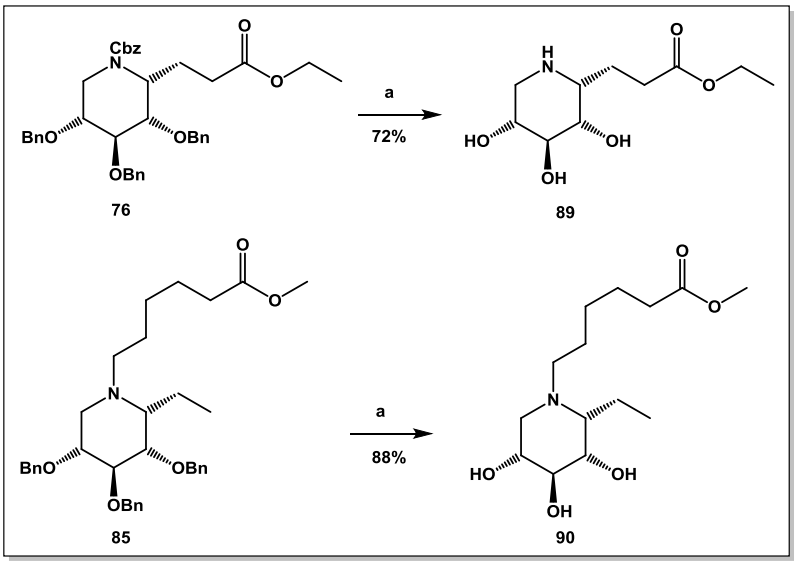
Bearing in mind that the ring nitrogen can also act as a nucleophile and cleave the reactive group of the probe, the histamine spacer part is attempted to be attached to the ring nitrogen to circumvent this problem.

Compound **38** is subjected to hydrogenolytic conditions in MeOH with Pd/$BaSO_4$ as catalyst to liberate the ring nitrogen as well as an ethyl chain attached to position C-1 (**84**). Alkylation with a formerly synthesized 6-iodohexylmethyl ester gives product **85**, which can be saponified to give compound **86**. Coupling to the histamine moiety **62** with the COMU reaction conditions described in Scheme 48, yields compound **87**. Hydrogenolytic deprotection with $Pd(OH)_2$ on activated charcoal provides the unprotected warhead **88** (Scheme 49).



Scheme 49: Synthesis of the 1-*C*-ethyl-*N*-alkylated warhead. a) 6-Iodohexylmethylester, DMF, Na_2CO_3, 60°C b) NaOH 3M, dioxane/H_2O c) COMU, DIEA, then histamine dihydrochloride d) H_2, $Pd(OH)_2$/C, MeOH/H_2O.

To evaluate the suitability of the synthesized molecules as warheads in LDPP probes, compound **76** and **85** are deprotected under hydrogenolytic conditions utilizing $Pd(OH)_2$ on activated charcoal as catalyst to give the unprotected compounds **89** and **90** (Scheme 50).



Scheme 50: Warhead precursor deprotection reactions. a) H_2, MeOH/H_2O, $Pd(OH)_2$/C.

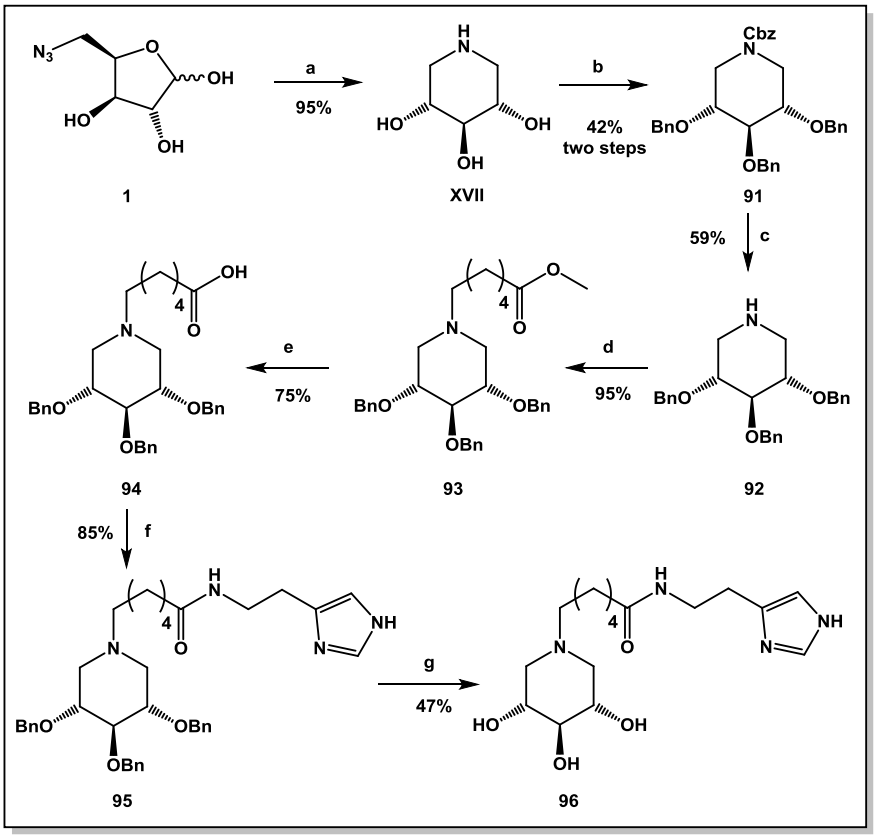
The unprotected warheads **82** and **88** as well as the deprotected compounds **89** and **90** exhibit excellent inhibition properties towards human lysosomal *β*-glucocerebrosidase, which clearly underlines the suitability of this compound class as warheads for LDPP probes .

3.6.4. Approach via reductive amination

Even though the SAW-Grignard reaction sequence offers a powerful approach towards the synthesis of warheads for LDPP probes, a synthesis via reductive amination of the corresponding azidodeoxy sugar seems more feasible, because it allows for a synthesis of probe warheads in much fewer steps.

Starting from readily available 5-azido-5-deoxy-α,β-D-xylofuranose (**1**), reductive amination can be performed under hydrogenolytic conditions with $Pd(OH)_2$ on activated charcoal to give 1,5-dideoxy-iminoxylitol (**XVII**). The ring nitrogen can be alkylated with alkyl halides such as 6-iodohexylmethylester. Due to purification problems caused by the similar polarity of the product and the obtained iodine salts in the reaction sequence, it is easier to perform a Cbz and benzyl protection to give compound **91** as well as a Cbz deprotection step to give the protected sugar compound **92** with a free ring nitrogen ready for alkylation purposes.

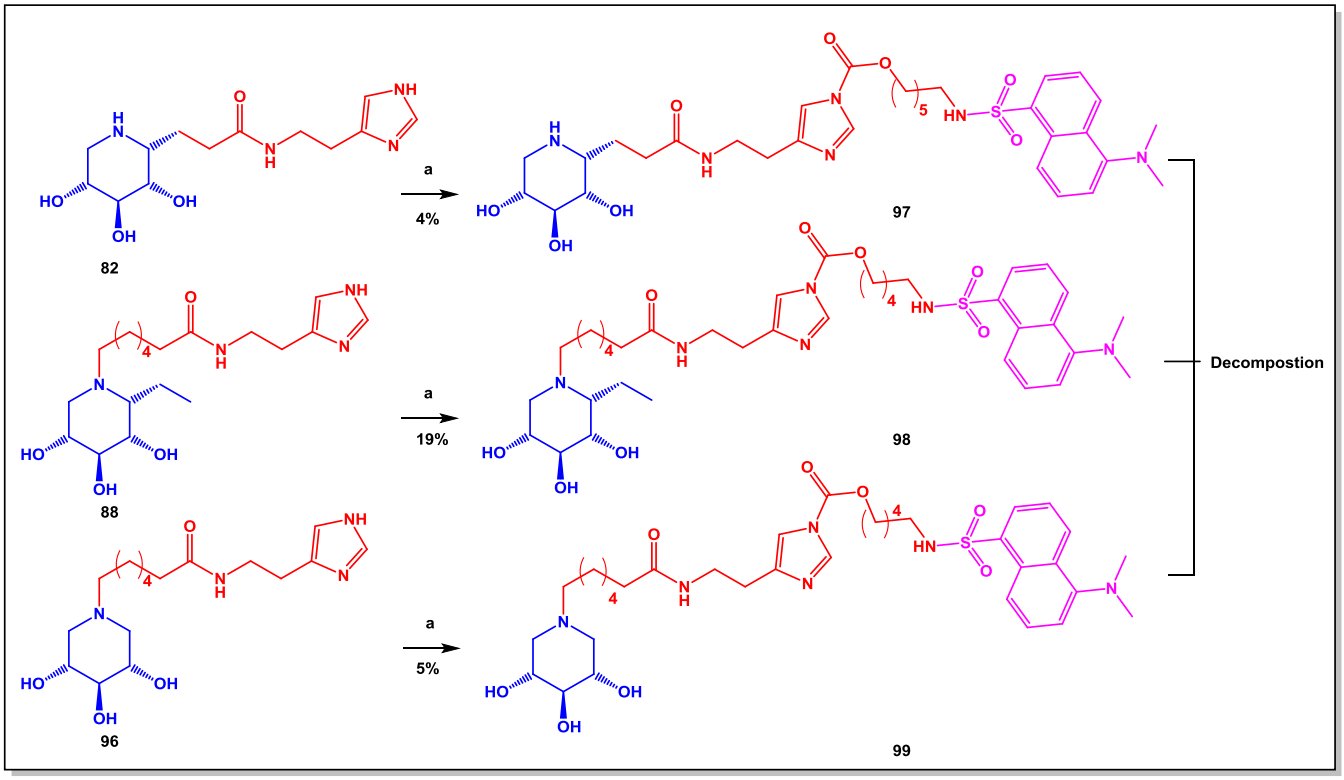
Compound **92** can be readily alkylated and obtained product **93** is saponified to give compound **94** which is subsequently coupled to the histamine moiety **62** via COMU coupling conditions as described earlier. Deprotection of product **95** under hydrogenolytic conditions with $Pd(OH)_2$ on activated charcoal gives unprotected warhead **96** (Scheme 51).



Scheme 51: Synthesis of the *N*-alkylated warhead. a) H_2, MeOH/H_2O, $Pd(OH)_2$/C b) CbzCl, MeOH, Et_3N, then NaH, DMF then BnBr c) H_2, MeOH, Pd/$BaSO_4$ d) 6-iodohexylmethylester, DMF, Na_2CO_3 e) NaOH 3M, dioxane/H_2O f) COMU, DMF, DIEA, then histamine dihydrochloride g) H_2, MeOH, H_2O, $Pd(OH)_2$/C.

With compounds **82, 88** and **96**, probe coupling reactions are performed. Indeed, TLC shows full conversion of the starting material in all cases, yielding a newly formed yellowish glowing spot observable under UV light. Silica gel chromatography, however, affords only products in low yields still containing impurities. Subsequent preparative TLC purification affords a pure product in very low yields and difficult to identify via NMR analysis. Additionally, HRMS analysis showed only signals of the starting materials employed in the synthesis.

It was reasoned that the acyl-imidazole linkage is too unstable for MALDI – TOF analysis, consequently only the histamine equipped warhead can be detected. TLCs of the obtained material also strongly suggest that the probe is cleaved by the slightly acidic character of the silica gel based TLC plate (Scheme 52).



Scheme 52: Probe coupling reactions. a) 82 or 88 or 96, DMF, DIEA, then 68.

3.7. Ligand directed tosyl chemistry probe

Hamachi *et.al.*[107] reported a tosyl chemistry based ligand directed protein profiling strategy employing a tosyl, rather than an acyl imidazole derived probe. This strategy should lead to probes, more stable in laboratory environments such as nucleophilic solvents, elevated temperatures or acid base catalyzed reactions. Therefore, a synthetic strategy is developed towards such probes employing a free amine at a protected sugar warhead, a 3-(chlorosulfonyl) benzoyl chloride as tosyl linker and an undecanol moiety as tag (Figure 38).

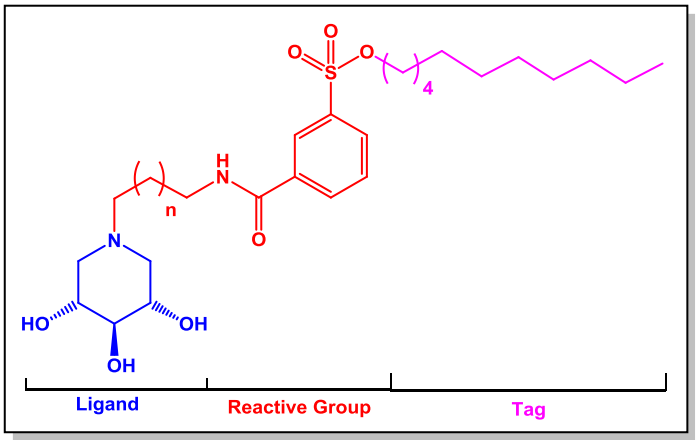
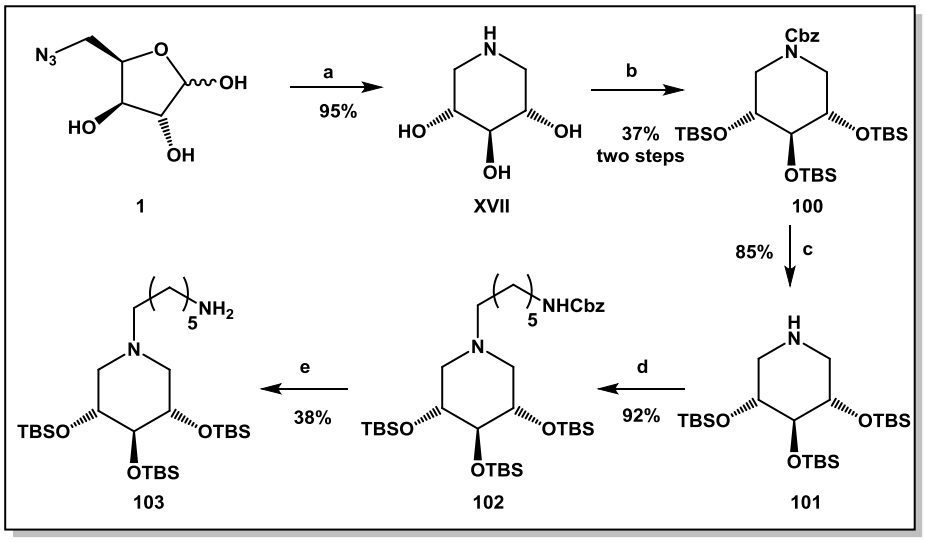


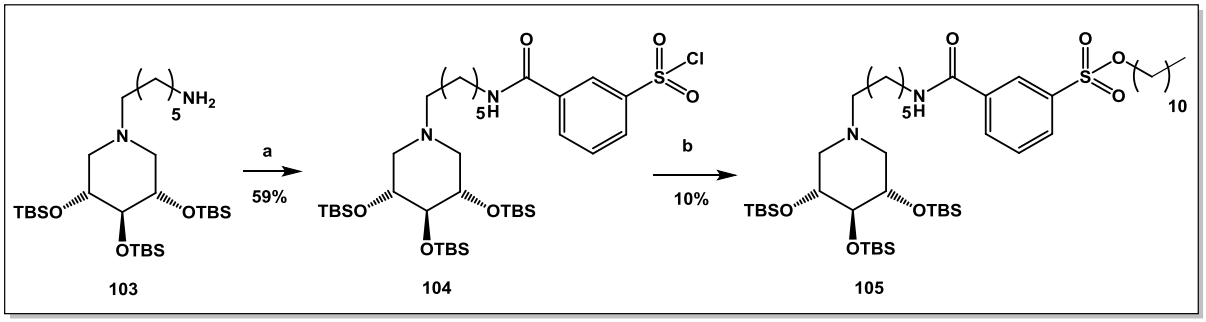
Figure 38: Tosyl chemistry based LDPP probe.[107]

5-Azido-5-deoxy-α,β-D-xylofuranose (**1**) is subjected to reductive amination conditions employing hydrogen atmosphere and $Pd(OH)_2$ on activated charcoal to give 1,5-dideoxy-1,5-iminoxylitol (**XVII**) in excellent yields. To circumvent purification problems, Cbz protection of the ring nitrogen and subsequent TBDSM protection of the remaining free hydroxyl groups to give compound **100** are conducted. Hydrogenolytic deprotection of the ring nitrogen employing $Pd/BaSO_4$ as catalyst affords compound **101** with the unprotected ring nitrogen ready for an alkylation reaction with a Cbz protected 6-iodohexylamine. Compound **102** is subjected to hydrogenolytic Cbz deprotection with $Pd/BaSO_4$ in MeOH to give the protected amine warhead **103** (Scheme 53).



Scheme 53: Synthesis oft he tosyl probe warhead. a) H_2, MeOH/H_2O, $Pd(OH)_2$/C b) CbzCl, MeOH, Et_3N then TBDMSCl, DMF, Imidazole, 60°C c) H_2, MeOH, $Pd/BaSO_4$ d) Cbz-NH-6-iodohexyl, DMF, Na_2CO_3 e) H_2, MeOH, $Pd/BaSO_4$.

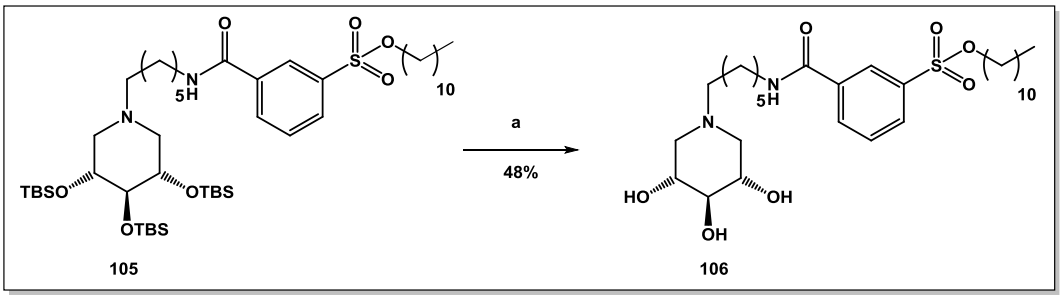
Coupling of the ligand directed tosyl probe is conducted employing 3-(chlorosulfonyl) benzoyl chloride as building block for the reactive group. Therefore, an excess of this reagent is dissolved in CH_2Cl_2 and cooled to 0°C. A reaction mixture of compound **103** with Et_3N in CH_2Cl_2, prepared in advance, is added dropwise. After consumption of the starting material, the solvent is removed under reduced pressure and excess of the reagent is removed employing a short silica gel column to obtain compound **104**. In the meantime, a solution of undecanol, Et_3N and DMAP is prepared in CH_2Cl_2 and added in 0.1 eq. steps to compound **104**. Each step is followed by TLC analysis of the reaction mixture. After consumption of compound **104**, the reaction mixture is concentrated immediately under reduced pressure. Compound **105** is obtained after column chromatography (Scheme 54).



Scheme 54: Synthesis of the tosyl derived LDPP probe. a) 3-(chlorosulfonyl) benzoyl chloride, CH_2Cl_2, Et_3N, 0°C b) undecanol, CH_2Cl_2, Et_3N, DMAP.

TBDMS deprotection of compound **105** is conducted in CH_3CN at room temperature at acidic conditions (pH 2) employing para-toluenesolfonic acid (pTSA). The reaction mixture is stirred for 48h, until the starting material is consumed. (TLC). Column chromatography affords compound **106** as a mixture of the product and pTSA. Further purification employing preparative TLC and extraction of the silica gel with MeOH affords pure compound **106**, ready for biological evaluation (Scheme 55).

Preliminary observations towards the stability of the tosyl derived LDPP probe indicate that MeOH is able to cleave the reactive site. After 72h, approx. half of compound **105** has reacted, when storing the probe in MeOH–d_4.



Scheme 55: Deprotection of compound 105. a) pTSA, CH_3CN.

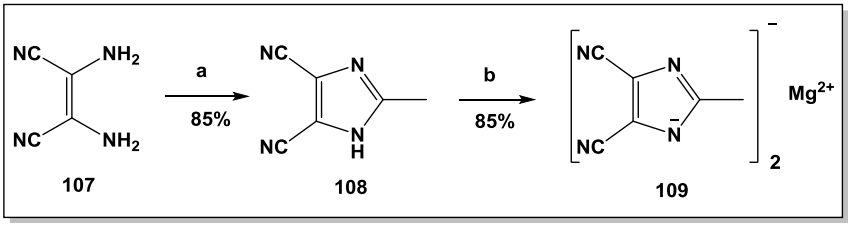
3.8. Spin off Varta micro innovation

Lithium-ion batteries are worldwide accepted as next generation battery systems. However, their tendency to form dendritical depositions causing severe fire hazards due to internal short circuits limits the use of Li-ion batteries for broad applicaton. The, in terms of electrochemical properties, similar Mg battery is considered a competitive alternative to lithium ion batteries, since Mg does not show tendencies to form dendrites.

To make use of these advantages however, several challenges have to be met, with both the electrolyte and the electrode systems. The surface passivation of Mg metal, for example, has challenged the development of electrolyte systems which require wide electrochemical windows and high compatibility with the electrode components. To this day, no application has been found for electrolytes and conductive salts with corrosive properties to crack the passivation layer, since their corrosive behavior corrodes the current collector on the positive electrode.

Our aim is to develop an electrolyte system based on heterocyclic anions with Hückel type stabilization as the conductive salt, which fulfills the requirements for secondary magnesium ion batteries.

Starting from diaminomaleonitrile **107**, the imidazole **108** is readily available from a reaction with triethylorthoacetate in acetonitrile. The corresponding Mg Hückel anion has been obtained in a reaction with magnesium-bis-(disopropylamide) $(DA)_2Mg$ to give the desired 4,5-dicyano-2-methyl-imidazole (**109**, Scheme 56).



Scheme 56: Synthesis of Hückel anion. a) Triethylorthoacetate, CH_3CN, reflux b) $(DA)_2Mg$, THF, reflux.

To prove the electrochemical suitablility and activity of compound **109**, cyclic voltammetry (CV) and constant current cycling (CCC) are conducted in 3-electrode Swagelok-cells with graphite anodes as working electrode, magnesium metal as counter and reference electrode, both polished prior to usage, and a 0.5 M solution of compound **109**/DMF as electrolyte.

The CV shows a high cathodic current in the first cycle, caused by the reduction of the electrolyte. In subsequent cycles however, the same electrochemical reactions appear, in a way that indicates a reversible magnesium intercalation and deintercalation (Figure 39).

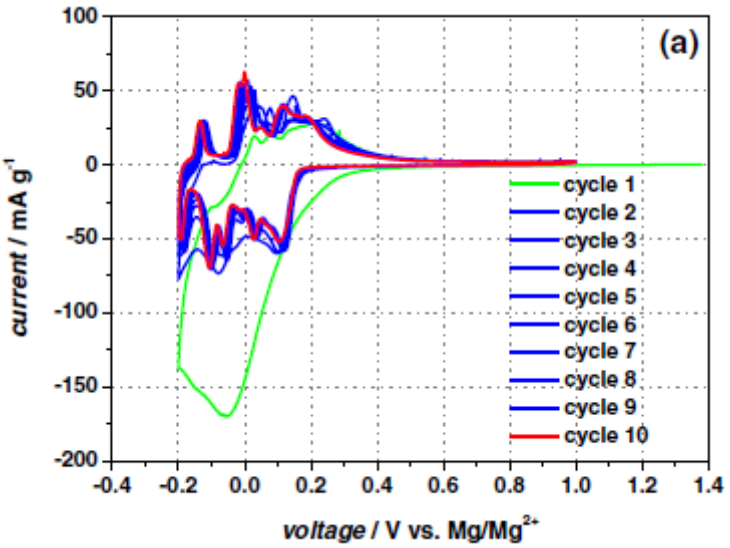


Figure 39: CV of a natural graphite anode obtained in 0.5M compound 109/DMF at a scan rate of 0.1mV*s^{-1}

Constant current cycling shows a high capacity loss in the first cycle but an increase of capacity and coulombic efficiency with subsequent cycling (Figure 40).

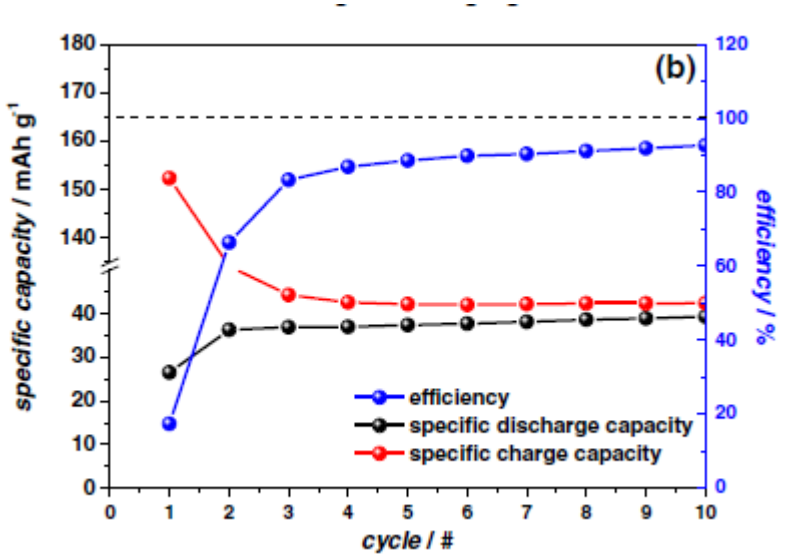


Figure 40: CCC of natural graphite in 0.5M of compound 109/DMF. C-rate is 0.1C.

Even though compound **109** seems to reveal highly reversible reduction and oxidation reactions, suggesting intercalation and deintercalation of magnesium into natural graphite, further investigations and evaluations of heterocyclic anions with Hückel type stabilization as conductive salts have to be conducted prior to their employment in secondary magnesium type batteries.

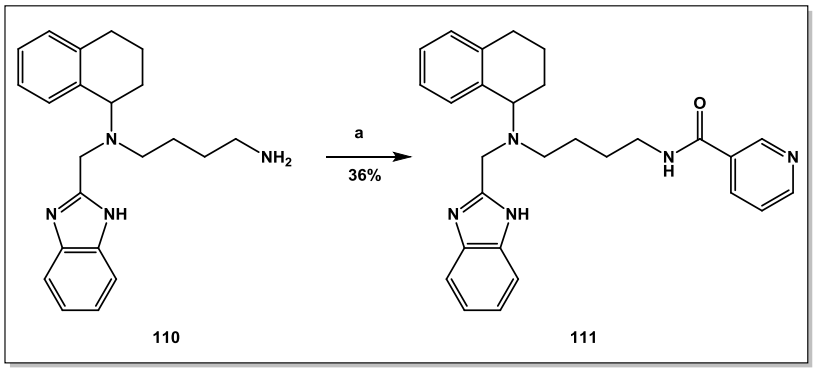
3.9. Spin off Center for Medical Research, Graz

Chemokine receptors belong to the family of 7-transmembrane receptors.[123] Three loops are in the extracellular- and three in the intracellular compartment, with all *N*-termini outside the cell surface. One intracellular loop is associated with heterotrimeric G proteins, initiating signal transduction when activated with a chemokine ligand, e.g. *CXCL12*. Of chemokines, *CXCR4* together with its ligand *CXCL12* plays a pivotal role in tumorigenesis, including proliferation, survival, migration, invasion and metastasis of more than 20 different types of cancer.

Small-molecule antagonists like commercially available AMD070 (**110**) are currently under investigation as potential therapeutic agents for lymphoma patients.[124]

To evaluate, whether inhibition of *CSCR4/CXCL12* is suitable for therapeutic intervention, we synthesized a novel AMD070 derivative for *in vitro* and *in vivo* studies on aggressive B cell lymphomas.

Commercially available AMD070 (**110**) is allowed to react with nicotinyl chloride hydrochloride under basic conditions with Et_3N to give compound **111** (Scheme 57).



Scheme 57: Synthesis of an AMD070 derivative. a) nicotinyl chloride hydrochloride, MeOH, Et_3N.

In vitro and *in vivo* studies with AMD070 and compound **111** on aggressive B cell lymphomas are currently conducted at the Center for Medical Research, Graz.

Preliminary kinetic investigation indicated that compound **111** exhibits better properties compared to commercially available compound **110** on aggressive B cell lymphomas. Based on this result further modifications will be installed at molecule **110**.

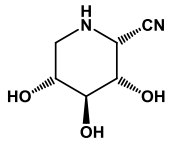
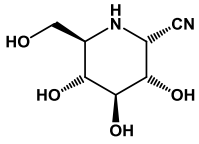
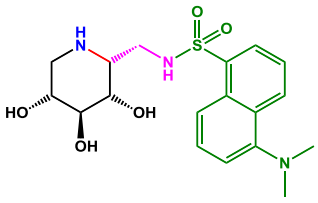
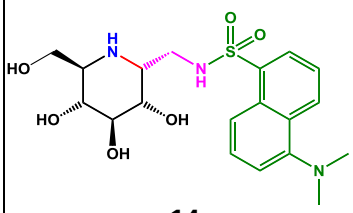
4. Biological evaluation

Assorted iminoalditol glycomimetics of this PhD thesis have been subjected to biological evaluation as inhibitors of selected glycoside hydrolases. These evaluations are performed in Prof. Withers' laboratories in Vancouver, Canada.

4.1. Preliminary studies

The kinetic evaluation of the compounds obtained in an earlier work[119] against β-glucosidase from *Agrobacterium* sp. (Abg) and human lysosomal β-glucocerebrosidase (GCase) is summarized in Table 4.

Table 4: Inhibition constants (K_i, µM) of compounds 2, 4, 13 and 14 with lysosomal *β*-glucocerebrosidase (GCase) and *β*-glucosidase *Agrobacterium* sp. (Abg).

Enzyme	Compound			
	 **2**	 **4**	 **13**	 **14**
Abg	220	172	36	91
GCase	34	716	0.0075	178

The *D-xylo* configurated compounds **2** and **13** show excellent inhibition properties for human GCase with K_i values of 34 µM and 7.5 nM, respectively. In contrast, the corresponding *D-gluco* configurated compounds **4** and **14** show modest inhibition with K_i values of 716 µM and 178 µM, respectively. In both cases the lipophilic dansyl group attached to position C-2 contributes greatly to the elevated affinity of compound **13** and compound **14**.[115]

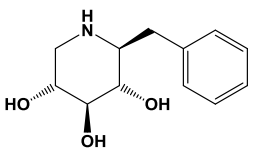
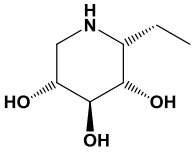
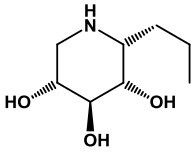
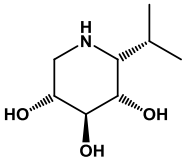
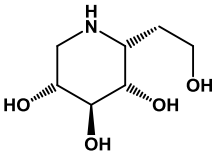
The leap of inhibition potency of compound **2** and **13** in comparison with compound **4** and **14**, were already observed by Martin *et.al.*[31] and can be explained by a

piperidine ring inversion from the classic $^4C_1$ conformation to a $^1C_4$ conformation in the *D-xylo* configurated compounds.

4.2. Grignard reagents as nucleophiles

Assorted 1-C-alkyl iminoxylitol glycomimetics were evaluated as inhibitors of selected glycoside hydrolases (Table 5).

Table 5: K_i values in µM of compounds 32-35 and 40 with Abg = *β*-glucosidase/*β*-galactosidase from *Agrobacterium* sp.; *β*-gal E.*coli* = lac Z *β* galactosidase from E.c*oli*; Fabrazyme = commercial recombinant human lysosomal *α* galactosidase; *α* glc = *α*-glucosidase from S. cerevisa; GCase = human *β*-glucocerebrosidase Gaucher; N.I. no inhibition or weak inhibition with estimated K_i values higher than 1mM.

Enzyme	Compound				
	 **32**	 **33**	 **34**	 **35**	 **40**
Abg	46.6	27.2	10.9	131.3	30.2
β-Gal (E.coli)	N.I.	N.I.	N.I.	N.I.	N.I.
Fabra-zyme	N.I.	N.I.	N.I.	N.I.	N.I.
α glc	N.I.	N.I.	N.I.	N.I.	N.I.
GCase	5.9	2.3	1.3	12.4	26.7

All compounds show a high inhibition potency with K_i values in the micromolar range against ß-glucosidases and no inhibition at all against α-glucosidase from s. *cerevisa*, ß-galactosidase from E. *coli* or the human lysosomal α galactosidase (Fabrazyme). In consistency with reports of Martin *et.al.*[31] all 1-*C*-aklyl iminoxylitol derivatives proved to be more active against the human β-glucocerebrosidase compared to the bacterial β-glucosidase from *Agrobacterium* sp. (Abg). This can be explained by the lipophilic character of the alkyl substituent, causing it to fit well into the lipophilic entrance of the active site of the enzyme. Compound **32**, with the alkyl substituent in

α-configuration at the pseudo anomeric position shows weaker inhibitions than the corresponding *C*-1-β-configured compounds.

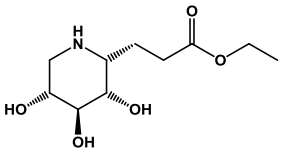
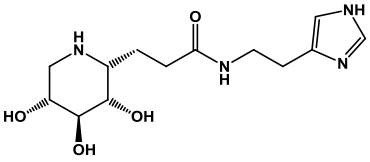
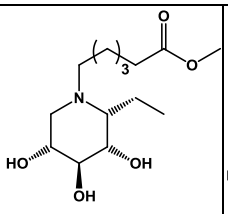
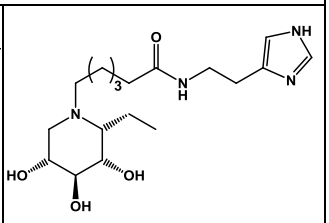
The propyl chain in compound **34** exhibits higher inhibitory activity than its ethyl chain counterpart compound **33**. This is consistent with the observation that enhanced lipophilicity of substituents at position C-1 leads to an increased inhibition of the human enzyme. As expected, the hydrophilic terminal hydroxyl group of compound **40** shows weaker interaction with the human GCase.

This new compound class shows excellent selectivity, since none of the other evaluated glycoside hydrolases show K_i-values below 1mM.[125]

4.3. Probe precursors

Selected samples of probe precursor molecules were biologically evaluated to prove their suitability as highly selective and powerful reversible, competitive inhibitors for selected glycoside hydrolases (Table 6).

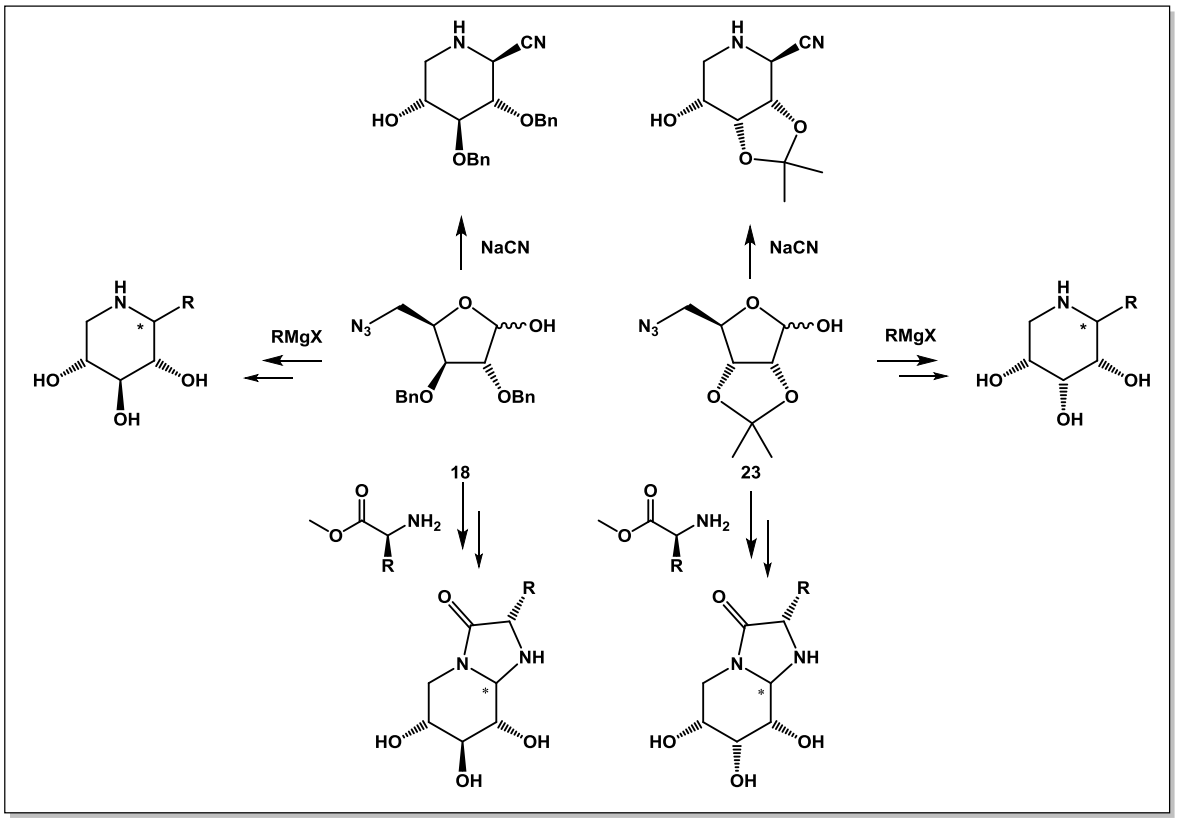
Table 6: Ki-values in µM of selected probe precursors with Abg = *ß*-glucosidase/*ß*-galactosidase from *Agrobacterium* sp.; β-gal E.*coli* = lac Z *β*-galactosidase from E.*coli*; Fabrazyme = commercial recombinant human lysosomal *α*-galactosidase; *α* glc = *α* glucosidase from S. cerevisa; GCase = human *β*-glucocerebrosidase Gaucher; N.I. no inhibition or weak inhibition with estimated Ki values higher than 1mM.

Enzyme	Compound			
	 **89**	 **82**	 **90**	 **88**
Abg	2.1	1.6	N.I.	N.I.
β-Gal (E.coli)	N.I.	N.I.	N.I.	N.I.
Fabrazyme	N.I.	N.I.	N.I.	N.I.
α glc	N.I.	N.I.	N.I.	N.I.
GCase	5.1	1.1	57	4.1

All compounds show excellent inhibition of β-glucosidases in the low µM range. All other glycoside hydrolases were not inhibited by the molecules, establishing that the synthesized molecules are highly selective and therefore suitable warheads for the proposed LDPP probes.

5. Conclusion and Outlook

Starting from readily available 5-azido-5-deoxy-2,3-di-*O*-benzyl-α,β-D-xylofuranose (**18**) or 5-azido-5-deoxy-2,3-*O*-isopropylidene-α,β-D-ribofuranose (**23**), several 1-C-elongated iminosugar-based inhibitors were synthesized with the Staudinger/aza-Wittig nucleophile reaction cascade. NaCN or Grignard reagents, as well as amino acids were employed as nucleophiles. The configuration of the newly formed stereocenter as well as the conformation of the piperidine ring was carefully determined via NMR spectroscopy and X-ray analysis (Scheme 58).

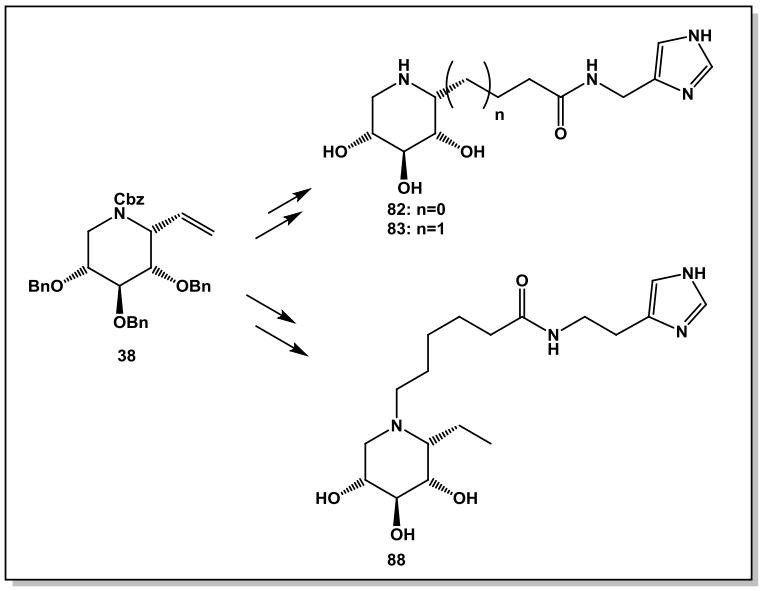


Scheme 58: SAW-nucleophile reaction cascade towards the synthesis of 1-C-elongated iminosugar based inhibitors.

A selected assortment of deprotected compounds was biologically evaluated with regard to their inhibition properties against several glycoside hydrolases. All compounds were found to exhibit excellent inhibitory properties as well as selectivity with human lysosomal β-glucocerebrosidase, the enzyme responsible for Gaucher disease.

These results clearly underline the suitability of this compound class as pharmacological chaperones for the treatment of Gaucher disease.

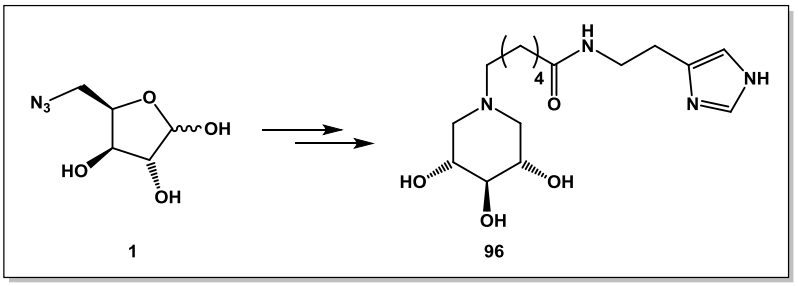
Furthermore, numerous synthetic routes towards acyl imidazole derived ligand directed protein profiling glycoprobes were established. Starting from compound **38**, which is readily available from the SAW-Grignard reaction cascade, follow up chemistry can be conducted either at position C-1 or the ring nitrogen of the iminosugar to give the deprotected iminosugar warheads **82**, **83** and **88** (Scheme 59).



Scheme 59: Synthetic routes towards acyl imidazole derived LDPP probes following SAW-Grignard follow-up chemistry.

Biological evaluation of selected, unprotected precursor molecules indicated high inhibitory properties with excellent selectivity against GCase. This clearly underlines the suitability of these molecules as warheads for ligand directed protein profiling glycoprobes.

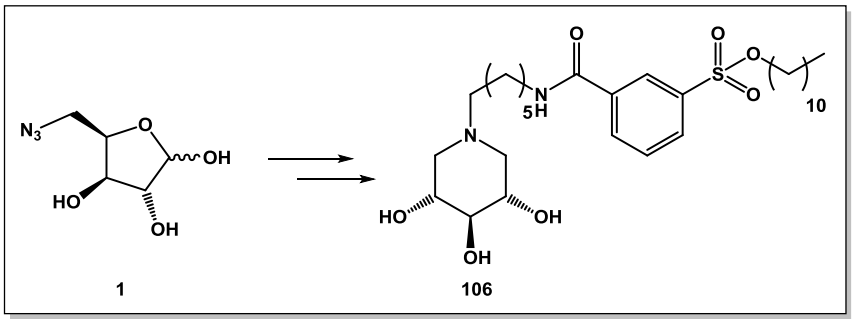
An alternative to the reaction cascade described above, reductive amination of 5-azido-5-deoxy-α,β-D-xylose (**1**) gave access to 1,5-dideoxy-1,5-iminoxylitol (**XVII**), which could also be alkylated at the ring nitrogen to give, after a few synthetic steps, an unprotected iminosugar warhead **96** for acyl imidazole derived LDPPs (Scheme 60).



Scheme 60: Synthesis of compound 96.

The coupling of the acyl imidazole moiety was successful, however decomposition of the molecule occurred.

The synthesis of a tosyl chemistry derived LDPP was established in a few steps, starting from 5-azio-5-deoxy-α,β-D-xylofuranose (**1**) as substrate and 3-(chlorosulfonyl) benzoyl chloride as reactive group (Scheme 61).



Scheme 61: Synthesis of a tosyl chemistry derived LDPP probe.

To ensure stability of the tosyl chemistry derived probe **106**, it has to be monitored carefully. The biological evaluation towards ligand directed protein profiling has yet to be conducted. Preliminary observations indicate that MeOH is able to cleave the reactive site of the probe over a period of 72h.

This synthetic approach gives access to a broad range of different probes, employing different configurations on the warhead, spacer arms designed for specific purposes and different tags for the identification of the labeled enzyme.

6. Experimental

6.1. General methods

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

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

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

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

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

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

NMR-Abbreviations:

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

6.2. General procedures

General procedure A – SAW – Grignard reaction in THF

PMe_3 (1M in toluene, 2 eq) was added to a solution of the respective azidodeoxyaldose in anhydrous THF under nitrogen atmosphere. Upon consumption of the starting material (detected on TLC, eluent: CH/EtOAc = 1/1, v/v) and formation of the iminium ion intermediate (detected on TLC, eluent: EtOAc/MeOH = 10/1, v/v) the Grignard reagent (5 eq) was added at 0°C. Upon consumption of the iminium ion intermediate the reaction was quenched with satd. NH_4Cl solution. The aqueous layer was extracted with EtOAc three times, the combined organic phases were dried over Na_2SO_4, filtered and concentrated *in vacuo* followed by purification utilizing silica gel chromatography employing eluents as indicated.

General procedure B – SAW – Grignard reaction with solvent exchange

PMe_3 (1M in toluene, 2 eq) was added to a solution of the respective azidodeoxyaldose in MeOH under nitrogen atmosphere. Upon consumption of the starting material (detected by TLC, eluent: CH/EtOAc = 1/1, v/v) and formation of the iminium ion intermediate (detected by TLC, eluent: EtOAc/MeOH = 10/1, v/v) the reaction mixture was concentrated under reduced pressure and the residue was coevaporated twice with toluene. The product was collected in anhydrous THF and the respective Grignard reagent (5 eq) was added at 0°C under nitrogen atmosphere. The reaction mixture was then treated as described in general procedure A.

General procedure C – Deprotection of benzylated products

The protected sugar compound was dissolved in MeOH/H_2O (1/1, v/v), $Pd(OH)_2/C$ was added and the reaction mixture was stirred under hydrogen atmosphere at ambient pressure. Upon consumption of the starting material (detected by TLC, eluent: EtOAc/MeOH = 10/1, v/v) the reaction mixture was filtered, concentrated under reduced pressure and the desired compound purified utilizing silica gel chromatography employing eluents as indicated.

General procedure D – Deprotection of isopropylidene protected products

The protected sugar compound was dissolved in H_2O to give a 10% solution. HCl conc. was added until pH 2 was reached and the reaction mixture was stirred until the starting material was consumed (detected by TLC, eluent: EtOAc/MeOH = 10/1, v/v). The reaction mixture was concentrated under reduced pressure and purified utilizing silica gel chromatography employing eluents as indicated.

General procedure E – Cbz protection

The corresponding starting material was dissolved in MeOH to give a 10% solution. Et_3N (2.2 eq) and benzyl chloroformiate (1.1 eq) were added at 0°C and the reaction mixture was allowed to warm to room temperature. Upon consumption of the starting material (detected by TLC employing eluents as indicated) the reaction mixture was concentrated under reduced pressure, diluted in CH_2Cl_2 and extracted with 2N HCl and satd $NaHCO_3$ solution. The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The obtained oil was purified utilizing silica gel chromatography employing eluents as indicated.

General procedure F – Bn protection

The corresponding starting material was dissolved in DMF to give a 10% solution. Sodium hydride (2.2 eq per OH-group) was added and the reaction mixture was stirred for 30 minutes at 0°C. Benzyl bromide (1.2eq per OH-group) was added and the reaction mixture was allowed to reach room temperature. Upon consumption of the starting material, the reaction mixture was quenched with MeOH and stirred for 30 minutes .The reaction mixture was then treated as described in general procedure E.

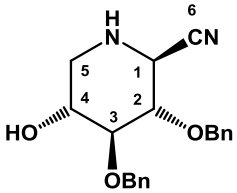
General procedure G – SAW amino acid reaction

PMe_3 (1M in toluene, 2 eq) was added to a solution of the respective azidodeoxyaldose in MeOH under nitrogen atmosphere. Upon consumption of the starting material (detected by TLC, eluent: CH/EtOAc = 1/1, v/v) and formation of the iminium ion intermediate (detected by TLC, eluent: EtOAc/MeOH = 10/1, v/v) the corresponding amino acid methyl ester hydrochloride, treated with strongly basic Merck III ion exchange residue in MeOH (5 mL) was added to the reaction mixture. Upon consumption of the iminium ion intermediate the reaction mixture was concentrated under reduced pressure and the obtained oil was purified utilizing silica gel chromatography employing the eluents as indicated.

6.3. Sodium cyanide as nucleophile

1(R)-1-C-Cyano-2,3-di-O-benzyl-1,5-dideoxy-1,5-imino-xylitol (24)

5-Azido-5-deoxy-2,3-di-*O*-benzyl-α,β-D-xylofuranose (**18,** 200 mg, 0.56 mmol, 1 eq) was dissolved in MeOH (20 mL) and treated with PMe_3 (1M in toluene, 1 mL, 1 mmol, 2 eq). After consumption of the starting material, sodium nitrile (140 mg, 2.81 mmol, 5 eq.) was added to the reaction mixture. After TLC (EtOAc/MeOH = 10/1, v/v) indicated the consumption of the iminium ion intermediate, the reaction mixture was concentrated under reduced pressure, taken up in EtOAc and extracted with satd. $NaHCO_3$ solution. After drying the organic phase over Na_2SO_4 and concentrating the reaction mixture under reduced pressure, the obtained yellow oil was purified, employing silica gel chromatography (CH/EtOAc = 1/1, v/v) and compound **24** was isolated with a yield of 26% containing major impurities.



Product **24**

MS: Calcd. for $[C_{20}H_{22}N_2O_3Na]$: m/z 361.1528

MS: Found: -

TLC: R_f = 0.25

(CH/EtOAc = 1/5, v/v)

CAM

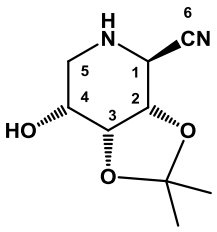
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.33-7.18 (m, 10H, Ph), 4.84-4.59 (m, 4H, C*H*$_2$Ph), 4.03 (d, 1H, $J_{1,2}$ 1.74 Hz, H-1), 3.54-3.48 (m, 3H, H-2, H-3, H-4), 3.05 (dd, 1H, $J_{5e,4}$ 3.27 Hz, $J_{5e,5a}$ 12.1 Hz, H-5e), 5.56 (dd, 1H, H-5a);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 138.2, 137.1 (C_q), 128.8-128.1 (Ph), 117.7 (C-6), 81.4 (C-2), 77.6 (C-3), 75.1 (*C*H_2Ph), 73.5 (*C*H_2Ph), 69.3 (C-4), 49.9 (C-1), 46.8 (C-5);

1(R)-1-C-Cyano-2,3-O-isopropylidene-1,5-dideoxy-1,5-iminoribitol (25)

5-Azido-5-deoxy-2,3-*O*-isopropylidene-α,β-D-ribofuranose (**23**, 200 mg, 0.93 mmol) was dissolved in MeOH (5 mL) and treated with PMe_3 (1M in toluene, 2 mL, 2 mmol, 2 eq). After consumption of the starting material, sodium nitrile (200 mg, 4.08 mmol, 4 eq.) was added to the reaction mixture. After TLC (EtOAc/MeOH = 10/1, v/v) indicated the consumption of the iminium ion intermediate, the reaction mixture was concentrated under reduced pressure. The obtained yellow oil was purified, employing silica gel chromatography (EtOAc) and compound **25** was isolated with a yield of 27% containing major impurities.



Product **25**

MS: Calcd. for [$C_9H_{14}N_2O_3Na$]: m/z 221.0902

MS: Found: -

TLC: R_f = 0.55

(EtOAc/MeOH = 10/1, v/v)

CAM

$[a]_D^{20}$ = -

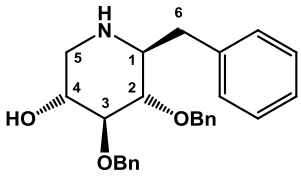
^{1}H NMR (300 MHz, MeOH-*d*$_4$) δ = 4.39 (dd, 1H, $J_{3,4}$ 4.64 Hz, H-3), 4.12 (dd, 1H, $J_{2,3}$ 4.50 Hz, $J_{1,2}$ 8.68 Hz, H-2), 3.78 (ddd, 1H, H-4), 3.49 (d, 1H, H-1), 2.81 (dd, 1H, $J_{5e,4}$ 5.58 Hz, $J_{5e,5a}$ 12.2 Hz, H-5e), 2.56 dd, 1H, $J_{5a,4}$ 11.0 Hz, H-5a), 1.46 (s, 3H, C*H*$_3$), 1.31 (s, 3H, C*H*$_3$);

^{13}C NMR (75.5 MHz, MeOH-*d*$_4$) δ = 120.4 (C-6), 111.7 (C_q), 76.9 (C-3), 76.8 (C-2), 67.1 (C-4), 51.0 (C-1), 47.1 (C-5), 28.4 (*C*H_3), 26.3 (*C*H_3);

6.4. Grignard reagents as nucleophiles

1(S)-1-C-Benzyl-2,3-di-O-benzyl-1,5-dideoxy-1,5-imino-xylitol (3,4-di-O-benzyl-1,2,6-trideoxy-1,5-imino-6-C-phenyl-L-glucitol, 26)

Following general procedure B, compound **18** (100 mg, 0.28 mmol, 1 eq) was dissolved in MeOH (5 mL) and treated with PMe_3 (1M in toluene, 560 µL, 0.56 mmol, 2 eq). After removal of MeOH *in vacuo* the cyclic iminium ion intermediate was dissolved in THF (5 mL) and benzylmagnesium bromide (2M in THF, 700 µL, 1.40 mmol, 5 eq) was added. Silica gel chromatography (EtOAc/MeOH = 10/1, v/v) gave compound **26** (97 mg) in a yield of 85 % as colourless oil. Recrystallization with MeOH/EtOAc (v/v 1/1) afforded compound **26** as colorless crystals.



Product **26**

MS: Calcd. for $[C_{26}H_{29}NO_3Na]$: m/z 426.2045

MS: Found: m/z 426.2044

TLC: R_f = 0.63

(EtOAc/MeOH = 10/1, v/v)

CAM

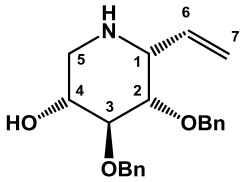
$[\alpha]_D^{20}$ = -39.0 (c 1.00, $CHCl_3$)

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.44-7.18 (m, 15H, Ph), 5.03 (d, 1H, CH_2Ph), 4.94 (d, 1H, CH_2Ph), 4.82 (d, 1H, CH_2Ph), 4.70 (d, 1H, CH_2Ph), 3.69 (ddd, 1H, $J_{4,5a}$ 9.8 Hz, H-4), 3.44 (dd, 1H, $J_{3,4}$ 8.3 Hz, $J_{2,3}$ 8.8 Hz, H-3) 3.25 (d, 1H, H-6), 3.21 (d, 1H, H-2), 3.02 (dd, 1H, $J_{5a,5e}$ 11.9 Hz, $J_{4,5e}$ 5.1 Hz, H-5e), 2.80 (ddd, 1H, $J_{1,2}$ 9.7 Hz, H-1), 2.44 (d, 1H, H-6), 2.38 (dd, 1H, $J_{4,5a}$ 10.6 Hz, H-5a);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 140.4, 139.8, 139.7 (3C, Ph), 130.3–127.7 (Ph), 89.1 (C-3), 84.0 (C-2), 76.4-76.2 (2C, CH_2Ph), 73.0 (C-4), 62.7 (C-1), 51.5 (C-5), 39.3 (C-6);

1(S)-1-C-Vinyl-2,3-di-O-benzyl-1,5-dideoxy-1,5-imino-xylitol (3,4-di-O-benzyl-1,2,6-trideoxy-1,5-imino-6-C-vinyl-D-iditol, 28)

Following general procedure B, compound **18** (200 mg, 0.70 mmol, 1 eq) was dissolved in MeOH (10 mL) and treated with PMe_3 (1M in toluene, 1.40 mL, 1.40 mmol, 2 eq). After removal of MeOH *in vacuo* the cyclic iminium ion intermediate was dissolved in THF (10 mL) and vinylmagnesium bromide (1M in THF, 3.50 mL, 3.50 mmol, 5 eq) was added. Silica gel chromatography (EtOAc/MeOH = 30/1, v/v) gave compound **28** (115 mg) in a yield of 60 % as colourless oil.



Product **28**

MS: Calcd. for $[C_{21}H_{25}NO_3Na]$: m/z 362.1732

MS: Found: m/z 362.1767

TLC: R_f = 0.2

(EtOAc/MeOH = 10/1, v/v),

CAM

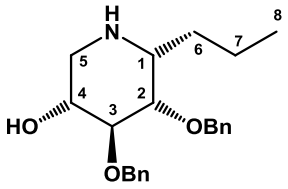
$[\alpha]_D^{20}$ = -36.9 (c 1.00, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4) (pH = 1) δ = 7.42-7.23 (m, 10H, Ph), 6.03 (ddd, 1H, H-6), 5.59-5.30 (m, 2H, H-7), 4.72 (d, 1H, CH_2Ph), 4.68 (d, 1H, CH_2Ph), 4.62 (d, 1H, CH_2Ph), 4.52 (d, 1H CH_2Ph), 4.10 (bs, 1H, H-4), 4.04 (d, 1H, $J_{1,2}$ 6.7 Hz, H-1, 3.95 (dd, 1H, $J_{3,4}$ 4.0 Hz, $J_{2,3}$ 3.8 Hz, H-3), 3.77 (bs, 1H, H-2), 3.43 (dd, 1H, $J_{5a,5e}$ 13.6 Hz, H-5e), 3.29 (dd, 1H, H-5a);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 139.1, 138.5 (2C, Ph), 131.9 (C-6), 129.7-129.27 (Ph), 122.7 (C-7), 759 (C-2), 73.9 (2C, CH_2Ph), 72.9 (C-3), 65.3 (C-4), 58.8 (C-1), 47.6 (C-5);

1(R)-1-C-Propyl-2,3-di-O-benzyl-1,5,dideoxy-1,5-imino-xylitol (3,4-di-O-benzyl-1,2,6-trideoxy-1,5-imino-6-C-propyl-D-iditol, 29)

Following general procedure B, compound **18** (100 mg, 0.28 mmol, 1 eq) was dissolved in MeOH (5 mL) and treated with PMe_3 (1M in toluene, 560 µL, 0.56 mmol, 2 eq). After removal of MeOH *in vacuo* the cyclic iminium ion intermediate was dissolved in THF (5 mL) and propylmagnesium bromide (2M in THF, 700 µL, 1.40 mmol, 5 eq) was added. Silica gel chromatography (EtOAc/MeOH = 15/1, v/v) gave compound **29** (48 mg) in a yield of 48 % as colourless oil.



Product **29**

MS: Calcd. for $[C_{22}H_{29}NO_3Na]$: m/z 378.2045

MS: Found: m/z 378.2044

TLC: R_f = 0.15

(EtOAc/MeOH = 10/1, v/v)

CAM

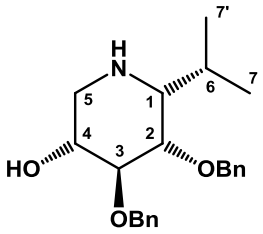
$[\alpha]_D^{20}$ = +4.1 (c 1.00, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4): δ = 7.39-7.29 (m, 10H, Ph), 4.71 (d, 2H, CH_2Ph), 4.66 (d, 2H, CH_2Ph), 4.62 (d, 2H, CH_2Ph), 4.46 (d, 2H, CH_2Ph), 3.76 (dd, 1H, $J_{2,3}$ 4.3 Hz, $J_{3,4}$ 4.5 Hz, H-3) 3.67 (m, 1H, H-4), 3.45 (dd, 1H, $J_{1,2}$ 3.1 Hz, H-2), 3.02 (dd, 1H, $J_{4,5e}$ 3.1 Hz, $J_{5a,5e}$ 13.6 Hz, H-5e), 2.94 (ddd, 1H, H-1), 2.82 (dd, 1H, $J_{4,5a}$ 4.0 Hz, H-5a) 1.60-1.40 (m, 2H, H-6), 1.37-1.11 (m, 2H, H-7), 0.90 (t, 3H, H-8);

^{13}C NMR (75.5 MHz, MeOH-d_4): δ = 140.0, 139.3 (2C, Ph), 129.6-128.9 (Ph), 77.4 (C-2), 76.4 (C-3), 74.1, 73.5 (2C, CH_2Ph), 69.1 (C-4), 55.2 (C-1), 48.0 (C-5), 32.9 (C-6), 20.2 (C-7), 14.6 (C-8);

1(R)-1-C-Isopropyl-2,3-di-O-benzyl-1,5-dideoxy-1,5-imino-xylitol (3,4-di-O-benzyl-1,2,6-trideoxy-1,5-imino-6-C-isopropyl-L-iditol, 30)

Following general procedure B, compound **18** (100 mg, 0.28 mmol, 1 eq) was dissolved in MeOH (5 mL) and treated with PMe_3 (1M in toluene, 560 µL, 0.56 mmol, 2 eq). After removal of MeOH *in vacuo* the cyclic iminium ion intermediate was dissolved in THF (5 mL) and isopropylmagnesium bromide (2M in THF, 700 µL, 1.40 mmol, 5 eq) was added. Silica gel chromatography (EtOAc/MeOH = 30/1, v/v) gave compound **30** (57 mg) in a yield of 57 % as colourless oil.



Product **30**

MS: Calcd. for $[C_{22}H_{29}NO_3Na]$: m/z 378.2045

MS: Found: m/z 378.2042

TLC: Rf = 0.32

(EtOAc/MeOH = 10/1, v/v),

CAM

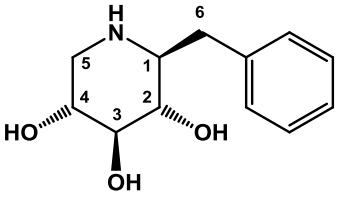
$[\alpha]_D^{20}$ = +3.5 (c 0.80, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.38-7.33 (m, 10H, Ph), 4.69 (d, 2H, CH_2Ph), 4.59 (d, 1H, CH_2Ph), 4.41 (d, 1H, CH_2Ph), 3.90 (bs, 1H, H-3), 3.70 (bs, 1H, H-4), 3.60 (bs, 1H, H-2), 3.07 (dd, 1H, $J_{4,5e}$ 3.07 Hz, $J_{5a,5e}$ 13.7 Hz, H-5e), 2.93 (dd, 1H, H-5a), 2.50 (bd, 1H, H-1), 1.87-1.76 (m, 1H, 6), 1.00, 0.79 (2xd, 6H, H-7, H-7');

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 139.8, 139.1 (2C, Ph), 129.7-128.8 (Ph) 75.5 (C-2), 73.9 (C-3), 73.4, 73.2 (2C, CH_2Ph), 67.7 (C-4), 61.4 (C-1), 49.2 (C-5), 29.9 (C-6), 20.5, 19.9 (2C, C-7, C-7');

(1S)-1-C-Benzyl-1,5-dideoxy-1,5-imino-xylitol (1,2,6-trideoxy-1,5-imino-6-C-phenyl-L-glucitol, 32)

Following general procedure C, $Pd(OH)_2$/C was added to a 10% solution of compound **26** (76 mg, 0.19 mmol, 1 eq) in MeOH/H_2O and stirred under a hydrogen atmosphere at ambient pressure. Purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 6/1/0.01, v/v/v) gave compound **32** (40 mg) in a yield of 95 % as colourless oil.



Product **32**

MS: Calcd. for [$C_{12}H_{17}NO_3Na$]: m/z 246.1106

MS: Found: m/z 246.1106

TLC: Rf = 0.44

($CHCl_3$/MeOH/concd NH_4OH = 3/1/0.01, v/v/v), CAM

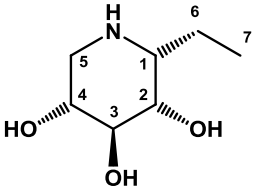
$[a]_D^{20}$ = -37.3 (c 1.00, MeOH)

^{1}H NMR (300 MHz, D_2O) δ = 7.39-7.24 (m, 5H, Ph) 3.70–3.61 (m, 1H, $J_{4,\ 5e}$ 5.0 Hz, $J_{4,4}$ 9.6 Hz, H-4), 3.48-3.37 (m, 3H, $J_{1,2}$ 7.4 Hz, $J_{2,3}$ 9.0 Hz, H-2, H-3, H-6), 3.16 (dd, 1H, $J_{4,5e}$ 4.9 Hz, $J_{5a,5e}$ 12.5 Hz, H-5e), 3.10-3.07 (m, 1H, H-1), 2.66 (dd, 1H, H-6), 2.57 (dd, 1H, $J_{4,5a}$ 11.8 Hz, H-5a);

^{13}C NMR (75.5 MHz, D_2O) δ = 134.6, 129.6, 129.3, 127.9 (6C, Ph), 76.1 (C-3), 71.3 (C-2), 66.9 (C-4), 60.3 (C-1), 46.3 (C-5), 35.2 (C-6);

1(R)-1-C-Ethyl-1,5-dideoxy-1,5-imino-xylitol (1,2,6-trideoxy-1,5-imino-6-C-ethyl-D-iditol, 33)

Following general procedure C, $Pd(OH)_2$/C was added to a 10% solution of compound **28** (50 mg, 0.15 mmol, 1 eq) in MeOH/H_2O (1/1, v/v) and stirred under a hydrogen atmosphere. Purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 2/1/0.25, v/v/v) gave compound **33** (20 mg) in a yield of 84 % as colourless oil.



Product **33**

MS: Calcd. for [$C_7H_{15}NO_3Na$]: m/z 184.0950

MS: Found: m/z 184.0949

TLC: Rf = 0.25

($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v), CAM

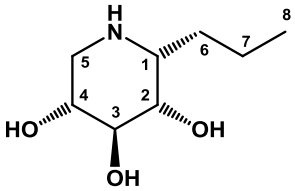
$[a]_D^{20}$ = -5.2 (c 0.66, H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 3.67-3.56 (m, 3H, H-2, H-3, H-4), 3.00 (dd, 1H, $J_{4,5e}$ 2.6 Hz, H-5a), 2.94 (dd, 1H, $J_{1,2}$ 2.3 Hz, H-1), 2.83 (dd, 1H, $J_{4,5a}$ 3.5 Hz, $J_{5a,5e}$ 13.5 Hz, H-5a), 1.50-1.29 (m, 2H, H-6), 0.67 (t, 3H, H-7);

^{13}C NMR (75.5 MHz, D_2O) δ = 68.3, 67.1 (3C, C-2, C-3, C-4), 56.5 (C-1), 44.9 (C-5) 20.4 (C-6), 9.0 (C-7);

1(R)1-C-Propyl-1,5,dideoxy-1,5-imino-xylitol (1,2,6-trideoxy-1,5-imino-6-C-propyl-D-iditol, 34)

Following general procedure C, $Pd(OH)_2/C$ was added to a 10% solution of compound **29** (60 mg, 1.70 mmol, 1 eq) in MeOH/H_2O (1/1, v/v) and stirred under a hydrogen atmosphere. Purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 3/1/0.25, v/v/v) gave compound **34** (24 mg) in a yield of 81 % as colourless oil. Recrystallization with HCl in MeOH/EtOAc (1/1, v/v) afforded the corresponding hydrochloride (**34**·HCl) as colorless crystals.



Product **34**

MS: Calcd. for [$C_8H_{17}NO_3Na$]: m/z 198.1106

MS: Found: m/z 198.1102

TLC: Rf = 0.5

($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v), CAM

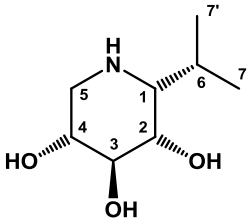
$[a]_D^{20}$ = +12.4 (c 0.40, H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 3.67-3.30 (m, 3H, H-2, H-3, H-4), 2.85-2.77 (ddd, 1H, $J_{1,1'}$ 7.0 Hz, $J_{1,2}$ 3.3 Hz, H-1) 2.75 (dd, 1H, $J_{4,5e}$ 3.78 Hz, $J_{5a,5e}$ 13.3 Hz, H-5e) 2.50 (dd, 1H, $J_{4,5a}$ 7.1 Hz, H-5a), 1.29-1.18 (m, 2H, H-6), 1.18-0.97 (m, 2H, H-7) 0.64 (t, 3H, H-8);

^{13}C NMR (75.5 MHz, D_2O) δ = 71.5, 71.4 (2C, C-2, C-3), 69.7 (C-4), 54.6 (C-1), 44.1 (C-5), 28.0 (C-6), 18.7 (C-7), 13.1 (C-8);

1(R)-1-C-Isopropyl-1,5-dideoxy-1,5-imino-xylitol (1,2,6-trideoxy-1,5-imino-6-C-isopropyl-L-iditol, 35)

Following general procedure C, $Pd(OH)_2/C$ was added to a 10% solution of compound **30** (61 mg, 1.70 mmol, 1 eq) in MeOH/H_2O (1/1, v/v) and stirred under a hydrogen atmosphere. Purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 5/1/0.25, v/v/v) gave compound **35** (30 mg) in a yield of 99 % as colourless oil.



Product **35**

MS: Calcd. for $[C_8H_{17}NO_3Na]$: m/z 198.1106

MS: Found: m/z 198.1105

TLC: Rf = 0.4

($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v), CAM

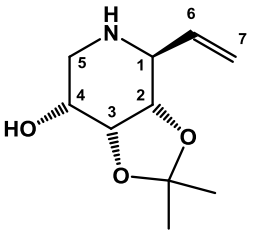
$[\alpha]_D^{20}$ = -9.3 (c 0.40, H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 3.66 (bs, 1H, $J_{2,3}$ 1.8 Hz, $J_{3,4}$ 3.3 Hz, H-3), 3.67 (bs, 1H, H-2) 3.44 (bs, 1H, H-4) 2.75 (dd, 1H, $J_{5a,5e}$ 14.0 Hz, $J_{4,5a}$ 2.3 Hz, H-5a), 2.64 (dd, 1H, $J_{4,5e}$ 1.9 Hz, H-5e), 2.49 (dd, 1H, $J_{1,6}$ 9.4 Hz, H-1) 1.87-1.75 (m, 1H, H-6), 1.00 (d, 3H, $J_{6,7}$ 6.7 Hz, H-7), 0.94 (d, 3H, $J_{6,7'}$ 6.7 Hz, H-7');

^{13}C NMR (75.5 MHz, D_2O) δ = 68.9, 68.6 (2C, C-2 C-3), 67.6 (C-4), 59.7 (C-1), 46.1 (C-5), 27.7 (C-6), 19.4, 18.8 (2C, C-7, C-7‘);

1(S)-1-C-Vinyl-2,3-isopropylidene-1,5-dideoxy-1,5-imino-ribitol (1,2,6-trideoxy-1,5-imino-3,4-O-isopropylidene-6-C-vinyl-L-allitol, 27)

Following general procedure B, compound **23** (95 mg, 0.44 mmol, 1 eq) was dissolved in MeOH (5 mL) and treated with PMe_3 (1M in toluene, 880 µL, 0.88 mmol, 2 eq). After removal of MeOH *in vacuo* the cyclic iminium ion intermediate was dissolved in THF (5 mL) and vinylmagnesium bromide (1M in THF, 2.20 mL, 2.2 mmol, 5 eq) was added. Silica gel chromatography (EtOAc/MeOH = 5/1, v/v) gave compound **27** (70 mg) in a yield of 80 % as colourless oil.



Product **27**

MS: Calcd. for [$C_{10}H_{17}NO_3Na$]: m/z 222.1106

MS: Found: -

TLC: Rf = 0.16

(EtOAc/MeOH = 10/1, v/v),

CAM

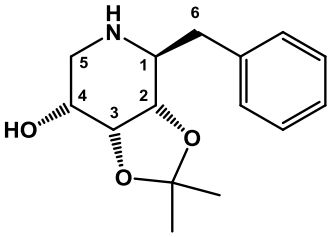
$[a]_D^{20}$ = -32.6 (c 1.00, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4) δ = 5.79 (ddd, 1H, H-6), 5.08 (dd, 2H, H-7), 4.25 (dd, 1H, $J_{2,3=3,4}$ 4.2 Hz, H-3), 3.70 (ddd, 1H, H-4), 3.61 (dd, 1H, $J_{1,2}$ 9.1 Hz, H-2), 2.93 (ddd, 1H, H-1), 2.78 (dd, 1H, $J_{5a,5e}$ 11.7 Hz, $J_{4,5e}$ 5.4 Hz, H-5e), 2.59 (dd, 1H, $J_{4,5a}$ 11.4 Hz, H-5a), 1.38, 1.22 (2C, 2xCH_3);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 138.5 (C-6), 117.0 (C-7), 110.8 (C_q), 79.1, 77.1 (2C, C-2, C-3), 67.9 (C-4), 61.4 (C-1), 47.8 (C-5), 28.7, 26.6 (2C, 2xCH_3);

1(S)-1-C-Benzyl-2,3-isopropylidene-1,5-dideoxy-1,5-imino-ribitol (6-C-benyzl-1,2,6-trideoxy-1,5-imino-3,4-O-isopropylidene-L-allitol, 31)

Following general procedure B, compound **23** (43 mg, 0.20 mmol, 1 eq) was dissolved in MeOH (2 mL) and treated with PMe_3 (1M in toluene, 400 µL, 0.40 mmol, 2 eq). After removal of MeOH *in vacuo* the cyclic iminium ion intermediate was dissolved in THF (2 mL) and benzylmagnesium bromide (2M in THF, 500 µL, 1.00 mmol, 5 eq) was added. Silica gel chromatography (EtOAc/MeOH = 20/1, v/v) gave compound **31** (27 mg) in a yield of 51 % as colourless oil.



Product **31**

MS: Calcd. for [$C_{15}H_{21}NO_3Na$]: m/z 286.1419

MS: Found: -

TLC: Rf = 0.46

(EtOAc/MeOH = 10/1, v/v),

CAM

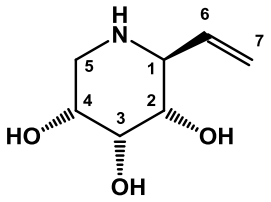
$[a]_D^{20}$ = -44.9 (c 1.00, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.32-7.18 (m, Ph), 4.40 (dd, 1H, $J_{3,4=2,3}$ 3.9 Hz, H-3), 3.86 (ddd, 1H, H-4), 3.78 (dd, 1H, $J_{1,2}$ 9.1 Hz, H-2), 3.10 (dd, 1H, H-6), 2.85 (dd, 1H, $J_{5a,5e}$ 11,5 Hz, $J_{4,5e}$ 5.4 Hz, H-5e), 2.75 (ddd, 1H, H-1), 2.59 (dd, 1H, $J_{4,5a}$ 11.2 Hz, H-5a), 2.42 (dd, 1H, H-1'), 1.55 (s, 3H, CH_3), 1.39 (s, 3H, CH_3);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 139.4, 130.3, 129.8, 129.7, 127.7 (Ph), 110.8 (C_q), 79.0, 77.1 (2C, C-2, C-3), 67.8 (C-4), 60.6 (C-1), 48.1 (C-5), 39.7 (C-6), 28.7, 26.7 (2C, 2xCH$_3$).

1(S)-1-C-Vinyl-1,5-dideoxy-1,5-imino-ribitol (6-C-vinyl-1,5,6-trideoxy-1,5-imino-L-allitol, 36)

Following general procedure D, compound **27** (18 mg, 0.10 mmol, 1 eq) gave after purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 5/1/0.25, v/v/v) compound **36** (15 mg) in a yield of 96 % as colourless oil.



Product **36**

MS: Calcd. for [$C_7H_{13}NO_3H$]: m/z 160.0974

MS: Found: 160.0975

TLC: Rf = 0.56

($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v), CAM

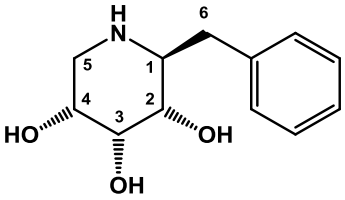
$[a]_D^{20}$ = -8.4 (c 1.05, H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 5.82-5.70 (m, 1H, H-6), 5.05 (dd, 2H, H-7), 4.11 (bs, 1H, H-3), 3.04 (ddd, 1H, H-4), 3.73-3.70 (m, 2H, H-1, H-2), 3.20 (dd, 1H, $J_{5a,5e}$ 12.3 Hz, $J_{4,5e}$ 4.1 Hz, H-5e), 3.06 (dd, 1H, $J_{4,5a}$ 11.8 Hz, H-5a);

^{13}C NMR (75.5 MHz, D_2O) δ = 129.6 (C-6), 124.8 (C-7), 69.7 (C-3), 68.3 (C-2), 64.7 (C-4), 56.2 (C-1), 41.5 (C-5);

1(S)-1-C-Benzyl-1,5-dideoxy-1,5-imino-ribitol (6-C-benzyl-1,5,6-trideoxy-1,5-imino-L-allitol, 37)

Following general procedure D, compound **31** (30 mg, 0.10 mmol, 1 eq) gave after purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 10/1/0.25, v/v/v) compound **37** (24 mg) in a yield of 94 % as colourless oil.



Product **37**

MS: Calcd. for $[C_{12}H_{17}NO_3H]$: m/z 224.1287

MS: Found: 224.1668

TLC: Rf = 0.70

($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v), CAM

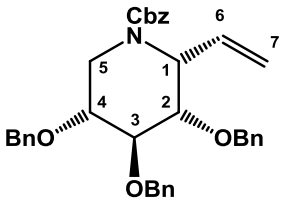
$[\alpha]_D^{20}$ = -18.0 (c 1.00, H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 7.20-7.10 (m, Ph), 4.02 (bs, 1H, H-3), 3.81 (ddd, 1H, $J_{4,3}$ 2.6 Hz, H-4), 3.61 (dd, 1H, $J_{2,3}$ 2.4 Hz, $J_{1,2}$ 10.4 Hz, H-2), 3.38 (ddd, 1H, H-1), 3.27 (dd, 1H, H-6), 3.00 (dd, 1H, $J_{5e,5a}$ 12.2 Hz, $J_{4,5e}$ 4.9 Hz, H-5e), 2.85 (dd, 1H, $J_{4,5a}$ 11.8 Hz, H-5a), 2.67 (dd, 1H, H-6);

^{13}C NMR (75.5 MHz, D_2O) δ = 134.6, 129.7, 129.4, 129.3, 127.9 (Ph), 69.8 (C-3), 68.6 (C-2), 64.5 (C-4), 55.3 (C-1), 42.1 (C-5), 34.7 (C-6);

1(R)-1-C-Vinyl-2,3,4-tri-O-benzyl-6-N-Cbz-1,5-dideoxy-1,5-imino-xylitol (3,4-di-O-benzyl-N-benzylcarboyl-1,2,6-trideoxy-1,5-imino-6-C-vinyl-D-iditol, 38)

Following general procedure E, compound **28** (180 mg, 0.59 mmol, 1 eq) gave a colourless oil (330 mg, 0.70 mmol) and was further treated as described in general procedure F. The obtained material was purified utilizing silica gel chromatography (CH/EtOAc = 10/1, v/v) to give compound **38** (300 mg) as colourless oil in a yield of 90%.



Product **38**

MS: Calcd. for [$C_{36}H_{37}NO_5Na$]: m/z 586.2570

MS: Found: 586.2901

TLC: Rf = 0.66

(CH/EtOAc = 3/1, v/v),

VAN

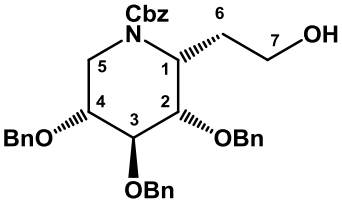
$[a]_D^{20}$ = +25.6 (c 1.12, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.47-7.30 (m, Ph), 6.17 (m, 1H, H-6), 5.45–5.16 (m, 4 ½ H, H-1, H-7, C*H*$_2$Ph), 5.05 (bs, ½ H, H-1), 54.9 (dd, 2H, C*H*$_2$Ph), 4.77 (dd, 4H, C*H*$_2$Ph), 4.44, 4.22 (2xdd, 1H, $J_{5e,5a}$ 13.4 Hz, $J_{5a,4}$ 5.4 Hz, H-5a) 3.72–3.54 (m, 3H, H-2, H-3, H-4) 3.03-2.93 (m, 1H, H-5e). Due to two pronounced rotameric populations (**38**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution of the ^{1}H-NMR spectrum.

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 155.7, (d, C=O), 138.9, 138.2, 138.1, 136.5 (4C, Ph), 131.0 (d, C-6), 130.9–127.6 (Ph), 118.2 (d, C-7), 82.6 (d, C-2), 79.6 (d, C-3), 78.3 (C-4), 75.8 (2C, *C*H$_2$Ph), 73.3, 73.1, 72.6 (2C, *C*H$_2$Ph), 67.7 (*C*H$_2$Ph), 55.0 (d, C-1), 42.1 (d, C-5). Due to two pronounced rotameric populations (**38**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed in ^{13}C-NMR spectrum.

1(R)-1-C-Hydroxyethyl-2,3,4-tri-O-benzyl-6-N-Cbz-1,5-dideoxy-1,5-imino-xylitol (3,4-di-O-benzyl-N-benzylcarboyl-1,2,6-trideoxy-6-C-hydroxyehtyl-1,5-imino-D-iditol, 39)

Compound **38** (125 mg, 0.22 mmol, 1 eq) was added to a solution of powdered $NaBH_4$ (50 mg, 1.33 mmol, 6 eq) in anhydrous THF (10 mL). Dimethylsulfate (DMS, 126 µl, 1.33 mmol, 6 eq) was added and the reaction mixture was stirred at room temperature until complete consumption of the starting material was detected by TLC (CH/EtOAc = 3/1, v/v). Subsequently H_2O (2 ml), 3M NaOH (4 ml) and H_2O_2 (30% solution, 4 ml) were added. The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified utilizing silica gel chromatography (CH/EtOAc = 10/1, v/v) to give compound **39** (77 mg) as colourless oil in a yield of 60 %.



Product **39**

MS: Calcd. for $[C_{36}H_{39}NO_6Na]$: m/z 604.2675

MS: Found: 604.2679

TLC: Rf = 0.30

(CH/EtOAc = 3/1, v/v),

VAN

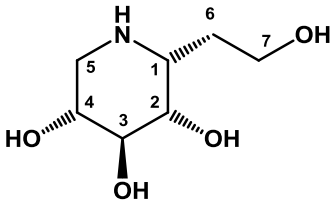
$[a]_D^{20}$ = +12.5 (c 1.00, $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.12-6.95 (m, Ph), 5.12-4.96 (m, 2H, CH_2Ph), 4.77 (dd, 2H, CH_2Ph), 4.68-4.53 (m, 5H, 2xCH_2Ph, H-1), 4.34, 4.10 (2xdd, 1H, $J_{5e,5a}$ 13.4 Hz, $J_{5a,4}$ 5.4 Hz, H-5a) 3.64–3.46 (m, 3H, H-2, H-3, H-7) 3.46–3.28 (m, 2H, H-4, H-7), 2.63 (dd, 1H, H-5e), 2.08–1.96, 1.66–1.46 (2xm, 2H, H-6). Due to two pronounced rotameric populations (**39**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution of the ^{1}H-NMR spectrum.

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 156.54 (d, C=O), 138.8, 138.1, 137,9, 136.2 (Ph), 128.7-127.7 (Ph), 82.0 (C-2), 79.2 (d, C-3), 77.9 (d, C-4), 75.7 (*C*H_2Ph), 73.6, 73.2, 72.8 (3C, *C*H_2Ph), 68.0 (d, *C*H_2Cbz), 58.3 (d, C-7), 49.7 (d, C-1), 41.5 (d, C-5), 27.2 (d, C-6). Due to two pronounced rotameric populations (**39**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed in ^{13}C-NMR spectrum.

1(R)-1-C-Hydroxyethyl-1,5-dideoxy-1,5-imino-xylitol (1,2,6-trideoxy-6-C-hydroxyehtyl-1,5-imino-D-iditol, 40)

Following general procedure C, $Pd(OH)_2$/C was added to a 10% solution of compound **39** (60 mg, 0.10 mmol, 1 eq) in MeOH/H_2O (1/1, v/v) and stirred under a hydrogen atmosphere. Silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 3/1/0.25, v/v/v) gave compound **40** (15 mg) in a yield of 82 % as colourless oil.



Product **40**

MS: Calcd. for [$C_7H_{15}NO_4Na$]: m/z 200.0899

MS: Found: 200.0892

TLC: Rf = 0.55

($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v), CAM

$[a]_D^{20}$ = -6.9 (c 1.00, H_2O)

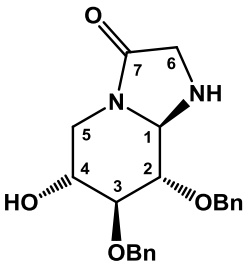
^{1}H NMR (300 MHz, D_2O) δ = 4.01-3.92 (m, 2H, H-3, H-4), 3.91–3.85 (dd, 1H, $J_{2,1}$ 2.4 Hz, $J_{2,3}$ 4.9 Hz, H-2) 3.70 (dd, 2H, H-6), 3.61 (dd, 1H, H-1) 3.42-3.35 (m, 1H, H-5a), 3.29–3.23 (m, 1H, H-5e), 1.95-1.85(m, 2H, H-7);

^{13}C NMR (75.5 MHz, D_2O) δ = 68.1, 67.2, 66.0 (3C, C-2, C-3, C-4), 57.8 (C-7), 53.5 (C-1), 45.3 (C-5), 29.8 (C-6);

6.5. Amino acids as nucleophiles

*(1*R*,2*S*,3*S*,4*R*)-2,3-di-*O*-Benzyl-4-hydroxyhexahydroimidazo[1,2-α]pyridine-*NH*-7-one (45)*

Following general procedure G, compound **18** (200 mg, 0.56 mmol, 1 eq) was dissolved in MeOH (5 mL) and treated with PMe_3 (1M in THF, 1.12 mL, 1.12 mmol, 2 eq). After consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v) glycin methyl ester (141 mg, 1.12 mmol, 2 eq) was added to the reaction mixture and gave, after purification utilizing silica gel chromatography (CH/EtOAc = 1/3, v/v), compound **45** (124 mg) in a yield of 60% as colorless oil.



Product **45**

MS: Calcd. for $[C_{21}H_{24}N_2O_4Na]$: m/z 391.1634

MS: Found: -

TLC: Rf = 0.55

(EtOAc/MeOH = 10/1, v/v)

VAN

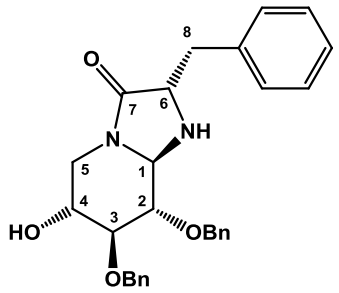
$[\alpha]_D^{20}$ = -77.6 (c 1.00, MeOH)

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.35-7.10 (m, 10H, Ph), 4.89-4.67 (m, 4H, C*H*$_2$Ph), 4.29 (d, 1H, $J_{1,2}$ 8.26 Hz, H-1) 4.13 (dd, 1H, $J_{5,4}$ 5.60 Hz, $J_{5e,5a}$ 12.9 Hz, H-5e), 3.47-3.38 (m, 2H, H-4, H-6) 3.30 (dd, 1H, $J_{3,4}$ 8.90 Hz, H-3), 3.28 (m, 1H, H-6), 3.12 (dd, 1H, $J_{2,3}$ 9.03 Hz, H-2), 2.51 (dd, 1H, $J_{5a,4}$ 10.7 Hz, H-5a);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 171.9 (C-7), 138.4-138.2 (C_q), 128.6-127.9 (Ph), 84.9 (C-3), 82.8 (C-2), 76.0 (C-1), 75.6 (*C*H_2Ph), 74.9 (*C*H_2Ph), 69.0 (C-4), 48.4 (C-6), 41.8 (C-5);

(1*R*,2*S*,3*S*,4*R*,6*S*)-6-Benzyl-2,3-di-*O*-benzyl-4-hydroxyhexahydroimidazo[1,2-α]pyridine-*NH*-7-one (46)

Following general procedure G, compound **18** (200 mg, 0.56 mmol, 1 eq) was dissolved in MeOH (5 mL) and treated with PMe_3 (1M in THF, 1.12 mL, 1.12 mmol, 2 eq). After consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v), phenylalanine methyl ester (230 mg, 1.13 mmol, 2 eq) was added to the reaction mixture and gave, after purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 20/1/0.01, v/v/v), compound **46** (150 mg) in a yield of 58% as colorless oil.



Product **46**

MS: Calcd. for $[C_{28}H_{30}N_2O_4H]$: m/z 459.2284

MS: Found: m/z 459.2135

TLC: Rf = 0.75

(EtOAc/MeOH = 10/1, v/v)

CAM

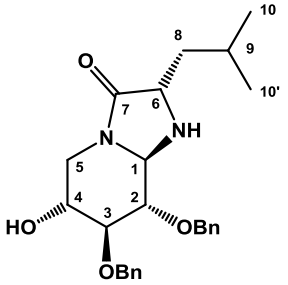
$[\alpha]_D^{20}$ = -21.9 (c 1.30, MeOH)

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.38-7.20 (m, 15H, Ph), 4.93-4.77 (m, 6H, CH_2Ph), 4.07 (d, 1H, $J_{1,2}$ 8.38 Hz, H-1), 4.06 (dd, 1H, $J_{5e,4}$ 5.23 Hz, $J_{5e,5a}$ 13.3 Hz, H-5e), 3.76 (ddd, 1H, H-6), 3.53 (ddd, 1H, $J_{4,3}$ 8.64 Hz, H-4), 3.34 (dd, $J_{3,2}$ 7.50 Hz, H-3), 3.22 (dd, 1H, H-2), 3.09 (dd, 1H, $J_{8,6}$ 4.08 Hz, $J_{8,8'}$ 14.06 Hz, H-8), 2.89 (dd, 1H, $J_{8',6}$ 7.11 Hz, H-8'), 2.56 (dd, 1H, $J_{5a,4}$ 11.2 Hz, H-5a);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 175.1 (C-7), 140.1-138.5 (C_q), 130.5-127.8 (Ph), 86.3 (C-3), 83.3 (C-2), 76.6 (*C*H_2Ph), 75.7 (*C*H_2Ph), 75.6 (C-1), 70.5 (C-4), 61.3 (C-6), 43.5 (C-5), 38.7 (C-8);

(1R,2S,3S,4R,6S)-2,3-di-O-Benzyl-4-hydroxy-6-isobutyl-hexahydroimidazo[1,2-α]pyridine-NH-7-one (47)

Following general procedure G, compound **18** (200 mg, 0.56 mmol, 1 eq) was dissolved in MeOH (5 mL) and treated with PMe_3 (1M in THF, 1.12 mL, 1.12 mmol, 2 eq). After consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v) leucine methyl ester (200 mg, 1.00 mmol, 2 eq) was added to the reaction mixture and gave, after purification utilizing silica gel chromatography (EtOAc/MeOH = 20/1, v/v) compound **47** (160 mg) in a yield of 69% as colorless oil.



Product **47**

MS: Calcd. for $[C_{25}H_{32}N_2O_4Na]$: m/z 447.2260

MS: Found: -

TLC: Rf = 0.70

(EtOAc/MeOH = 10/1, v/v)

CAM

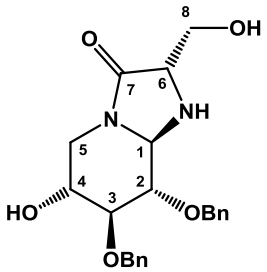
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.31-7.10 (m, 10H, Ph), 4.38-4.69 (m, 4H, CH_2Ph), 4.31 (d, 1H, $J_{1,2}$ 8.32 Hz, H-1), 3.96 (dd, 1H, $J_{5,4}$ 5.60 Hz, $J_{5e,5a}$ 12,8 Hz, H-5e), 3.47-3.35 (m, 2H, H-4, H-6), 3.33 (dd, 1H, $J_{3,4}$ 9.00 Hz, H-3), 3.16 (dd, 1H, $J_{2,3}$ 8.39 Hz, H-2), 2.62 (dd, 1H, $J_{5a,4}$ 10.0 Hz, H-5a), 1.74 (ddd, 1H, H-9), 1.53 (ddd, 1H, $J_{8,6}$ 3.86 Hz, $J_{8,8'}$ 13.7 Hz, H-8), 1.29 (ddd, 1H, $J_{8',6}$ 5.57 Hz, H-8'), 0.86 (m, 6H, H-10, H-10');

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 176.8 (C-7), 140.4, 140.2 (C_q), 129.3-128.5 (Ph), 86.4 (C-3), 82.8 (C-2), 76.6 (CH_2Ph), 75.8 (CH_2Ph), 75.7 (C-1), 70.6 (C-4), 58.1 (C-6), 43.6 (C-5), 42.3 (C-8), 26.4 (C-9), 23.6 (C-10), 22.0 (C-10');

(1R,2S,3S,4R,6S)-2,3-di-O-Benzyl-4-hydroxy-6-(hydroxymethyl)-hexahydroimidazo[1,2-α]pyridine-NH-7-one (48)

Following general procedure G, compound **18** (200 mg, 0.56 mmol, 1 eq) was dissolved in MeOH (5 mL) and treated with PMe_3 (1M in THF, 1.12 mL, 1.12 mmol, 2 eq). After consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v) serine methyl ester (170 mg, 1.12 mmol, 2 eq) was added to the reaction mixture and gave, after purification utilizing silica gel chromatography (EtOAc) compound **48** (140 mg) in a yield of 63% as colorless oil.



Product **48**

MS: Calcd. for [$C_{22}H_{26}N_2O_5Na$]: m/z 421.1739

MS: Found: -

TLC: Rf = 0.45

(EtOAc/MeOH = 10/1, v/v)

CAM

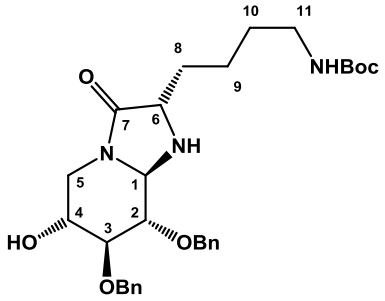
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.40-7.24 (m, 10H, Ph), 5.04-4.80 (m, 4H, CH_2Ph), 4.48 (d, 1H, $J_{1,2}$ 7.36 Hz, H-1), 4.12 (dd, 1H, $J_{5.4}$ 5.49 Hz, $J_{5e,5a}$ 12.6 Hz, H-5e), 3.81 (dd, 1H, $J_{8,6}$ 3.23 Hz, $J_{8,8'}$ 11.0 Hz, H-8), 3.74 (dd, 1H, H-6), 3.65 (dd, 1H, $J_{8',6}$ 2,85 Hz, H-8'), 3.58 (ddd, 1H, H-4), 3.47 (dd, 1H, $J_{3,4}$ 8.31 Hz, H-3), 3.44 (dd, 1H, $J_{2,3}$ 8.80 Hz, H-2), 2.72 (dd, 1H, H-5a);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 173.3 (C-7), 140.2, 140.2 (C_q), 123.2-128.5 (Ph), 86.3 (C-3), 85.5 (C-2), 76.5 (*C*H_2Ph), 75.9 (*C*H_2Ph), 75.5 (C-1), 70.5 (C-4), 62.9 (C-8), 62.6 (C-6), 43.5 (C-5);

(1*R*,2*S*,3*S*,4*R*,6*S*)-2,3-di-*O*-Benzyl-4-hydroxy-6-(11-*NH*-Boc-butyl)-hexahydroimidazo[1,2-α]pyridine-*NH*-7-one (49)

Following general procedure G, compound **18** (200 mg, 0.56 mmol, 1 eq) was dissolved in MeOH (5 mL) and treated with PMe_3 (1M in THF, 1.12 mL, 1.12 mmol, 2 eq). After consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v) Boc-lysine methyl ester (330 mg, 1.12 mmol, 2 eq) was added to the reaction mixture and gave, after purification utilizing silica gel chromatography (CH/EtOAc = ½, v/v) compound **49** (200 mg) in a yield of 66% as colorless oil.



Product **49**

MS: Calcd. for $[C_{30}H_{41}N_3O_6Na]$: m/z 562.2893

MS: Found: -

TLC: Rf = 0.60

(EtOAc/MeOH = 10/1, v/v)

CAM

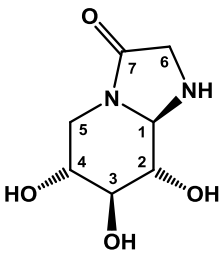
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.34-7.16 (m, 10H, Ph), 4.92-4.74 (m, 4H, CH_2Ph), 4.37 (d, 1H, $J_{1,2}$ 8.40 Hz, H-1), 4.03 (dd, 1H, $J_{5,4}$ 5.52 Hz, $J_{5e,5a}$ 12.9 Hz, H-5e), 3.53-3.45 (m, 2H, H-4, H-6), 3.39 (dd, 1H, $J_{3,4}$ 8.64 Hz, H-3), 3.21 (dd, 1H, $J_{2,3}$ 8.64 Hz, H-2), 3.00 (ddd, 2H, H-8), 2.67 (dd, 1H, $J_{5a\text{-}4}$ 10.4 Hz, H-5a), 1.78-1.22 (m, 15H, H-9, H-10, H-11, 3xCH_3-Boc);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 175.9 (C-7), 140.1,140.0 (C_q), 129.3-128.5 (Ph), 86.4 (C-3), 83.3 (C-2), 79.8 (C_q-Boc), 76.6 (*C*H_2Ph), 75.8 (*C*H_2Ph), 75.7 (C-1), 70.6 (C-4), 59.8 (C-6), 43.5 (C-5), 41.0 (C-8), 32.6, 30.8, 28.8, 23.9 (C-9, C-10, C-11, *C*H_3-Boc);

(1R,2S,3S,4R)-2,3,4-Trihydroxyhexahydroimidazo[1,2-α]pyridine*-NH-*7-one (53)

Following general procedure C, $Pd(OH)_2/C$ was added to a 10% solution of compound **45** (90 mg, 0.24 mmol) in $MeOH/H_2O$ (1/1, v/v) and stirred under a hydrogen atmosphere. Purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 10/1/0.01, v/v/v) gave compound **53** (45 mg) in a yield of 99 % as colourless oil.



Product **53**

MS: Calcd. for $[C_7H_{12}N_2O_4Na]$: m/z 211.0695

MS: Found: -

TLC: Rf = 0.40

($CHCl_3$/MeOH/concd NH_4OH = 3/1/0.01, v/v/v)

CAM

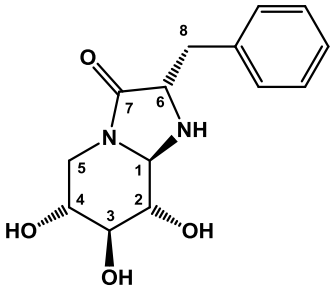
$[\alpha]_D^{20}$ = - 14 (c 0.75, H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 4.43 (d, 1H, $J_{1,2}$ 8.62 Hz, H-1), 4.04 (dd, 1H, $J_{5,4}$ 5.14 Hz, $J_{5e,5a}$ 12.8 Hz, H-5e), 3.58 (dd, 1H, H-6), 3.50 (ddd, 1H, H-4), 3.48 (dd, 1H, H-6'), 3.41 (dd, 1H, $J_{3,4}$ 8.92 Hz, H-3), 3.29 (dd, 1H, $J_{2,3}$ 8.52 Hz, H-2), 2,75 (dd, 1H, $J_{5a,4}$ 11.0 Hz, H-5a);

^{13}C NMR (75.5 MHz, D_2O) δ = 173.6 (C-7), 76.1 (C-3), 75.3 (C-1), 73.6 (C-2), 68.2 (C-4), 47.9 (C-6), 41.9 (C-5);

(1R,2S,3S,4R,6S)-6-Benzyl-2,3,4-trihydroxyhexahydroimidazo[1,2-α]pyridine*-NH-*7-one (54)

Following general procedure C, $Pd(OH)_2/C$ was added to a 10% solution of compound **46** (30 mg, 0.07 mmol) in $MeOH/H_2O$ (1/1, v/v) and stirred under a hydrogen atmosphere. Purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 10/1/0.01, v/v/v) gave compound **54** (15 mg) in a yield of 82 % as colourless oil.



Product **54**

MS: Calcd. for [$C_{14}H_{18}N_2O_4Na$]: m/z 301.1164

MS: Found: m/z 301.1647

TLC: Rf = 0.75

($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v)

CAM

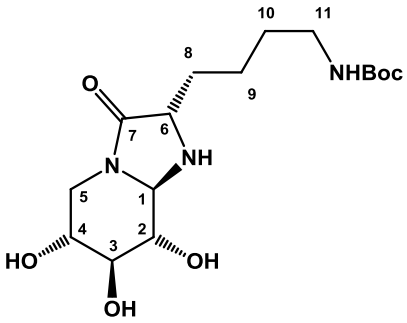
$[a]_D^{20}$ = - 33.5 (c 1.00, H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 7.34-7.20 (m, 5H, Ph), 3.96 (dd, 1H, $J_{5,4}$ 5.73 Hz, $J_{5e,5a}$ 13.0 Hz, H-5e), 3.89 (m, 1H, H-6), 3.83 (d, 1H, $J_{1,2}$ 8.77 Hz, H-1), 3.39 (ddd, 1H, $J_{4,3}$ 8.88 Hz, H-4), 3.22 (dd, 1H, $J_{3,2}$ 9.23 Hz, H-3), 3.13 (dd, 1H, H-2), 3.03 (dd, 1H, H-8), 2.93 (dd, 1H, H-8'), 2.53 (dd, 1H, $J_{5a,4}$ 10.7 Hz, H-5a);

^{13}C NMR (75.5 MHz, D_2O) δ = 174.5 (C-7), 136.3 (C_q), 129.4-127.3 (Ph), 76.0 (C-3), 74.0 (C-1), 73.2 (C-2), 68.1 (C-4), 59.8 (C-6), 41.9 (C-5), 36.6 (C-8);

(1*R*,2*S*,3*S*,4*R*,6*S*)-2,3,4-Trihydroxy-6-(11-*NH*-Boc-butyl)-hexahydroimidazo[1,2-α]pyridine-*NH*-7-one (55)

Following general procedure C, $Pd(OH)_2$/C was added to a 10% solution of compound **49** (200 mg, 0.37 mmol) in MeOH/H_2O (1/1, v/v) and stirred under a hydrogen atmosphere. Purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 3/1/0.01, v/v/v) gave compound **55** (100 mg) in a yield of 75 % as colourless oil.



Product **55**

MS: Calcd. for [$C_{16}H_{29}N_3O_6Na$]: m/z 382.1954

MS: Found: -

TLC: Rf = 0.80

($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v)

CAM

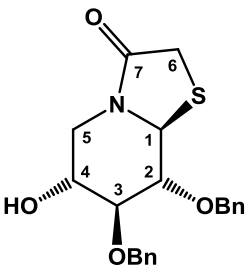
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, D_2O) δ = 4.09 (d, 1H, $J_{1,2}$ 8.7 Hz, H-1), 3.77 (dd, 1H, $J_{5,4}$ 5.1 Hz, $J_{5e,5a}$ 12.9 Hz, H-5e), 3.39-3.29 (m, 1H, H-6), 3.18 (ddd, 1H, $J_{4,3}$ 8.4 Hz, $J_{4,5a}$ 10.2 Hz, H-4), 3.12 (dd, 1H, $J_{3,2}$ 9.1 Hz, H-3), 2.96 (dd, 1H, H-2), 2.79-2.71 (m, 2H, H-8), 2.50 (dd, 1H, H-5a), 1.54-0.99 (m, 15H, H-9, H-10, H-11, 3x*CH$_3$-Boc);*

^{13}C NMR (75.5 MHz, D_2O) δ = 175.3 (C-7), 80.9 (C_q-Boc), 76.1 (C-3), 74.1 (C-1), 73.7 (C-2), 68.3 (C-4), 58.4 (C-5), 57.4 (C-8), 30.7, 29.8, 27.7 21.9 (C-9, C-10, C-11, *C*H_3-Boc);

(1*R*,2*S*,3*S*,4*R*)-2,3-di-*O*-Benzyl-4-hydroxytetra-5*H*-thiazolo[3,2-α]pyridine-7-one (42)

Compound **18** (210 mg, 0.60 mmol, 1 eq) was dissolved in MeOH (10 mL) and PMe_3 (1.2 mL, 1M, 1.2 mmol, 2 eq) was added to the reaction mixture. After consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v), mercaptoacetic acid (270 µL, 3.0 mmol, 5 eq), was added to the reaction mixture. After consumption of the iminium ion intermediate (detected by TLC: EtOAc/MeOH = 10/1, v/v) the reaction mixture was concentrated under reduced pressure and purified employing column chromatography (EtOAc) to give compound **42** (57 mg) as colorless oil with a yield of 25%.



Product **42**

MS: Calcd. for [$C_{21}H_{23}NO_4SNa$]: m/z 408.1245

MS: Found: -

TLC: Rf = 0.75

(EtOAc)

CAM

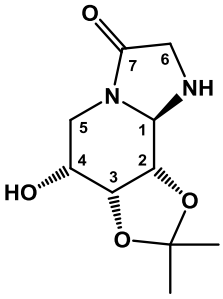
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.31-7.19 (m, 10H, Ph), 4.88-4.58 (m, 4H, C*H*$_2$Ph), 4.41 (d, 1H, $J_{1,2}$ 8.05 Hz, H-1), 4.27 (dd, 1H, $J_{5e,4}$ 5.58 Hz, $J_{5e,5a}$ 13.0 Hz, H-5e), 3.54-3.45 (m, 3H, H-4, H-6), 3.36 (dd, 1H, $J_{3,4}$ 8.55 Hz, H-3), 3.31 (dd, 1H, $J_{2,3}$ 8.80 Hz, H-2), 2.55 (dd, 1H, $J_{5a,4}$ 11.1 Hz, H-5a);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 169.7 (C-7), 138.2, 137.7 (C_q), 128.8-128.0 (Ph), 86.4 (C-3), 85.2 (C-2), 75.8 (*C*H_2Ph), 75.7 (*C*H_2Ph), 69.1 (C-4), 62.2 (C-1), 44.5 (C-5), 32.0 (C-6);

(1*R*,2*S*,3*R*,4*R*)-2,3-*O*-Isopropylidene-4-hydroxyhexahydroimidazo[1,2-α]pyridine*-NH-*7-one (50)

Compound **23** (250 mg, 1.17 mmol, 1 eq) was dissolved in MeOH (10 mL) and PMe_3 (2.1 mL, 1M, 2.1 mmol, 1.8 eq) was added to the reaction mixture. After consumption of the starting material (detected by TLC: CH/EtOAc = 1/1, v/v), glycine methyl ester hydrochloride (499 mg, 3.98 mmol, 3.4 eq), treated with basic ion exchange residue Merck III prior to addition, was added to the reaction mixture. After consumption of the iminium ion intermediate (detected by TLC: EtOAc/MeOH = 10/1, v/v) the reaction mixture was concentrated under reduced pressure and purified utilizing column chromatography (EtOAc) to give compound **50** (146 mg) as colorless oil with a yield of 46%.



Product **50**

MS: Calcd. for [$C_{10}H_{16}N_2O_4Na$]: m/z 251.1008

MS: Found: -

TLC: Rf = 0.33

(EtOAc/MeOH = 10/1, v/v)

CAM

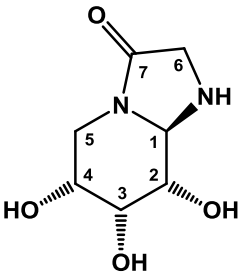
$[\alpha]_D^{20}$ = - 22.8 (c 1 MeOH)

^{1}H NMR (300 MHz, MeOH-d_4) δ = 4.64 (d, 1H, $J_{1,2}$ 7.61 Hz, H-1), 4.23 (dd, 1H, $J_{3,2}$ 3.32 Hz, $J_{3,4}$ 3.46 Hz, H-3), 4.02-3.94 (m, 2H, H-2, H-4), 3.58 (dd, 1H, $J_{5,4}$ 4.57 Hz, $J_{5e,5a}$ 13.1 Hz, H-5e), 3.33 (dd, 1H, H-6), 3.21 (dd, 1H, H-6'), 3.15 (dd, 1H, $J_{5a,4}$ 6.7 Hz, H-5a), 1.44 (s, 3H, CH_3-Isoprop.), 1.29 (CH_3-Isoprop);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 175.1 (C-7), 111.5 (C_q), 79.4 (C-2), 77.3 (C-3), 75.7 (C-1), 65.6 (C-4), 49.5 (C-6), 42.5 (C-5), 27.3 (CH$_3$-isoprop), 25.5 (CH$_3$-isoprop);

(1R,2S,3R,4R)-2,3,4-Trihydroxyhexahydroimidazo[1,2-α]pyridine*-NH-*7-one (56)

Following general procedure D, compound **50** (146 mg, 0.78 mmol, 1 eq) gave after purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd. NH_4OH = 3/1/0.25, v/v/v), a 177 mg mixture of compound **56** and NH_4Cl.



Product **56**

MS: Calcd. for [$C_7H_{12}N_2O_4Na$]: m/z 211.0695

MS: Found: -

TLC: Rf = 0.25

($CHCl_3$/MeOH/concd. NH_4OH = 1/1/0.25, v/v/v)

CAM

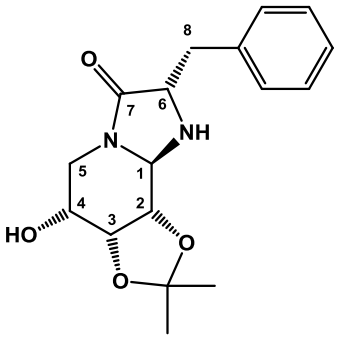
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, D_2O) δ = 4.64 (d, 1H, $J_{1,2}$ 9.6 Hz, H-1), 4.10 (dd, 1H, $J_{3,4}$ 2.4 Hz, H-3), 3.87 (dd, 1H, $J_{5e,4}$ 5.7 Hz, $J_{5e,5a}$ 12.5 Hz, H-5e), 3.75 (ddd, 1H, $J_{4,5a}$ 8.8 Hz, H-4), 3.58 (dd, 2H, H-6), 3.56 (dd, 1H, $J_{2,3}$ 2.4 Hz, H-2), 2.99 (dd, 1H, H-5a);

^{13}C NMR (75.5 MHz, D_2O) δ = 173.5 (C-7), 72.1 (C-2), 71.0 (C-3), 71.0 (C-1), 66.0 (C-4), 48.0 (C-6), 39.0 (C-5);

(1*R*,2*S*,3*R*,4*R*,6*S*)-6-Benzyl-2,3-*O*-isopropylidene-4-hydroxyhexahydroimidazo[1,2-α]pyridine-*NH*-7-one (51)

Compound **23** (210 mg, 0.97 mmol, 1 eq) was dissolved in MeOH (10 mL) and PMe_3 (1M in toluene, 2.1 mL, 2.1 mmol, 2.2 eq) was added to the reaction mixture. After consumption of the starting material (detected by TLC: CH/EtOAc = 1/1, v/v), phenylalanine methyl ester hydrochloride (580 mg, 2.70 mmol, 2.8 eq), treated with basic ion exchange residue Merck III prior to addition was added to the reaction mixture. After consumption of the iminium ion intermediate (detected by TLC: EtOAc/MeOH = 10/1, v/v) the reaction mixture was concentrated under reduced pressure and purified employing column chromatography (EtOAc) to give compound **51** (107 mg) as colorless oil with a yield of 36%.



Product **51**

MS: Calcd. for [$C_{17}H_{22}N_2O_4Na$]: m/z 341.1477

MS: Found: -

TLC: Rf = 0.55

(EtOAc/MeOH = 10/1, v/v)

CAM

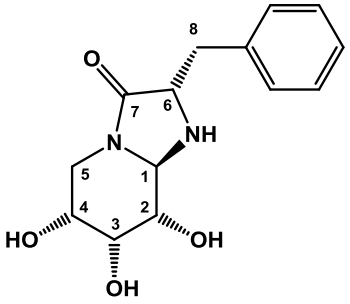
$[\alpha]_D^{20}$ = - 27.9 (c 0.99 MeOH)

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.35-7.28 (m, 5H, Ph), 4.29 (dd, 1H, $J_{3,2}$ 3.5 Hz, $J_{3,4}$ 3.5 Hz, H-3), 4.19 (d, 1H, $J_{1,2}$ 7.4 Hz, H-1), 3.97 (dd, 1H, H-6), 3.81 (ddd, 1H, $J_{4,5e}$ 4.4 Hz, H-4), 3.74 (dd, 1H, $J_{5e,5a}$ 12.8 Hz, H-5e), 3.34 (dd, 1H, H-2), 3.11-3.03 (m, 1H, H-8), 3.03-2.97 (m, 1H, H-8'), 1.44 (s, 3H, CH_3-isoprop), 1.35 (s, 3H, CH_3-isoprop);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 176.2 (C-7), 138.4 (C_q), 130.4-128.0 (Ph), 111.4 (C_q-isoprop.), 79.5 (C-3), 77.4 (C-2), 74.3 (C-1), 66.0 (C-4), 61.8 (C-6), 41.8 (C-5), 38.7 (C-8), 27.4 (*C*H_3-isoprop), 25.8 (*C*H_3-isoprop);

(2S,3R,4R,1R,6S)-6-Benzyl-2,3,4-trihydroxyhexahydroimidazo[1,2-α]pyridine-NH-7-one (57)

Following general procedure D, compound **51** (110 mg, 0.31 mmol, 1 eq) gave after purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd. NH_4OH = 3/1/0.01, v/v/v) compound **57** (42 mg) as colorless solid in a yield of 45%.



Product **57**

MS: Calcd. for $[C_{14}H_{18}N_2O_4Na]$: m/z 301.1164

MS: Found: -

TLC: Rf = 0.90

($CHCl_3$/MeOH/concd. NH_4OH = 1/1/0.25, v/v/v)

CAM

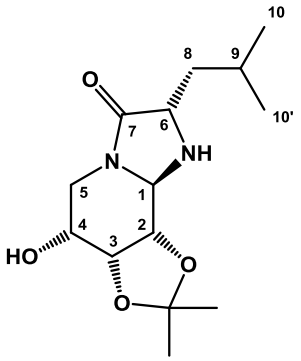
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, D_2O) δ = 7.39-7.28 (m, 5H, Ph), 4.13 (d, 1H, $J_{1,2}$ 8.9 Hz, H-1), 3.97 (dd, $J_{3,2}$ 2.9 Hz, H-3), 3.89-3.03 (m, 1H, H-6), 3.79-3.73 (m, 1H, H-5e), 3.60 (ddd, 1H, $J_{4,5e}$ 5.4 Hz, $J_{4,5a}$ 7.8 Hz, H-4), 3.31 (dd, 1H, H-2), 3.03 (dd, 1H, H-8), 2.91 (dd, 1H, H-8'), 2.80 (dd, 1H, $J_{5a,5e}$ 11.7 Hz, H-5a);

^{13}C NMR (75.5 MHz, D_2O) δ = 174.5 (C-7), 136.5 (C_q), 129.4-127.3 (Ph), 71.9 (C-3), 71.2 (C-2), 70.8 (C-1), 66.0 (C-4), 60.0 (C-6), 39.0 (C-5), 36.6 (C-8);

(1R,2S,3R,4R,6S)-2,3-O-isopropylidene-4-hydroxy-6-isobutyl-hexahydroimidazo[1,2-α]pyridine-NH-7-one (52)

Compound **23** (200 mg, 0.94 mmol, 1 eq) was dissolved in MeOH (10 mL) and PMe_3 (1M in toluene, 2.1 mL, 2.1 mmol, 2.2 eq) was added to the reaction mixture. After consumption of the starting material (detected by TLC: CH/EtOAc = 1/1, v/v), leucine methyl ester hydrochloride (210 mg, 1.18 mmol, 1.3 eq), treated with basic ion exchange residue Merck III prior to addition was added to the reaction mixture. After consumption of the iminium ion intermediate (detected by TLC: EtOAc/MeOH = 10/1, v/v) the reaction mixture was concentrated under reduced pressure and purified utilizing column chromatography (EtOAc) to give compound **52** (85 mg) as colorless oil with a yield of 34%.



Product **52**

MS: Calcd. for [$C_{14}H_{24}N_2O_4Na$]: m/z 307.1634

MS: Found: -

TLC: Rf = 0.55

(EtOAc/MeOH = 10/1, v/v)

CAM

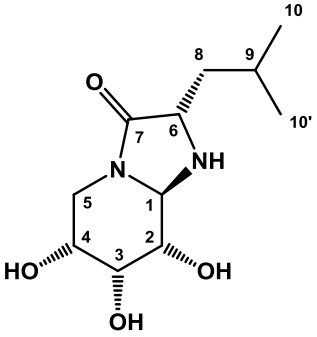
$[a]_D^{20}$ = - 30.7 (c 0.99 MeOH)

^{1}H NMR (300 MHz, MeOH-*d*$_4$) δ = 4.48 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 4.08 (dd, 1H, $J_{3,2}$ 3.4 Hz, $J_{3,4}$ 6.3 Hz, H-3), 3.89-3.78 (m, 2H, H-4, H-6), 3.48 (dd, 1H, $J_{5e,5a}$ 12.6 Hz, $J_{5e,4}$ 4.3 Hz, H-5e), 3.27 (dd, 1H, H-2), 3.01 (dd, 1H, $J_{5a,4}$ 6.5 Hz, H-5a), 1.63, 1.32, 1.16 (s, 9H, 2x C*H*$_3$-isoprop, C-10), 0.74 (s, 4H, C-9, C-10');

^{13}C NMR (75.5 MHz, MeOH-*d*$_4$) δ = 177.2 (C-7), 111.7 (C_q), 79.2 (C-3), 77.5 (C-2), 74.1 (C-1), 65.7 (C-4), 58.4 (C-6), 42.8 (C-8), 42.2 (C-5), 27.4 (*C*H$_3$-isoprop), 26.2 (C-9), 25.6 (*C*H$_3$-isoprop), 23.8 (C-10), 21.9 (C-10');

(1*R*,2*S*,3*R*,4*R*,6*S*)-2,3,4-Hydroxy-6-isobutyl-hexahydroimidazo[1,2-α]pyridine-*NH*-7-one (58)

Following general procedure D, compound **52** (85 mg, 0.28 mmol, 1 eq) gave, after purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd. NH_4OH = 3/1/0.01, v/v/v), 428 mg of a mixture of compound **58** and NH_4Cl.



Product **58**

MS: Calcd. for [$C_{11}H_{20}N_2O_4Na$]: m/z 267.1321

MS: Found: -

TLC: Rf = 0.90

($CHCl_3$/MeOH/concd. NH_4OH = 1/1/0.25, v/v/v)

CAM

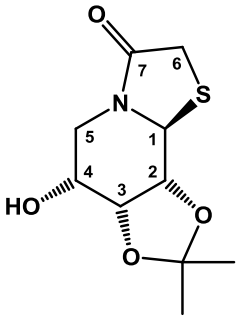
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, D_2O) δ = 4.65 (d, 1H, $J_{1,2}$ 9.3 Hz, H-1), 4.11 (dd, 1H, H-3), 3.92 (dd, 1H, $J_{5e,4}$ 5.5 Hz, $J_{5e,5a}$ 12.1 Hz, H-5e), 3.72-3.59 (m, 2H, H-4, H-6), 3.47 (dd, 1H, $J_{2,3}$ 2.0 Hz, H-2), 3.07 (dd, 1H, H-5a), 1.95-1.82 (m, 1H, H-9), 1.72-1.60 (m, 1H, H-8), 1.59-1.46 (m, 1H, H-8'), 1.04-0.94 (m, 6H, H-10, H-10');

^{13}C NMR (75.5 MHz, D_2O) δ = 175.3 (C-7), 74.0 (C-3), 72.4 (C-2), 72.3 (C-1), 68.0 (C-4), 58.2 (C-6), 41.9 (C-8), 40.7 (C-5), 26.2 (C-9), 23.7 (C-10), 21.9 (C-10');

(1R,2S,3R,4R)-2,3-O-isopropylidene-4-hydroxytetra-5H-thiazolo[3,2-α]pyridine-7-one (44)

Compound **23** (200 mg, 0.93 mmol, 1 eq) was dissolved in MeOH (10 mL) and PMe_3 (1M in toluene, 2.1 mL, 2.1 mmol, 2.3 eq) was added to the reaction mixture. Upon consumption of the starting material (detected by TLC: CH/EtOAc = 1/1, v/v) mercapto acetic acid (110 µL, 1.58 mmol, 1.7 eq) was added and the reaction mixture was stirred until no iminium ion intermediate was detected by TLC (EtOAc/MeOH = 10/1, v/v). The reaction mixture was concentrated under reduced pressure and purified utilizing silica gel chromatography (EtOAc) to give compound **44** (80 mg) with a yield of 35% as colorless oil.



Product **44**

MS: Calcd. for [$C_{10}H_{12}NO_4SNa$]: m/z 268.0619

MS: Found: -

TLC: Rf = 0.75

(EtOAc/MeOH = 10/1, v/v)

CAM

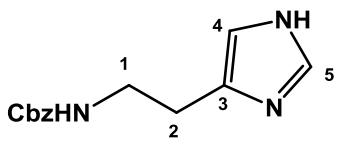
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, MeOH-d_4) δ = 4.82 (d, 1H, $J_{1,2}$ 8.5 Hz, H-1), 4.40 (dd, 1H, $J_{3,2}$ 3.8 Hz, $J_{3,4}$ 3.7 Hz, H-3), 4.25 (dd, 1H, H-2), 4.07 (ddd, 1H, $J_{4.5e}$ 4.8 Hz, H-4), 3.85 (dd, 1H, $J_{5e,5a}$ 13.0 Hz, H-5e), 3.65 (s, 2H, H-6), 3.25 (dd, 1H, $J_{5a,4}$ 8.0 Hz, H-5a), 1.79 (s, 3H, CH_3-isoprop), 1.75 (CH_3-isoprop);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 172.6 (C-7), 111.8 (C_q), 82.3 (C-2), 77.7 (C-3), 65.6 (C-4), 62.3 (C-1), 44.6 (C-5), 32.8 (C-6), 27.7 (*C*H_3-isoprop), 25.9 (*C*H_3-isoprop);

Benzyl(*NH-*imidazol-3-yl)carbamate (63)

Following general procedure E, histamine dihydrochloride (**62**, 1 g, 5.40 mmol, 1 eq) was dissolved in MeOH (10 mL). Et_3N (1.50 mL, 10.9 mmol, 2 eq) and Cbz chloride (840 µL, 6.00 mmol, 1.1 eq) were added. Purification utilizing silica gel chromatography (EtOAc/MeOH = 20/1, v/v) gave compound **63** (500 mg) as colorless solid with a yield of 33%.



Product **63**

MS: Calcd. for [$C_{13}H_{15}N_3O_2Na$]: m/z 268.1062

MS: Found: -

TLC: Rf = 0.5

(EtOAc/MeOH/ = 10/1, v/v)

CAM

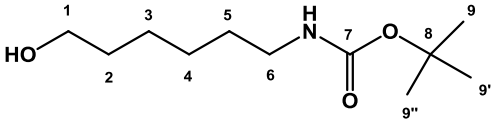
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.73 (s, 1H, H-5), 7.31 (m, 5H, Ph), 6.88 (s, 1H, H-4), 5.05 (s, 2H, CH_2Ph), 3.37 (t, 2H, H-1), 2.78 (t, 2H, H-2);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 158.8 (C=O), 138.4 (C_q), 135.8 (C-5), 135.4 (C-3), 129.4, 128.9, 128.7 (Ph), 118.0 (C-4), 67.3 (*C*H_2Ph), 41.6 (C-1), 28.0 (C-2);

Boc protected spacer moiety, compound 60

6-Aminohexanol (**59**, 1 g, 8.50 mmol, 1 eq) was dissolved in CH_2Cl_2 (10 mL). Et_3N (2.4 mL, 17.1 mmol, 2 eq) and *tert*.-butanolate anhydride (2.00 g, 9.40 mmol, 1.1 eq) were added to the reaction mixture. Upon consumption of the starting material (detected by TLC: CH/EtOAc = 1/1, v/v) the reaction mixture was extracted with 2N HCl and sat. $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The obtained sirup **60** (1 g) was employed in the next step without further purification.



Product **60**

MS: Calcd. for $[C_{11}H_{23}NO_3Na]$: m/z 240.1576

MS: Found: -

TLC: Rf = 0.45

(CH/EtOAc/ = 1/1, v/v)

Van

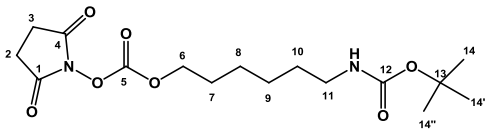
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, MeOH-d_4) δ = 3.03 (t, 2H, H-1), 2.29 (t, 2H, H-6), 1.62-1.30 (m, 17H, H-2, H-3, H-4, H-5, CH_3-9, CH_3-9', CH_3-9''');

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 177.7 (C-7), 79.8 (C-8), 62.7 (C-1), 41.2, 35.0, 30.7, 27.4, 25.8 (C-2, C-3, C-4, C-5, C-6), 27.4 (C-9, C-9', C9''');

Succimidyl activated and Boc protected spacer moiety, compound 61

Compound **60** (1 g, 4.60 mmol, 1 eq) was dissolved in CH_3CN (35 mL), Et_3N (1.3 mL, 9.2 mmol, 2 eq) and *N,N'*-disuccinimidyl carbonate (1.4 g, 5.10 mmol, 1.1 eq) were added to the reaction mixture and it was allowed to reach 40°C. After TLC indicated full conversion of the starting material (CH/EtOAc = 1/1, v/v), the reaction mixture was concentrated under reduced pressure and the obtained sirup was taken up in EtOAc. The organic phase was extracted with sat. $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated under reduced pressure. Compound **61** (1.7 g) was obtained as colorless solid and employed in the next step without further purification.



Product **61**

MS: Calcd. for [$C_{17}H_{27}NO_7Na$]: m/z 380.1685

MS: Found: -

TLC: Rf = 0.40

(CH/EtOAc/ = 1/1, v/v)

CAM

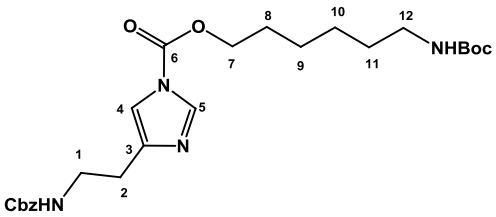
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 4.25 (t, 2H, H-6), 3.10-2.96 (m, 2H, H-11), 2.79-2.75 (m, 2H, H-2), 1.99-1.95 (m, 2H, H-3), 1.77-1.24 (m, 17H, H-7, H-8, H-9, H-10, C*H*$_3$-14, C*H*$_3$-14', C*H*$_3$-14''');

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 168.7 (C-1, C-4), 156.0 (C-12), 151.7 (C-5), 79.0 (C-13), 71.4 (C-6), 40.1 (C-11), 29.8, 28.3, 26.3, 25.1 (C-7, C-8, C-9, C-10), 28.4 (C-14, C-14', C-14''), 25.5 (C-2, C-3);

Cbz and Boc protected acyl imidazole moiety, compound 64

Compound **63** (500 mg, 1.80 mmol, 1 eq) was dissolved in DMF (20 mL) and pyridine (435 µL, 5.30 mmol, 3 eq). Compound **61** (910 mg, 2.70 mmol, 1.5 eq) was added to the reaction mixture and stirred at ambient temperature. After consumption of the starting material (detected by TLC: EtOAc/MeOH = 1/1, v/v) the reaction mixture was concentrated under reduced pressure, taken up in CH_2Cl_2 and extracted with H_2O and sat. NaCl solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Purification of the obtained syrup utilizing silica gel chromatography (CH/EtOAc = 1/1, v/v) gave compound **64** (760 mg) as colorless solid with a yield of 87%.



Product **64**

MS: Calcd. for [$C_{25}H_{36}N_4O_6Na$]: m/z 511.2533

MS: Found: -

TLC: Rf = 0.75

(EtOAc/MeOH = 10/1, v/v)

CAM

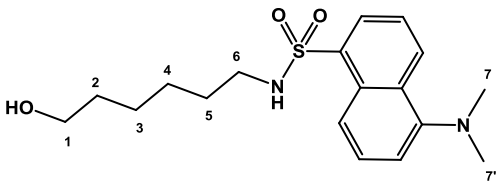
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.00 (s, 1H, H-5), 7.31-7.18 (m, 5H, Ph), 7.11 (s, 1H, H-4), 5.06-4.96 (m, 2H, C*H*$_2$Ph), 4.31 (t, 2H, H-7), 3.48-3.39 (m, 2H, H-1), 3.09-2.98 (m, 2H, H-12), 2.73-2.64 (m, 2H, H-2), 1.76-1.24 (m, 17H, H-8, H-9, H-10, H-11, 3x(C*H*$_3$)-Boc);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 156.0 (2x C=O), 148.6 (C-6), 141.4 (C_q), 136.6 (C-3, C-5), 128.5-128.0 (Ph), 113.8 (C-4), 78.8 (C_q-Boc), 68.3 (*C*H$_2$Ph), 66.7 (C-7), 60.5 (C-2), 40.3 (C-12), 30.0, 28.1, 26.3, 25.4 (C-2, C-8, C-9, C-10, C-11), 28.4 (3x*C*H$_3$-Boc);

Dansylated spacer moiety, compound 67

6-Aminohexanol (**59**, 110 mg, 0.85 mmol, 1 eq) was dissolved in MeOH (5 mL) and Et_3N (250 µL, 1.8 mmol, 2.1 eq). Dansylchloride (**66**, 240 mg, 1.90 mmol, 1.1 eq) was added and the reaction mixture was stirred at ambient temperature. After consumption of the starting material (detected by TLC: EtOAc/MeOH = 10/1, v/v) the reaction mixture was concentrated under reduced pressure and purified utilizing silica gel chromatography (EtOAc/MeOH = 10/1, v/v). Compound **67** (330 mg) was obtained as greenish oil with a yield of 96%.



Product **67**

MS: Calcd. for [$C_{18}H_{26}N_2O_3SNa$]: m/z 373.1562

MS: Found: -

TLC: Rf = 0.80

(EtOAc/MeOH = 10/1, v/v)

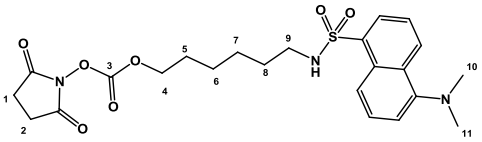
CAM

^{1}H NMR (300 MHz, MeOH-d_4) δ = 8.54 (d, 1H, C*H*-dansyl), 8.36 (d, 1H, C*H*-dansyl), 8.18 (d, 1H, C*H*-dansyl), 7.56 (q, 2H, C*H*-dansyl), 7.25 (d, 1H, C*H*-dansyl), 3.39 (t, 2H, H-1), 2.86 (s, 6H, H-7, H-7‘), 2.83 (t, 2H, H-6), 1.29 (m, 4H, H-2, H-3), 1.09 (m, 4H, H-4, H-5);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 153.2, 137.2, 131.2, 131.0 (C_q-dansyl), 131.1, 130.1, 129.0, 124.3, 120.6, 116.4 (*C*H-dansyl), 62.7 (C-1), 45.8 (C-7, C-7’), 43.8 (C-6), 33.3, 30.5, 27.3, 26.3 (C-2, C-3, C-4, C-5);

Succimidyl activated dansyl moiety, compound 68

Compound **67** (100 mg, 0.30 mmol, 1 eq) was dissolved in CH_3CN (3 mL) and Et_3N (79 µL, 0.60 mmol, 2 eq). *N,N’*-disuccimidyl carbonate (80 mg, 0.31 mmol, 1.1 eq) was added and the reaction mixture was stirred at 40°C. After TLC indicated full conversion of the starting material (CH/EtOAc = 1/2, v/v) the reaction mixture was concentrated under reduced pressure, taken up in CH_2Cl_2 and extracted with 2N HCl and satd. $NaHCO_3$ solution. Purification utilizing silica gel chromatography (CH/EtOAc = 3/1, v/v) gave compound **68** (110 mg) with a yield of 80% as greenish solid.



Product **68**

MS: Calcd. for [$C_{23}H_{29}N_3O_7SNa$]: m/z 514.1624

MS: Found: -

TLC: Rf = 0.66

(CH/EtOAc = 1/2, v/v)

CAM

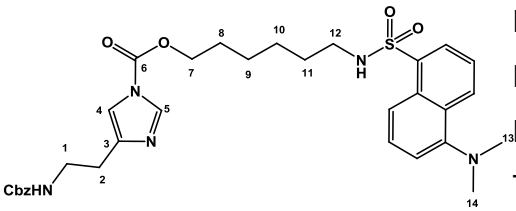
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.53 (d, 1H, C*H*-dansyl), 8.28 (d, 1H, C*H*-dansyl), 8.22 (d, 1H, C*H*-dansyl), 7.53 (q, 2H, C*H*-dansyl), 7.18 (d, 1H, C*H*-dansyl), 4.93 (t, 1H, H-4), 4.18 (t, 2H, H-9), 3.08 (t, 1H, H-4‘), 2.88 (s, 6H, H-10, H-11), 2.84-2.82 (m, 4H, H-1, H-2), 1.53 (t, 2H, H-5), 1.34 (t, 2H, H-6), 1.15 (m, 4H, H-7, H-8);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 168.9 (C-3), 151.6, 135.0, 129.9 (C_q-dansyl), 130.4, 129.7, 128.4, 123.4, 118.9, 115.3 (*C*H-dansyl), 71.3 (C-4), 45.5 (C-10, C-11), 43.1 (C-9), 29.3, 28.2, 25.6, 24.9 (C-5, C-6, C-7, C-8), 25.6 (C-1, C-2);

Cbz protected and dansylated acyl imidazole moiety, compound 69

Compound **68** (110 mg, 0.23 mmol, 1 eq) and compound **63** (65 mg, 0.23 mmol, 1 eq) were dissolved in DMF (5 mL) and pyridine (40 µL, 0.46 mmol, 2 eq). The reaction mixture was stirred at ambient temperature until the starting materials vanished on TLC (EtOAc/MeOH = 10/1, v/v). The reaction mixture was concentrated under reduced pressure, taken up in CH_2Cl_2 and extracted with H_2O and satd. NaCl solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to give compound **69** (120 mg) as greenish oil with a yield of 84 %.



Product **69**

MS: Calcd. for [$C_{32}H_{39}N_5O_6SNa$]: m/z 644.2519

MS: Found: -

TLC: Rf = 0.20

(CH/EtOAc = 1/1, v/v)

CAM

$[a]_D^{20}$ = -

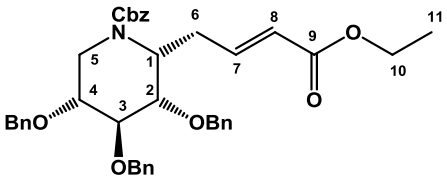
^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.53 (d, 1H, C*H*-dansyl), 8.28 (d, 1H, C*H*-dansyl), 8.22 (d, 1H, C*H*-dansyl), 8.00 (s, 1H, H-5) 7.50 (q, 2H, C*H*-dansyl), 7.29-7.17 (m, 5H, Cbz), 7.16 (d, 1H, C*H*-dansyl), 7.15 (s, 1H, H-4), 5.08-4.93 (m, 2H, CH_2-Cbz), 4.25 (t, 2H, H-7), 3.47-3.36 (m, 2H, H-1), 2.89-2.62 (m, 10H, H-2, H-12, H-13, H-14), 1.59-1.19 (m, 8H, H-8, H-9, H-10, H-11);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 156.5 (C=O), 152.6 (C_q-dansyl), 148.6 (C-6), 136.7 (C-3, C-4), 136.0, 130.0 (C_q-dansyl), 130.4, 129.7 (*C*H-dansyl), 128.5-128.0 (Ph), 128.4, 123.4, 118.9, 115.3 (*C*H-dansyl), 113.8 (C-5), 68.2 (*C*H_2-Cbz), 66.7 (C-3, C-9), 45.5 (C-13, C-14), 43.0 (C-12), 40.4 (C-2), 29.5, 28.2, 25.9, 25.1 (C-8, C-9, C-10, C-11);

1(R)-1-C-(Ethylbut-7-enoatyl)-2,3,4-tri-O-benzyl-5-N-Cbz-1,5-dideoxy-1,5-imino-xylitol (75)

Compound **39** (330 mg, 0.57 mmol, 1 eq) was dissolved in CH_2Cl_2 (10 mL) and treated with Dess Martin Periodinane (360 mg, 0.85 mmol, 1.5 eq). After consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v) the reaction mixture was extracted with 2N HCl and satd. $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The obtained yellow oil was added dropwise to a formerly prepared solution of KOtBu (190 mg, 1.7 mmol, 3 eq) and triethylphosphonoacetate (340 µL, 1.7 mmol, 3 eq) in THF (30 mL).

Upon consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v), CH_2Cl_2 was added and the reaction mixture was extracted with 2N HCl and satd. $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Purification utilizing silica gel chromatography (CH/EtOAc = 10/1, v/v) gave compound **75** (180 mg) as colorless oil with a yield of 49%.



Product **75**

MS: Calcd. for [$C_{40}H_{43}NO_7Na$]: m/z 672.2937

MS: Found: -

TLC: Rf = 0.75

(CH/EtOAc = 2/1, v/v)

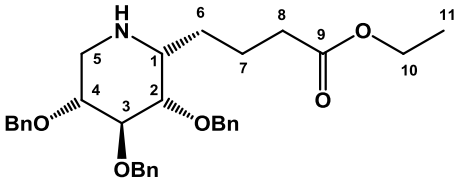
CAM

$[a]_D^{20}$ = -

Due to two pronounced rotameric populations (**75**) of the *N*-Cbz group as well as a mixture of E/Z of the double bond, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution, which results in an unimpretable ^{1}H-NMR spectrum. The respective peaks however are observed in the suspected region. Follow up chemistry, namely Cbz deprotection with concominant reduction of the double bond yielding compound **75a**, reveals that compound **75** was formed as expected.

1(R)-1-C-(Ethylbutyratyl)-2,3,4-tri-O-benzyl-1,5-dideoxy-1,5-imino-xylitol (75a)

Compound **75** (180 mg, 0.28 mmol, 1 eq) was dissolved in MeOH (18 mL). Pd/$BaSO_4$ was added and the reaction mixture was stirred under hydrogen atmosphere until TLC indicated full conversion of the starting material (CH/EtOAc = 2/1, v/v). The reaction mixture was filtered and concentrated under reduced pressure. Compound **75a** (137 mg) was obtained with a yield of 94 % and employed in the next step without further purification.



Product **75a**

MS: Calcd. for [$C_{32}H_{39}NO_5Na$]: m/z 540.2726

MS: Found: -

TLC: Rf = 0.15

(CH/EtOAc = 2/1, v/v)

VAN

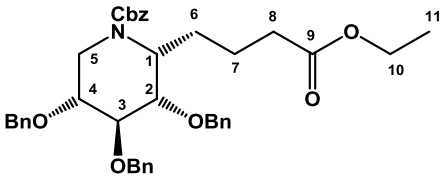
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.28-7.15 (m, 15H, Ph), 4.59-4.36 (m, 6H, CH_2Ph), 4.03 (q, 2H, H-10), 3.72 (dd, 1H, $J_{3,2}$ 4.3 Hz, $J_{3,4}$ 4.5 Hz, H-3), 3.49 (dd, 1H, $J_{2,1}$ 3.8 Hz, H-2), 3.45 (ddd, 1H, $J_{4,5}$ 3.2 Hz, H-4), 3.13-3.06 (m, 2H, H-1, H-5), 2.24-2.16 (m, 2H, H-8), 1.79-1.69 (m, 2H, H-7), 1.17 (t, 3H, H-11);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 173.4 (C-9), 137.9, 137.8 (C_q), 128.7-127-8 (Ph), 77.4 (C-4), 75.3 (C-2), 74.1 (C-3), 73.6, 72.4, 72.1 (*C*H_2Ph), 60.4 (C-10), 55.4 (C-1), 44.4 (C-5), 34.1 (C-8), 27.6 (C-6), 21.2 (C-7), 14.4 (C-11);

1*(R)*-1*-C*-(Ethylbutyratyl)-2,3,4-tri*-O*-benzyl-5*-N*-Cbz-1,5-dideoxy-1,5-imino-xylitol (77)

Compound **75a** (140 mg, 0.26 mmol, 1 eq) was dissolved in MeOH (10 mL), Et_3N (88 µL, 0.62 mmol, 2,4 eq) and CbzCl (45 µL, 0.32 mmol, 1.2 eq) were added and the reaction mixture was stirred at ambient temperature. After consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v) the reaction mixture was concentrated under reduced pressure, taken up in CH_2Cl_2 and extracted with 2N HCl and sat. $NaHCO_3$ solution. After drying the organic phase over Na_2SO_4 it was concentrated under reduced pressure and purified utilizing silica gel chromatography (CH/EtOAc = 10/1, v/v). Compound **77** (110 mg) was obtained as colourless oil with a yield of 65 %.



Product **77**

MS: Calcd. for [$C_{40}H_{45}NO_7Na$]: m/z 674.3094

MS: Found: -

TLC: Rf = 0.70

(CH/EtOAc = 2/1, v/v)

VAN

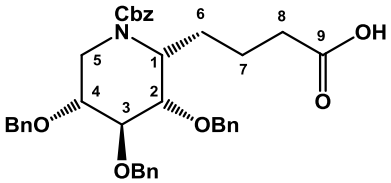
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.26-7.14 (m, 20H, Ph), 5.04-4.95 (m, 2H, C*H*$_2$Cbz), 4.77-4.69 (m, 2H, C*H*$_2$Ph), 4.60-4.47 (m, 4½H, 2xC*H*$_2$Ph, H-1), 4.31 (dd, ½H, $J_{5,4}$ 5.7 Hz, $J_{5e,5a}$ 13.4 Hz, H-5e), 4.26-4.20 (m, ½H, H-1), 4.10-3.97 (m, 2½H, H-10-H-5e), 3.54 (dd, 1H, $J_{3,2}$ 9.5 Hz, $J_{3,4}$ 9.0 Hz, H-3), 3.40 (dd, 1H, $J_{2,1}$ 5.8 Hz, H-2), 3.37-3.24 (m, 1H, H-4), 2.63-dd, 1H, $J_{5a,4}$ 10.9 Hz, H-5a), 2.20 (m, 2H, H-8), 1.75-1.62 (m, 2H, H-7), 1.56-1.42 (m, 2H, H-6), 1.13 (t, 3H, H-11); Due to two pronounced rotameric populations (**77**) of the N-Cbz group, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution of the ^{1}H NMR spectrum.

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 173.3 (d, C-9), 155.6 (d, Cbz), 139.0, 138.2, 136.6 (C_q), 128.7-127.6 (Ph), 82.2 (d, C-3), 79.9 (d, C-2), 78.3 (C-4), 75.8, 73.3, 73.2 (*C*H$_2$Ph), 67.7 (d, *C*H$_2$Cbz), 60.4 (d, C-10), 52.8 (d, C-1), 40.8 (d, C-5), 33.9 (C-8), 24.0 (d, C-7), 21.1 (d, C-6), 14.4 (C-11);

1(*R*)-1-C-(Butyric acidyl)-2,3,4-tri-O-benzyl-5-N-Cbz-1,5-dideoxy-1,5-imino-xylitol (79)

Compound **77** (110 mg, 0.17 mmol, 1 eq) was dissolved in dioxane/H_2O (10 mL, 1/1, v/v) and treated with 5 drops of a 3M NaOH solution. After consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v) the solution was acidified with 2N HCl solution and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The obtained oil was purified utilizing silica gel chromatography (EtOAc) and gave compound **79** (80 mg) with a yield of 76 % as colorless oil.



Product **79**

MS: Calcd. for [$C_{38}H_{41}NO_7Na$]: m/z 646.2781

MS: Found: -

TLC: Rf = 0.10

(CH/EtOAc = 2/1, v/v)

CAM

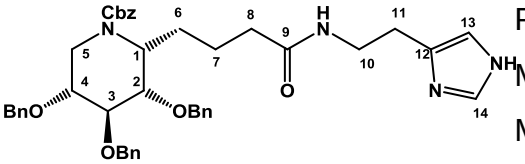
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.26-7.14 (m, 20H, Ph), 5.05-4.97 (m, 2H, CH_2Cbz), 4.78-4.69 (m, 2H, CH_2Ph), 4.61-4.46 (m, 4½H, 2xCH_2Ph, H-1), 4.32 (dd, ½H, $J_{5,4}$ 5.5 Hz, $J_{5e,5a}$ 13.3 Hz, H-5e), 4.25-4.18 (m, ½H, H-1), 4.07 (dd, ½H, H-5e), 3.55 (dd, 1H, $J_{3,2}$ 9.3 Hz, $J_{3,4}$ 8.9 Hz, H-3), 3.42 (dd, 1H, $J_{2,1}$ 5.7 Hz, H-2), 3.38-3.26 (m, 1H, H-4), 2.63 (dd, 1H, $J_{5a,4}$ 10.4 Hz, H-5a), 2.37-2.15 (m, 2H, H-8), 1.78-1.65 (m, 2H, H-7), 1.54-1.44 (m, 2H, H-6); Due to two pronounced rotameric populations (**79**) of the N-Cbz group, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution of the ^{1}H NMR spectrum.

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 179.1 (d, C-9), 155.7 (d, Cbz), 138.9, 138.1, 136.5 (C_q), 128.7-127.7 (Ph), 82.1 (d, C-3), 79.9 (d, C-2), 78.3 (C-4), 75.8, 73.3, 72.9 (*C*H_2Ph), 67.7 (d, *C*H_2Cbz), 52.8 (d, C-1), 40.8 (d, C-5), 33.5 (C-8), 23.8 (d, C-7), 20.8 (d, C-6); Due to two pronounced rotameric populations (**79**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed in the ^{13}C NMR spectrum.

1(R)-1-C-(10-N-(NH-imidazol-12-yl)ethyl)butyramidyl)-2,3,4-tri-O-benzyl-5-N-Cbz-1,5-dideoxy-1,5-imino-xylitol (81)

Compound **79** (80 mg, 0.13 mmol, 1 eq) was dissolved in DMF (15 mL). COMU (110 mg, 0.26 mmol, 2 eq) and DIEA (90 µL, 0.52 mmol, 4 eq) were added to the reaction mixture and stirred for 30 minutes at ambient temperature. Then, histamine dihydrochloride (36 mg, 0.20 mmol, 1.5 eq) was added to the reaction mixture and stirred until the starting material vanished on TLC (EtOAc/MeOH = 10/1, v/v). The reaction mixture was concentrated under reduced pressure and purified twice utilizing silica gel chromatography (EtOAc/MeOH = 20/1, v/v and $CHCl_3$/MeOH/concd NH_4OH = 6/1/0.01, v/v/v). Compound **81** (75 mg) was obtained in a yield of 81 % as yellowish solid.



Product **81**

MS: Calcd. for [$C_{43}H_{48}N_4O_6Na$]: m/z 739.3472

MS: Found: -

TLC: Rf = 0.80

($CHCl_3$/MeOH/concd NH_4OH = 6/1/0.01, v/v/v)

CAM

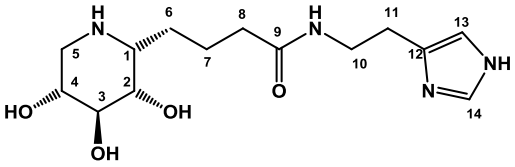
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.67 (s, 1H, H-14), 7.30-7.11 (m, 20H, Ph), 6.72 (s, 1H, H-13), 5.05-4.93 (m, 2H, C*H*$_2$Cbz), 4.77-4.68 (m, 2H, C*H*$_2$Ph), 4.61-4.44 (m, 4½H, 2xC*H*$_2$Ph, H-1), 4.29 (dd, ½H, $J_{5,4}$ 5.9 Hz, $J_{5e,5a}$ 13.5 Hz, H-5e), 4.28-4.20 (m, ½H, H-1), 4.09 (dd, ½H, H-5e), 3.53 (dd, 1H, $J_{3,2}$ 9.1 Hz, $J_{3,4}$ 9.1 Hz, H-3), 3.43-3.26 (m, 4H, H-2, H-4, H-10), 2.75-2.61 (m, 3H, H-5a, H-11), 2.18-1.94 (m, 2H, H-8), 1.72-1.30 (m, 4H, H-6, H-7); Due to two pronounced rotameric populations (**81**) of the N-Cbz group, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution of the ^{1}H NMR spectrum.

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 173.3 (d, C-9), 155.7 (d, Cbz), 138.9, 138.1, (C_q) 138.0 (C-12) 136.4 (C_q-Ph), 134.3 (C-14), 128.7-127.6 (Ph), 116.3 (d, C-13), 82.0 (d, C-3), 79.8 (d, C-2), 78.2 (C-4), 75.7, 73.2, 72.9 (*C*H_2Ph), 67.6 (d, *C*H_2Cbz), 52.8 (d, C-1), 41.0 (d, C-5), 39.0 (C-10), 35.9 (C-8), 26.4 (C-11), 24.0 (d, C-7), 21.8 (d, C-6); Due to two pronounced rotameric populations (**81**) of the N-Cbz group, signal splitting in the respective NMR spectra has been observed in the ^{13}C NMR spectrum.

1(*R*)-1-*C*-(10-*N*-(*NH*-Imidazol-12-yl)ethyl)butyramidyl)-1,5-dideoxy-1,5-imino-xylitol (83)

Compound **81** (75 mg, 1.0 mmol, 1 eq) was dissolved in MeOH (5 mL) and stirred with $Pd(OH)_2$ on activated charcoal under hydrogen atmosphere. After consumption of the starting material (detected by TLC: $CHCl_3$/MeOH/concd NH_4OH = 6/1/0.01, v/v/v) the reaction mixture is filtered and concentrated under reduced pressure. The crude material **83** was found to contain major impurities and could not be purified.



Product **83**

MS: Calcd. for [$C_{14}H_{24}N_4O_4Na$]: m/z 335.1695

MS: Found: -

TLC: Rf = 0.45

($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v)

CAM

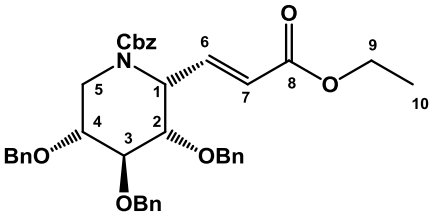
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, D_2O) δ = 8.49 (s, 1H, H-14), 7.22 (s, 1H, H-12), 4.02-3.92 (m, 3H, H-2, H-3, H-4), 3.50-2.23 (m, 5H, H-1, H-5, H-10), 2.92-2.83 (m, 2H, H-11), 2.30-2.21 (m, 2H, H-8), 1.78-1.56 (m, 4H, H-6, H-7);

^{13}C NMR (75.5 MHz, D_2O) δ = 175.9 (C-9), 133.5 (C-14), 131.3 (C-12), 116.5 (C-13), 67.5 (C-3), 67.0 (C-2), 66.0 (C-4), 54.7 (C-1), 45.3 (C-5), 38.0 (C-10), 35.0 (C-8), 27.1 (C-11), 24.3 (C-7), 20.8 (C-6);

***1*(R)*-1*-C*-(Ethylprop-6-enoatyl)-2,3,4-tri*-O*-benzyl-5*-N*-Cbz-1,5-dideoxy-1,5-imino-xylitol* (74)**

Compound **38** (550 mg, 0.98 mmol, 1 eq) was dissolved in CH_2Cl_2/MeOH (100 mL, 1/1, v/v) and stirred under ozone atmosphere at -30°C until no starting material was detected on TLC (CH/EtOAc = 2/1, v/v). N_2 was bubbled through the reaction mixture to remove ozone traces and dimethylsulfide (200 µL) was added to the reaction mixture. It was stirred for 45 minutes, followed by concentration under reduced pressure. The resulting colorless oil was added dropwise to a prepared solution of KOtBu (330 mg, 2.90 mmol, 3 eq) and triethylphosphonoacetate (580 µL, 2.90 mmol, 3 eq) in THF (50 mL). Upon consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v), CH_2Cl_2 was added and extracted with 2N HCl and satd. $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Purification utilizing silica gel chromatography (CH/EtOAc = 10/1, v/v) gave compound **74** (500 mg) with a yield of 78% as colorless oil.



Product **74**

MS: Calcd. for [$C_{39}H_{41}NO_7Na$]: m/z 358.2781

MS: Found: -

TLC: Rf = 0.55

(CH/EtOAc = 2/1, v/v)

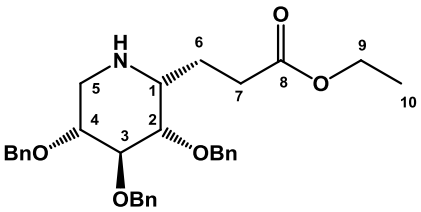
VAN

$[a]_D^{20}$ = -

Due to two pronounced rotameric populations (**74**) of the *N*-Cbz group as well as a mixture of E/Z of the double bond, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution, which results in an unimpretable ^{1}H-NMR spectrum. The respective peaks however are observed in the suspected region. Follow up chemistry, namely Cbz deprotection with concominant reduction of the double bond yielding compound **74a**, reveals that compound **74** was formed as expected.

1(R)-1-C-(Ethylpropionatyl)-2,3,4-tri-O-benzyl-1,5-dideoxy-1,5-imino-xylitol (74a)

Compound **74** (1.4 g, 2.2 mmol, 1 eq) was dissolved in MeOH (30 mL), Pd/$BaSO_4$ was added and the reaction mixture stirred under hydrogen atmosphere until the starting material vanished on TLC (CH/EtOAc = 2/1, v/v). The reaction mixture was filtered and concentrated under reduced pressure. Compound **74a** (500 mg) was purified utilizing silica gel chromatography (CH/EtOAc = 1/1, v/v) and isolated with a yield of 45% as colorless oil.



Product **74a**

MS: Calcd. for [$C_{31}H_{37}NO_5Na$]: m/z 526.2569

MS: Found: -

TLC: Rf = 0.10

(CH/EtOAc = 1/1, v/v)

CAM

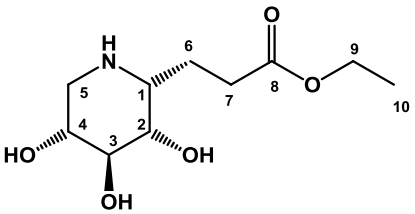
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.32-7.10 (m, 15H, Ph), 4.53-4.40 (m, 6H, C*H*$_2$Ph), 4.05 (q, 2H, H-9), 3.68 (dd, 1H, $J_{3,2}$ 5.7 Hz, $J_{3,4}$ 5.5 Hz, H-3), 3.33 (dd, 1H, 1H, $J_{2,1}$ 4.5 Hz, H-2), 3.32 (ddd, 1H, $J_{4,5}$ 5.6 Hz, H-4), 2.96-2.87 (m, 2H, H-1, H-5e), 2.81 (dd, 1H, $J_{5a,5e}$ 13.5 Hz, H-5a), 2.40-2.19 (m, 2H, H-7), 1.85-1.75 (m, 2H, H-6), 1.17 (t, 3H, H-10);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 173.9 (C-8), 138.7, 138.6, 138,5 (3x C_q), 128.5-127.7 (Ph), 78.2 (C-4), 77.4 (C-2), 76.5 (C-3), 73.9, 72.4, 72.0 (3x *C*H_2Ph), 60.4 (C-9), 54.5 (C-1), 44.6 (C-5), 31.5 (C-7), 24.0 (C-6), 14.4 (C-10);

***1*(R)-1-C-(Ethylpropionatyl)-1,5-dideoxy-1,5-imino-xylitol (89)**

Compound **76** (150 mg) was dissolved in MeOH/H_2O (1/1, v/v) and $Pd(OH)_2$ on activated charcoal was added to the solution. The reaction mixture was stirred under hydrogen atmosphere until the starting material vanished on TLC (CH/EtOAc = 1/2, v/v). The reaction mixture was filtered, concentrated under reduced pressure and the obtained oil was purified utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 3/1/0.01, v/v/v). Compound **89** (50 mg) was obtained with a yield of 72% as colourless oil.



Product **89**

MS: Calcd. for [$C_{10}H_{19}NO_5Na$]: m/z 256.1161

MS: Found: -

TLC: Rf = 0.80

(CHC_3/MeOH/concd NH_4OH = 1/1/0.25, v/v/v)

CAM

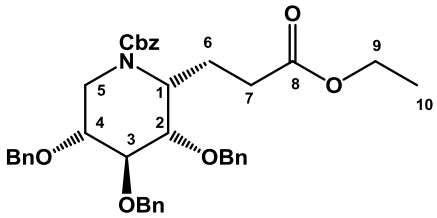
$[a]_D^{20}$ = -13.8 (c 1.2 H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 4.10 (q, 2H, H-9), 3.99-3.94 (m, 2H, H-3, H-4), 3.90-3.86 (m, 1H, H-2), 3.46 (ddd, 1H, $J_{1,2}$ 1.3 Hz, H-1), 3.36 (dd, 1H, $J_{5,4}$ 1.6 Hz, $J_{5e,5a}$ 13.8 Hz, H-5e), 3.24 (dd, 1H, H-5a), 2.58-2.40 (m, 2H, H-7), 2.08-1.91 (m, 2H, H-6), 1.18 (t, 3H, H-10);

^{13}C NMR (75.5 MHz, D_2O) δ = 174.9 (C-8), 67.5 (C-2), 67.0 (C-3), 66.1 (C-4), 62.1 (C-9), 54.3 (C-1), 45.5 (C-5), 29.4 (C-7), 23.0 (C-6), 13.4 (C-10);

1(R)-1-C-(Ethylpropionatyl)-2,3,4-tri-O-benzyl-5-N-Cbz-1,5-dideoxy-1,5-imino-xylitol (76)

Compound **74** (750 mg, 1.40 mmol, 1 eq) was dissolved in MeOH (20 mL) and Et_3N (480 µL, 3.40 mmol, 2.4 eq). CbzCl (250 µL, 1.70 mmol, 1.2 eq) was added and the reaction mixture was stirred at ambient temperature. Upon consumption of the starting material (detected by TLC: CH/EtOAc = 1/1, v/v) the reaction mixture was concentrated under reduced pressure, taken up in CH_2Cl_2 and extracted with 2N HCl and sat. $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Compound **76** (270 mg) was obtained after purification utilizing silica gel chromatography (CH/EtOAc = 10/1, v/v) with a yield of 24% as colorless oil.



Product **76**

MS: Calcd. for [$C_{39}H_{43}NO_7Na$]: m/z 660.2937

MS: Found: -

TLC: Rf = 0.45

(CH/EtOAc = 3/1, v/v)

CAM

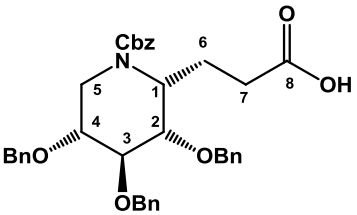
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.29-7.12 (m, 20H, Ph), 5.03-4.94 (m, 2H, C*H*$_2$Cbz), 4.80-4.74 (m, 2H, C*H*$_2$Ph), 4.65-4.50 (m, 4½H, 2xC*H*$_2$Ph, H-1), 4.36-4.26 (m, 1H, H-1, H-5e), 4.09-3.90 (m, 2½H, H-9, H-5e), 3.58 (dd, 1H, $J_{3,2}$ 9.0 Hz, $J_{3,4}$ 9.2 Hz, H-3), 3.42 (dd, 1H, $J_{2,1}$ 6.1 Hz, H-2), 3.32 (ddd, 1H, $J_{4,5}$ 5.5 Hz, H-4), 2.65 (dd, 1H, $J_{5a,5e}$ 13.1 Hz, H-5a), 2.22-2.07 (m, 2H, H-7), 1.93-1.72 (m, 2H, H-6), 1.12 (t, 3H, H-10); Due to two pronounced rotameric populations (**76**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution of the ^{1}H NMR spectrum.

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 173.1 (d, C-8), 155.6 (d, Cbz), 138.9, 138.2, 136.4 (C_q-Ph), 128.7-127.0 (Ph), 82.0 (d, C-3), 79.6 (d, C-2), 78.2 (C-4), 75.8, 73.2, 72.8 (d, *C*H$_2$Ph), 67.7 (d, *C*H$_2$Cbz), 60.5 (d, C-9), 52.7 (d, C-1), 40.9 (d, C-5), 30.7 (C-7), 19.9 (d, C-6), 14.3 (C-10); Due to two pronounced rotameric populations (**76**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed in the ^{13}C NMR spectrum.

1(R)-1-C-(Ethylpropionic acidyl)-2,3,4-tri-O-benzyl-5-N-Cbz-1,5-dideoxy-1,5-imino-xylitol (78)

Compound **76** (220 mg, 0.35 mmol, 1 eq) was dissolved in dioxane/H_2O (20 mL, 1/1, v/v) and a 3M NaOH solution was added dropwise (1 mL). After consumption of the starting material (detected by TLC: CH/EtOAc = 3/1, v/v) the reaction mixture was acidified with 2N HCl and extracted with EtOAc. The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. Compound **78** (230 mg) was obtained as slightly impure oil with a yield of 109% and employed in the next step without further purification.



Product **78**

MS: Calcd. for [$C_{37}H_{39}NO_7Na$]: m/z 632.2624

MS: Found: -

TLC: Rf = 0.60

(EtOAc)

CAM

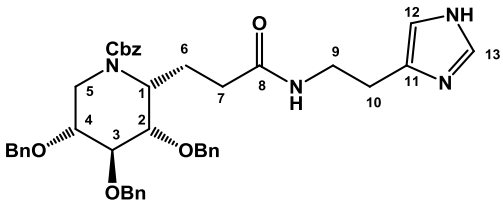
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.27-7.08 (m, 20H, Ph), 5.02-4.90 (m, 2H, CH_2Cbz), 4.81-4.72 (m, 2H, CH_2Ph), 4.57-4.35 (m, 5H, 2xCH_2Ph, H-1), 4.19 (dd, ½H, $J_{5,4}$ 5.2 Hz, $J_{5e,5a}$ 13.3 Hz, H-5e), 4.03 (dd, ½H, H-5e), 3.51 (dd, 1H, $J_{3,2}$ 8.5 Hz, $J_{3,4}$ 9.1 Hz, H-3), 3.35-3.18 (m, 2H, H-2, H-4), 2.64 (dd, 1H, H-5a), 2.18-1.63 (m, 4H, H-6, H-7); Due to two pronounced rotameric populations (**78**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution of the ^{1}H NMR spectrum.

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 179.1 (d, C-9), 155.7 (d, Cbz), 138.9, 138.1, 136.5 (C_q-Ph), 128.7-127.7 (Ph), 82.1 (d, C-3), 79.9 (d, C-2), 78.3 (C-4), 75.8, 73.3, 72.9 (*C*H_2-Ph), 67.7 (d, *C*H_2-Cbz), 52.8 (d, C-1), 40.8 (d, C-5), 33.5 (C-8), 23.8 (d, C-7), 20.8 (d, C-6); Due to two pronounced rotameric populations (**78**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed in the ^{13}C NMR spectrum.

1(R)-1-C-(9-N(NH-imidazo-11-yl)ethyl)butyramidyl)-2,3,4-tri-O-benzyl-N-Cbz-1,5-dideoxy-1,5-iminoxylitol (80)

Compound **78** (340 mg, 0.57 mmol, 1 eq) was dissolved in DMF (20 mL). COMU (490 mg, 1.14 mmol, 2 eq) and DIEA (400 µL, 2.33 mmol, 4 eq) were added and the reaction mixture was stirred for 30 minutes at ambient temperature. Histamine dihydrochloride (160 mg, 0.86 mmol, 1.5 eq) was added and the reaction mixture was stirred until the starting material vanished on TLC (EtOAc/MeOH = 10/1, v/v). The reaction mixture was concentrated under reduced pressure and purified utilizing silica gel chromatography (EtOAc/MeOH = 10/1, v/v) to give compound **80** (300 mg) as yellow oil in a yield of 75 %.



Product **80**

MS: Calcd. for [$C_{42}H_{46}N_4O_6Na$]: m/z 725.3315

MS: Found: -

TLC: Rf = 0.60

($CHCl_3$/MeOH/concd. NH_4OH = 6/1/0.01, v/v/v)

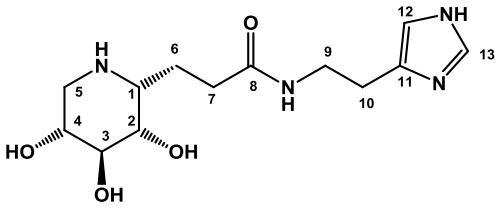
CAM

$[\alpha]_D^{20}$ = -

Due to two pronounced rotameric populations (**80**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed leading to poor resolution of the ^{1}H NMR and ^{13}C NMR spectrum, rendering interpretation impossible.

1(R)-1-C-(9-N-(NH-Imidazo-11-yl)ethyl)propylamidyl)-1,5-dideoxy-1,5-imino-xylitol (82)

Compound **80** (300 mg, 0.43 mmol, 1 eq) was dissolved in MeOH/H_2O (15 mL, 1/1, v/v), $Pd(OH)_2$ activated charcoal was added and the reaction mixture was stirred under hydrogen atmosphere. Upon consumption of the starting material (detected by TLC: $CHCl_3$/MeOH/concd NH_4OH = 6/1/0.01, v/v/v) the reaction mixture was filtered and concentrated under reduced pressure. After purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 3/1/0.25, v/v/v) compound **82** (100 mg) was obtained as colourless oil with a yield of 78%.



Product **82**

MS: Calcd. for [$C_{13}H_{22}N_4O_4Na$]: m/z 321.1539

MS: Found: -

TLC: Rf = 0.50

($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v)

CAM

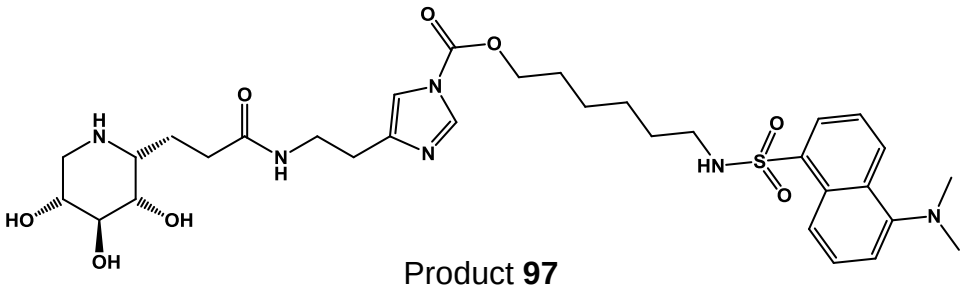
$[a]_D^{20}$ = - 6.5 (c 1 H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 7.83 (s, 1H, H-13), 6.92 (s, 1H, H-12), 3.77 (ddd, 1H, $J_{4,3}$ 3.6 Hz, $J_{4,5e}$ 4.7 Hz, $J_{4,5a}$ 2.6 Hz, H-4), 3.75 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{2,1}$ 4.9 Hz, H-2), 3.69 (dd, 1H, H-3), 3.36 (t, 2H, H-9), 3.14 (ddd, 1H, H-1), 3.11 (dd, 1H, H-5a), 2.97 (dd, 1H, $J_{5e,5a}$ 13.6 Hz, H-5e), 2.72 (t, 2H, H-10), 2.22 (t, 2H, H-7), 1.89-1.70 (m, 2H, H-6);

^{13}C NMR (75.5 MHz, D_2O) δ = 175.1 (C-8), 135.1 (C-13), 133.6 (C-11), 116.8 (C-12), 68.9 (C-4, C-2), 67.0 (C-3), 59.5 (C-1), 54.6 (C-5), 38.7 (C-9), 31.7 (C-7), 25.4 (C-10), 23.3 (C-6);

1*-C-*Acyl imidazole derived LDPP probe, compound (97)

Compound **82** (100 mg, 0.34 mmol, 1 eq) was dissolved in DMF (10 mL). DIEA (117 µL, 0.67 mmol, 2 eq) and compound **68** (180 mg, 0.36 mmol, 1 eq) were added to the reaction mixture and it was stirred until no starting material was detected on TLC ($CHCl_3$/MeOH/concd. NH_4OH = 1/1/0.25, v/v/v). The reaction mixture was concentrated under reduced pressure and purified utilizing silica gel chromatography (EtOAc/MeOH = 10/1, v/v) and subsequent preparative TLC ($CHCl_3$/MeOH/concd NH_4OH = 4/1/0.005, v/v/v). The silica gel was extracted with MeOH dest., filtered and concentrated under reduced pressure to afford compound **97** (10 mg) in a yield of 4.4%.



Product **97**

MS: Calcd. for $[C_{32}H_{46}N_6O_6SNa]$:m/z 697.2996

MS: Found: -

TLC: Rf = 0.55

($CHCl_3$/MeOH/concd NH_4OH = 6/1/0.01, v/v/v)

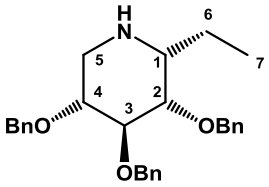
CAM

$[a]_D^{20}$ = -

The small amounts of obtained compound **97** rendered interpretation of the NMR respective NMR spectra impossible. The spectrum is shown in chapter 7.

1(R)-1-C-Ethyl-1,5-dideoxy-1,5-imino-xylitol (84)

Compound **38** (1.2 g, 2.13 mmol, 1 eq) was dissolved in MeOH (20 mL). $Pd/BaSO_4$ was added and the reaction mixture was stirred under hydrogen atmosphere. Upon consumption of the starting material (detected by TLC: CH/EtOAc = 3/1, v/v) the reaction mixture was filtered and concentrated under reduced pressure. Compound **84** (740 mg) was obtained with a yield of 81% as colorless oil.



Product **84**

MS: Calcd. for $[C_{28}H_{33}NO_3Na]$: m/z 321.1539

MS: Found: -

TLC: Rf = 0.2

(CH/EtOAc = 3/1, v/v)

CAM

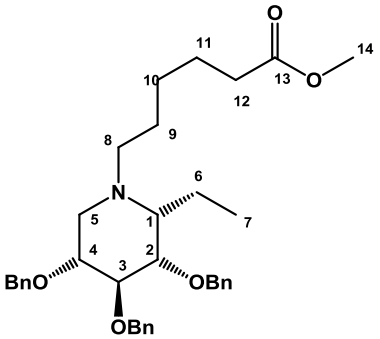
$[\alpha]_D^{20}$ = - 1.1 (c 1.0 $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.35-7.07 (m, 15H, Ph), 4.65-4.35 (m, 6H, C*H*$_2$Ph), 3.67 (dd, 1H, $J_{3,4=3,2}$ 5.9 Hz, H-3), 3.40-3.29 (m, 2H, H-2, H-4), 2.91 (dd, 1H, $J_{5e,4}$ 3.9 Hz, $J_{5e,5a}$ 13.7 Hz, H-5e), 2.85-2.75 (m, 2H, H-5a, H-1), 1.55-1.42 (m, 2H, H-6), 0.81 (t, 3H, H-7);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 138.7 (3x C_q), 128.4-127.6 (Ph), 78.1 (C-2), 76.9 (C-3), 76.6 (C-4), 73.8, 72.2, 71.9 (3x *C*H_2-Ph), 56.5 (C-1), 44.4 (C-5), 21.2 (C-6), 10.8 (C-7);

1(R)-1-C-Ethyl-5-N-(methylhexanoatyl)-2,3,4-tri-O-benzyl-1,5-dideoxy-1,5-imino-xylitol (85)

Compound **84** (740 mg, 1.72 mmol, 1 eq) was dissolved in DMF (20 mL). 6-iodohexylmethylester (660 mg, 2.60 mmol, 1.5 eq) and Na_2CO_3 (545 mg, 5.15 mmol, 3 eq) were added and the reaction mixture was stirred at 60°C. Upon consumption of the starting material (detected by TLC: CH/EtOAc = 2/1, v/v) the reaction mixture was concentrated under reduced pressure, taken up in CH_2Cl_2 and extracted with 2N HCl and satd. $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Purification utilizing silica gel chromatography gave compound **85** (640 mg) in a yield of 67% as colourless oil.



Product **85**

MS: Calcd. for $[C_{35}H_{45}NO_5Na]$: m/z 582.3195

MS: Found: -

TLC: Rf = 0.55

(CH/EtOAc = 2/1, v/v)

VAN

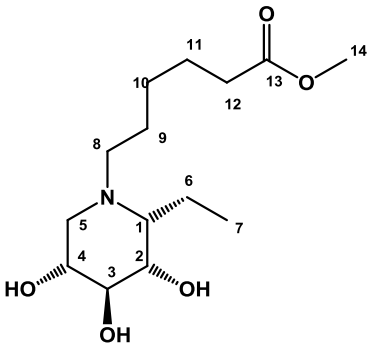
$[\alpha]_D^{20}$ = +12.7 (c 1.1 $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.40-7.0 (m, 15H, Ph), 4.84-4.70 (m, 2H, C*H*$_2$Ph), 4.65-4.50 (m, 4H, C*H*$_2$Ph), 3.60 (s, 3H, H-14), 3.57-3.43 (m, 3H, H-2, H-3, H-4), 2.78-2.61 (m, 2H, H-1, H-5e), 2.52-2.34 (m, 3H, H-5a, H-8), 2.22 (t, 2H, H-12), 1.63-1.13 (m, 8H, H-6, H-9, H-10, H-11), 0.87 (t, 3H, H-7);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 174.3 (C-13), 139.2, 138.8, 138.7 (3x C_q), 128.4-127.4 (Ph), 83.2 (C-4), 80.6 (C-2), 78.3 (C-3), 75.5, 73.1, 72.8 (3x *C*H_2Ph), 61.6 (C-1), 54.2 (C-8), 51.5 (C-14), 48.5 (C-5), 34.2 (C-12), 28.4 (C-9), 26.7 (C-10), 24.9 (C-11), 16.5 (C-6), 13.5 (C-7);

***1*(R)-*1*-C-*Ethyl-5*-N-*(methylhexanoatyl)-1,5-dideoxy-1,5-imino-xylitol (90)**

Compound **85** (350 mg, 0.6 mmol, 1 eq) was treated as described in general procedure C. Purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd. NH_4OH = 10/1/0.01, v/v/v) gave compound **90** (160 mg) with a yield of 88% as colorless oil.



Product **90**

MS: Calcd. for $[C_{14}H_{27}NO_5Na]$: m/z 312.1787

MS: Found: -

TLC: Rf = 0.66

($CHCl_3$/MeOH/concd. NH_4OH = 3/1/0.01, v/v/v)

CAM

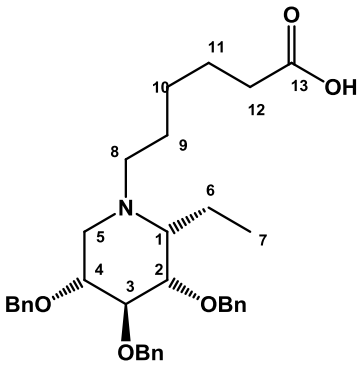
$[\alpha]_D^{20}$ = +7.4 (c 1.0 MeOH)

^{1}H NMR (300 MHz, MeOH-d_4) δ = 4.05-3.89 (m, 3H, H-2, H-3, H-4), 3.69 (s, 3H, H-14), 3.57-3.46 (m, 1H, H-5e), 3.43-3.29 (m, 2H, H-1, H-5a), 3.28-3.15 (m, 2H, H-8), 2.14 (t, 2H, H-12), 2.03-1.85 (m, 2H, H-6), 1.83-1.65 (m, 4H, H-9, H-11), 1.52-1.37 (m, 2H, H-10), 1.06 (t, 3H, H-7);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 175.7 (C-13), 69.4 (C-4), 69.2 (C-3, C-2), 63.7 (C-1), 54.3 (C-5), 53.7 (C-8), 52.1 (C-14), 34.5 (C-12), 27.2 (C-10), 25.5 (C-11), 23.4 (C-9), 19.8 (C-6), 10.3 (C-7);

***1*(R)-*1*-C-*ethyl-5*-N-*(Hexanoicacidyl)-2,3,4-tri*-O-*benzyl-1,5-dideoxy-1,5-iminoxylitol (86)**

Compound **85** (60 mg, 0.11 mmol, 1 eq) was dissolved in dioxane/H_2O (5 mL, 1/1, v/v). 3M NaOH solution (10 drops) was added and the reaction mixture was stirred until the starting material vanished on TLC (CH/EtOAc = 2/1, v/v). EtOAc was added and the reaction mixture was extracted with 2N HCl and satd. $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Compound **86** (40 mg) was obtained as colourless oil with a yield of 69%.



Product **86**

MS: Calcd. for [$C_{34}H_{43}NO_5Na$]: m/z 568.3039

MS: Found: -

TLC: Rf = 0.45

(CH/EtOAc = 1/1, v/v)

CAM

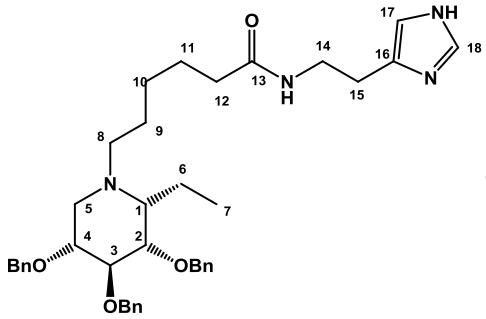
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.35-7.06 (m, 15H, Ph), 4.83-4.43 (m, 6H, C*H*$_2$Ph), 3.71-3.50 (m, 3H, H-2, H-3, H-4), 3.05-3.34 (m, 2H, H-1, H-5e), 2.68-2.42 (m, 3H, H-5a, H-8), 2.20 (t, 2H, H-12), 1.70-1.11 (m, 8H, H-6, H-9, H-10, H-11), 0.89 (t, 3H, H-7);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 138.8, 138.5, 138.4 (3x C_q), 128.6-127.5 (Ph), 81.0, 79.3, 77.3 (C-2, C-3, C-4), 75.2, 73.2, 73,0 (3x *C*H_2-Ph), 61.0 (C-1), 53.4 (C-8), 48.3 (C-5), 34.6 (C-12), 27.0, 26.6, 24.9 (C-9, C-10, C-11), 16.4 (C-6), 13.4 (C-7);

1*(R)-*1*-C-*5*-N-*(14*-N-*(Imidazo-16-yl)ethyl)hexylamidyl)-2,3,4-tri*-O-*benzyl-1,5-dideoxy-1,5-imino-xylitol (87)

Compound **86** (400 mg, 0.73 mmol, 1 eq) was dissolved in DMF (20 mL). COMU (704 mg, 1.46 mmol, 2 eq) and DIEA (509 µL, 2.92 mmol, 4 eq) were added and the reaction mixture was stirred for 30 minutes. Histamine dihydrochloride (203 mg, 1.10 mmol, 1.5 eq) was added to the reaction mixture and stirred until no starting material was detected on TLC (EtOAc/MeOH = 10/1, v/v). The reaction mixture was concentrated under reduced pressure and purified utilizing silica gel chromatography ($CHCl_3$/MeOH/concd. NH_4OH = 12/1/0.01, v/v/v) to give compound **87** (140 mg) as yellow solid with small impurities in a yield of 30%.



Product **87**

MS: Calcd. for [$C_{34}H_{43}NO_5Na$]: m/z 568.3039

MS: Found: -

TLC: Rf = 0.45

(CH/EtOAc = 1/1, v/v)

CAM

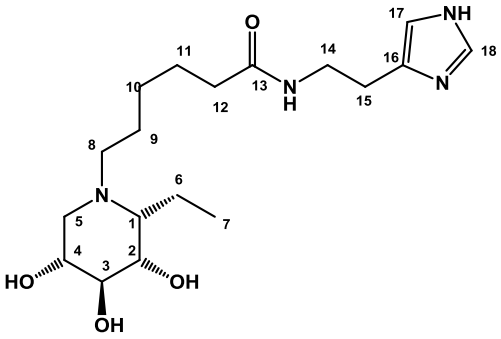
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.67 (s, 1H, H-18), 7.40-7.26 (m, 15H, Ph), 6.90 (s, 1H, H-17), 4.82-4.52 (m, 6H, CH_2Ph), 3.82-3.65 (m, 3H, H-2, H-3, H-4), 3.45 (t, 2H, H-14), 3.12-3.00 (m, 2H, H-1, H-5e), 2.92-2.73 (m, 3H, H-5a, H-12), 2.19 (t, 2H, H-8), 1.79-1.23 (m, 8H, H-6, H-9, H-10, H-11), 0.93 (t, 3H, H-7);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 176.1 (C-13), 139.9, 139.7, 139.6 (3x C_q), 136.0 (C-18), 135.6 (C-16), 129.5-128.9 (Ph), 118.2 (C-17), 78.5, 77.1 (C-2, C-3, C-4), 75.5, 73.7, 73.6 (3x CH_2-Ph), 63.0 (C-1), 54.6 (C-12), 50.5 (C-5), 40.3 (C-14), 37.0 (C-8), 27.7, 27.5, 27.0, 26.7 (C-9, C-10, C-11, C-15), 18.1 (C-6), 13.2 (C-7);

1(R)-1-C-5-N-(14-N-(Imidazo-16-yl)ethyl)hexylamidyl)-1,5-dideoxy-1,5-imino-xylitol (88)

Following general procedure C, $Pd(OH)_2$/C was added to a 10% solution of compound **87** (140 mg, 0.22 mmol, 1 eq) in MeOH/H_2O (1/1, v/v) and stirred under a hydrogen atmosphere. Purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 3/1/0.25, v/v/v) gave compound **88** (70 mg) in a yield of 86 % as colourless oil.



Product **88**

MS: Calcd. for [$C_{18}H_{32}N_4O_4Na$]: m/z 391.2321

MS: Found: -

TLC: Rf = 0.60

($CHCl_3$/MeOH/concd NH_4OH = 2/1/0.25, v/v/v)

CAM

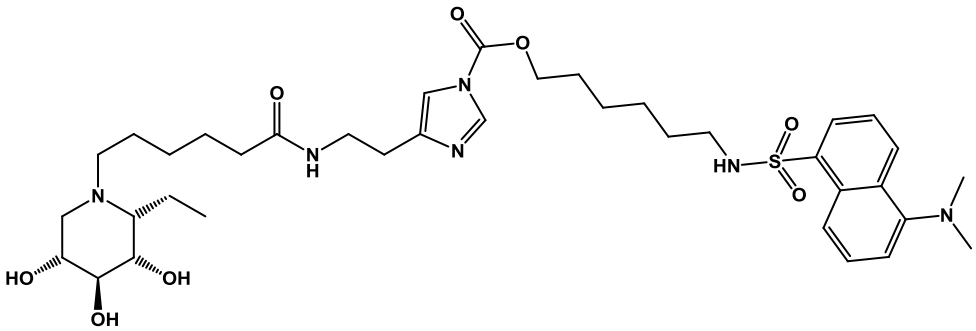
$[a]_D^{20}$ = +5.8 (c 1.04 H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 7.95 (s, 1H, H-18), 6.96 (s, 1H, H-17), 3.87 (ddd, 1H, $J_{4.5e}$ 3.1 Hz, $J_{4,5a}$ 5.7 Hz, $J_{4,3}$ 5.6 Hz, H-4), 3.82 (dd, 1H, $J_{2,1}$ 3.1 Hz, $J_{2,3}$ 4.9 Hz, H-2), 3.75 (dd, 1H, H-3), 3.38 (t, 2H, H-14), 3.27-3.16 (m, 2H, H-1, H-5e), 3.00 (dd, 1H, $J_{5a,5e}$ 12.5 Hz, H-5a), 2.93 (t, 2H, H-12), 2.76 (t, 2H, H-15), 2.13 (t, 2H, H-8), 1.79-1.42 (m, 6H, H-6, H-9, H-11), 1.29-1.10 (m, 2H, H-10), 0.92 (t, 3H, H-7);

^{13}C NMR (75.5 MHz, D_2O) δ = 176.6 (C-13), 142.0 (C-18), 134.7 (C-16), 120.6 (C-7), 69.4 (C-3), 68.6 (C-4), 67.6 (C-2), 62.4 (C-1), 52.7 (C-12), 51.8 (C-5), 38.4 (C-14), 35.4 (C-8), 25.4 (C-10), 25.2 (C-9), 24.9 (C-15), 22.9 (C-11), 17.1 (C-6), 10.8 (C-7);

1*-C-*Ethyl*-N-*acyl imidazole LDPP probe, compound 98

Compound **88** (50 mg, 0.14 mmol, 1 eq) dissolved in DMF (5 mL) and treated as described at compound **97** with DIEA (70 µL, 0.41 mmol, 3 eq) and compound **68** (100 mg, 0.20 mmol, 1.5 eq). Purification utilizing silica gel chromatography (MeOH) and subsequent preparative TLC ($CHCl_3$/MeOH/concd. NH_4OH = 5/1/0.005, v/v/v) afforded compound **98** (20 mg) in a yield of 19%.



Product **98**

MS: Calcd. for [$C_{37}H_{56}N_6O_8SNa$]:m/z 767.3778

MS: Found: -

TLC: Rf = 0.40

($CHCl_3$/MeOH/concd NH_4OH = 3/1/0.01, v/v/v)

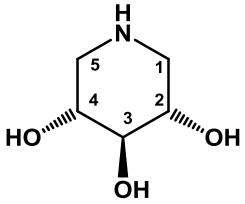
CAM

$[\alpha]_D^{20}$ = -

The small amounts of obtained compound **98** rendered interpretation of the respective NMR spectra impossible. The spectrum is shown in chapter 7.

1,5-Dideoxy-1,5-imino-xylitol (XVII)

Compound **1** (1.8 g, 10.3 mmol, 1 eq.) was dissolved in MeOH/H_2O (15 mL, 1/1, v/v), $Pd(OH)_2$ on activated charcoal was added and the reaction mixture was stirred under hydrogen atmosphere until full consumption of the starting material was observed on TLC ($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v). The reaction mixture was filtered and concentrated under reduced pressure. Compound **XVII** (1.3 g) was obtained as colorless oil in 95% yield and used without further purification.



Product **XVII**

MS: Calcd. for [$C_5H_{11}NO_3Na$]: m/z 156.0637

MS: Found: -

TLC: Rf = 0.33

($CHCl_3$/MeOH/concd NH_4OH = 1/1/0.25, v/v/v)

CAM

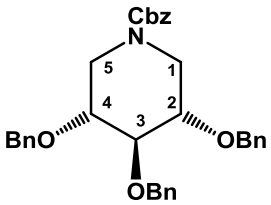
$[\alpha]_D^{20}$ = - 3.14 (c 1.14 H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 3.50 (ddd, 2H, $J_{4,5e\,=\,2,1e}$ 4.9 Hz, $J_{4,3=2,3}$ 8.9 Hz, $J_{4,5a=2,1a}$ 10.8 Hz, H-2, H-4), 3.27 (dd, 1H, H-3), 3.13 (dd, 2H, $J_{5e,5a=1e,1a}$ 12.5 Hz, H-5e, H-1e), 2.51 (dd, 2H, H-5a, H-1a);

^{13}C NMR (75.5 MHz, D_2O) δ = 77.0 (C-3), 69.4 (C-2, C-4), 48.1 (C-1, C-5);

5*-N-*Cbz-2,3,4-Tri*-O-*benzyl-1,5-dideoxy-1,5-imino-xylitol (91)

Following general procedure E, compound **XVII** (880 mg, 6.60 mmol, 1 eq) gave a yellow oil (2.8 g, 10.5 mmol) and was further treated as described in general procedure F. The obtained material was purified employing silica gel chromatography (CH/EtOAc = 10/1, v/v) to give coumpound **91** (1.5 g) as colourless oil in a yield of 42%.



Product **91**

MS: Calcd. for [$C_{34}H_{35}NO_5Na$]: m/z 560.2413

MS: Found: -

TLC: Rf = 0.66

(CH/EtOAc = 2/1, v/v)

CAM

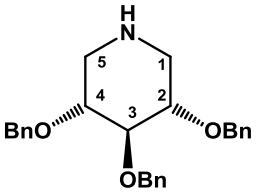
$[a]_D^{20}$ = - 0.75 (c 1.19 $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.27-7.08 (m, 20H, Ph), 5.10-4.95 (m, 2H, C*H*$_2$Cbz), 4.83-4.72 (m, 2H, C*H*$_2$Ph), 4.70-4.50 (m, 4H, 2xC*H*$_2$Ph), 4.40-4.02 (m, 2H, H-5e, H-1e), 3.48-3.26 (m, 3H, H-2, H-3, H-4), 2.70-2.57 (m, 2H, H-5a, H-1a); Due to two pronounced rotameric populations (**91**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution of the ^{1}H NMR spectrum.

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 155.1 (Cbz), 138.8, 138.2, 136.5 (C_q-Ph), 128.6-127.7 (Ph), 85.4 (C-3), 77.6 (C-2, C-4), 75.5 (*C*H_2Ph), 73.0 (2x *C*H_2Ph), 67.5 (CH_2Cbz), 46.1 (C-1, C-5); Due to two pronounced rotameric populations (**91**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed in the ^{13}C NMR spectrum.

***2,3,4-Tri*-O-*benzyl*-1,5-dideoxy-1,5-imino-xylitol (92)**

Compound **91** (1.8 g, 3.35 mmol, 1 eq) was dissolved in MeOH (30 mL). Pd/$BaSO_4$ was added to the reaction mixture and it was stirred under hydrogen atmosphere until no starting material was detected on TLC (CH/EtOAc = 2/1, v/v). The reaction mixture was filtered and concentrated under reduced pressure. Purification employing column chromatography (EtOAc) gave coumpound **92** (800 mg) as colourless oil in a yield of 59%.



Product **92**

MS: Calcd. for [$C_{26}H_{29}NO_3Na$]: m/z 426.2045

MS: Found: -

TLC: Rf = 0.1

(EtOAc)

CAM

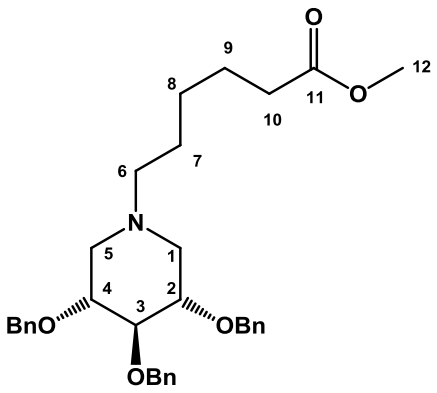
$[a]_D^{20}$ = - 0.02 (c 1.3 $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.40-7.14 (m, 15H, Ph), 4.82-4.77 (m, 2H, C*H*$_2$-Ph), 4.67-4.53 (m, 4H, 2x C*H*$_2$-Ph), 3.42 (dd, 1H, $J_{3,2=3,4}$ 8.0 Hz, H-3), 3.36 (ddd, 2H, $J_{4,5e=2,1e}$ 4.9 Hz, $J_{4,5a=2-1a}$ 10.2 Hz, H-2, H-4), 3.12 (dd, 2H, $J_{5e,5a=1e,1a}$ 12.4 Hz, H-1e, H-5e), 2,41 (dd, 2H, H-1a, H-5a);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 139.1, 138.7 (3x C_q-Ph) 128.4-127.5 (Ph), 85.4 8 (C-3), 79.9 (C-2, C-4), 75.5 (*C*H_2-Ph), 72.9 (2x *C*H_2-Ph), 49.2 (C-1, C-5);

***5*-N-*(Methylhexanoatyl)*-2,3,4-tri-O-benzyl-1,5-dideoxy-1,5-imino-xylitol (93)**

Compound **92** (1.3 g, 3.22 mmol, 1 eq) was dissolved in DMF (20 mL), 6-iodohexylmethyl ester (1.04 g, 3.86 mmol, 1.2 eq) and Na_2CO_3 (819 mg, 7.73 mmol, 2.4 eq) were added and the reaction mixture was heated to 60°C. After consumption of the starting material (detected on TLC: EtOAc) the reaction mixture was concentrated under reduced pressure, taken up in CH_2Cl_2 and extracted with 2N HCl and satd $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified employing silica gel chromatography (CH/EtOAc = 4/1, v/v). Compound **93** (1.62 g) was obtained as colorless oil in a yield of 95%.



Product **93**

MS: Calcd. for [$C_{33}H_{41}NO_5Na$]: m/z 554.2882

MS: Found: -

TLC: Rf = 0.66

(CH/EtOAc = 1/1, v/v)

CAM

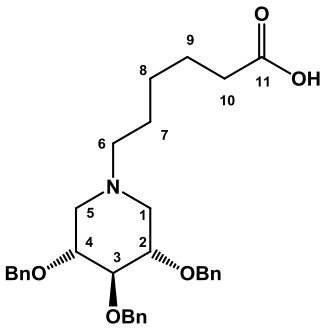
$[a]_D^{20}$ = -0.45 (c 1.00 $CHCl_3$)

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.32-7.16 (m, 15H, Ph), 4.86-4.77 (m, 2H, CH_2Ph), 4.70-4.53 (m, 4H, 2x CH_2Ph), 3.52 (s, 3H, H-12), 3.49 (ddd, 2H, $J_{4,5e=2,1e}$ 4.9 Hz, $J_{4,5a=2-1a}$ 9.6 Hz, $J_{4,3=2,3}$ 8.6 Hz, H-2, H-4), 3.30 (dd, 1H, H-3), 2.96 (dd, 2H, $J_{5e,5a=1e,1a}$ 10.6 Hz, H-1e, H-5e), 2.28 (t, 2H, H-6), 2.23 (t, 2H, H-10), 1.84 (dd, 2H, H-5a, H-1a), 1.55 (q, 2H, H-9), 1.37 (q, 2H, H-7), 1.20 (q, 2H, H-8);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 147.1 (C-11), 139.1, 138.7 (3x C_q) 128.4-127.6 (Ph), 86.5 (C-3), 78.9 (C-2, C-4), 75.4 (CH_2Ph), 73.1 (2x CH_2Ph), 57.6 (C-6), 56.3 (C-1, C-5), 51.5 (C-12), 34.0 (C-10), 26.9 (C-8), 26.6 (C-7), 24.8 (C-9);

***5*-N-*(Hexanoic acidyl)*-2,3,4-*tri*-O-*benzyl*-1,5-dideoxy-1,5-imino-xylitol (94)**

Compound **93** (1.60 g, 3.00 mmol, 1 eq) was dissolved in dioxane/H_2O (20 mL, 1/1, v/v). 3M NaOH solution (2 mL) was added and the reaction mixture was heated to 40°C. After consumption of the starting material (detected on TLC: CH/EtOAc = 1/1, v/v) EtOAc and 2N HCl was added to the reaction mixture. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Purification employing silica gel chromatography (CH/EtOAc = 1/1, v/v) gave compound **94** (1.18 g) as colorless crystals in a yield of 75%.



Product **94**

MS: Calcd. for [$C_{32}H_{39}NO_5Na$]: m/z 540.2726

MS: Found: -

TLC: Rf = 0.5

(EtOAc/MeOH = 1/1, v/v)

CAM

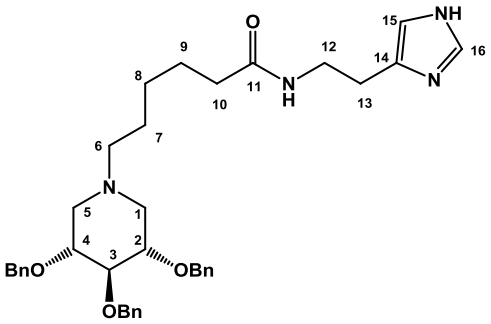
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.47-7.24 (m, 15H, Ph), 4.76-4.59 (m, 6H, CH_2-Ph), 3.93-3.85 (m, 3H, H-3, H-2, H-4), 3.49-3.28 (m, 4H, H-1, H-5), 3.13 (t, 2H, H-6), 2.35 (t, 2H, H-10), 1.82-1.60 (m, 4H, H-7, H-9), 1.49-1.35 (m, 2H, H-8);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 177.2 (C-11), 139.1, 138.9 (3x C_q-Ph), 129.5-129.1 (Ph), 74.4 (C-3), 74.2 (C-2, C-4), 73.5 (CH_2-Ph), 58.3 (C-6), 53.5 (C-1, C-5), 34.5 (C-10), 27.1 (C-8), 25.4 (C-9), 24.5 (C-7);

5*-N-*(12*-N-*(Imidazo-14-yl)ethyl)hexylamidyl)-2,3,4-tri*-O-*benzyl-1,5-dideoxy-1,5-imino-xylitol (95)

Compound **94** (1.20 g, 2.30 mmol, 1 eq) was dissolved in DMF (40 mL). Histamine dihydrochloride (630 mg, 3.40 mmol, 1.5 eq), COMU (1.95 g, 4.60 mmol, 2 eq) and DIEA (1.6 mL, 9.11 mmol, 4 eq) were added to the reaction mixture and stirred until the starting material vanished on TLC (EtOAc/MeOH = 10/1, v/v). The reaction mixture was concentrated under reduced pressure and purified employing silica gel chromatography ($CHCl_3$/MeOH/concd. NH_4OH = 12/1/0.01, v/v/v). Compound **95** (1.4 g) was obtained as yellow oil with a yield of 85%.



Product **95**

MS: Calcd. for [$C_{37}H_{45}N_3O_5Na$]: m/z 634.3257

MS: Found: -

TLC: Rf = 0.33

($CHCl_3$/MeOH/concd. NH_4OH = 6/1/0.01, v/v/v)

CAM

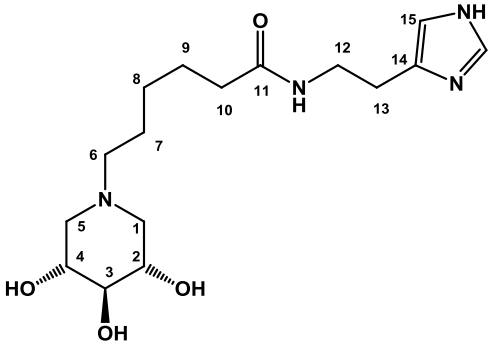
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, MeOH-d_4) δ = 7.75 (s, 1H, H-16), 7.31-7.10 (m, 15H, Ph), 6.84 (s, 1H, H-15), 4.70-4.65 (m, 2H, CH_2Ph), 4.56-4.51 (m, 4H, 2x CH_2Ph), 3.51 (ddd, 2H, $J_{4,3=2,3}$ 8.5 Hz, $J_{4,5e=2,1e}$ 4.2 Hz, $J_{4,5a=2,1a}$ 9.0 Hz, H-2, H-4), 3.36 (dd, 1H, H-3), 3.32 (t, 2H, H-12), 3.05 (dd, 2H, $J_{5e,5a=1e,1a}$ 11.0 Hz, H-5e, H-1e), 2.69 (t, 2H, H-13), 2.43 (t, 2H, H-6), 2.13 (dd, 2H, H-5a, H-1a), 2.06 (t, 2H, H-10), 1.53-1.43 (m, 2H, H-9), 1.41-1.31 (m, 2H, H-7), 1.21-1.10 (m, 2H, H-8);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 176.1 (C-11), 140.1, 139.7 (C_q), 135.8 (C-16), 135.4 (C-14), 129.5-128.7 (Ph), 118.1 (C-15), 84.1 (C-3), 78.3 (C-2, C-4), 75.9 (*C*H_2Ph), 73.8 (2x *C*H_2Ph), 58.6 (C-6), 56.3 (C-1, C-5), 40.0 (C-12), 36.9 (C-10), 27.8 (C-8), 27.4 (C-9), 26.8 (C-7), 26.7 (C-13);

***5*-N-*(12*-N-*(Imidazo-14-yl)ethyl)hexalamidyl)-1,5-dideoxy-1,5-imino-xylitol (96)**

Following general procedure C, $Pd(OH)_2$/C was added to a 10% solution of compound **95** (1.4 g, 2.29 mmol, 1eq) in MeOH/H_2O (1/1, v/v) and stirred under a hydrogen atmosphere. Purification employing silica gel chromatography ($CHCl_3$/MeOH/concd NH_4OH = 5/1/0.25, v/v/v) gave compound **96** (370 mg) in a yield of 47% as colourless oil.



Product **96**

MS: Calcd. for [$C_{16}H_{27}N_3O_5Na$]: m/z 364.1848

MS: Found: -

TLC: Rf = 0.75

($CHCl_3$/MeOH/concd. NH_4OH = 1/1/0.25, v/v/v)

CAM

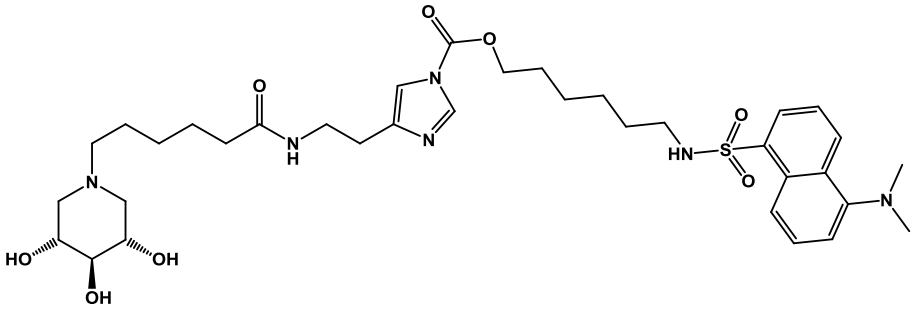
$[a]_D^{20}$ = 0.0 (c 1.11 H_2O)

^{1}H NMR (300 MHz, D_2O) δ = 7.57 (s, 1H, H-16), 6.80 (s, 1H, H-15), 3.44 (ddd, 2H, $J_{4,3=2,3}$ 9.1 Hz, $J_{4,5e=2,1e}$ 4.9 Hz, $J_{4,5a=2,1a}$ 10.6 Hz, H-2, H-4), 3.33 (t, 2H, H-12), 3.10 (dd, 1H, H-3), 2.91 (dd, 2H, $J_{5e,5a=1e,1a}$ 10.9 Hz, H-5e, H-1e), 2.67 (t, 2H, H-13), 2.26 (t, 2H, H-6), 2.07 (t, 2H, H-10), 1.89 (dd, 2H, H-5a, H-1a), 1.48-1.37 (m, 2H, H-9), 1.37-1.26 (m, 2H, H-7), 1.11-0.93 (m, 2H, H-8);

^{13}C NMR (75.5 MHz, D_2O) δ = 176.8 (C-11), 135.7 (C-16), 134.6 (C-14), 117.0 (C-15), 78.4 (C-3), 69.5 (C-2, C-4), 56.8 (C-1, C-5), 56.7 (C-6), 38.8 (C-12), 33.6 (C-10), 25.9 (C-13), 25.9 (C-9), 25.1 (C-7), 25.1 (C-8);

N-*Acyl imidazole derived LDPP probe, compound 99*

Compound **96** (100 mg, 0.29 mmol, 1 eq) was dissolved in DMF (5 mL) and treated as described at compound **97** with DIEA (122 µL, 0.70 mmol, 2.4 eq) and compound **68** (340 mg, 0.69 mmol, 2.3 eq). Purification employing silica gel chromatography (MeOH) and subsequent preparative TLC ($CHCl_3$/MeOH/concd. NH_4OH = 5/1/0.01) afforded compound **99** (10 mg) with a yield of 4.8%.



Product **99**

MS: Calcd. for [$C_{35}H_{52}N_6O_8SNa$]: m/z 739.3465

MS: Found: -

TLC: Rf = 0.80

($CHCl_3$/MeOH/concd. NH_4OH = 3/1/0.01, v/v/v)

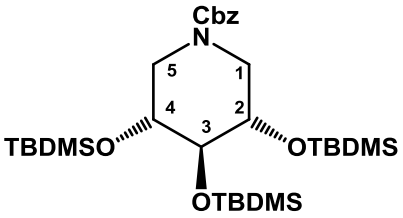
CAM

$[\alpha]_D^{20}$ = -

The small amounts of obtained compound **X** rendered interpretation of the NMR respective NMR spectra impossible. The spectrum is shown in chapter 7.

***5*-N-*Cbz*-2,3,4-*Tri*-O-*TBDMS*-1,5-dideoxy-1,5-imino-xylitol (100)**

Compound **XVII** (860 mg, 3.20 mmol, 1 eq) was dissolved in DMF (9 mL). TBDMS chloride (2.2 g, 14.5 mmol, 4.5 eq) and imidazole (2 g, 29.0 mmol, 9 eq) were added and the reaction mixture was stirred at 60°C until no starting material was detected on TLC ($CHCl_3$/MeOH/concd. NH_4OH = 6/1/0.01, v/v/v). The reaction mixture was concentrated under reduced pressure, taken up in CH_2Cl_2 and extracted with 2N HCl and satd. $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Purification utilizing silica gel chromatography (CH/EtOAc = 40/1, v/v) gave compound **100** (720 mg) as colorless oil with a yield of 37%.



Product **100**

MS: Calcd. for $[C_{31}H_{59}NO_5Si_3Na]$:m/z 632.3599

MS: Found: -

TLC: Rf = 0.75

(CH/EtOAc = 5/1, v/v)

VAN

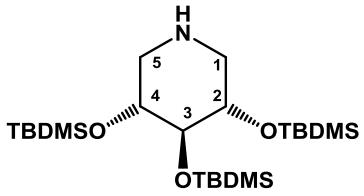
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.39-7.12 (m, 5H, Cbz), 5.18-5.04 (m, 2H, C*H*$_2$Cbz), 3.58-3.33 (m, 7H, H-1, H-2, H-3, H-4, H-5), 0.96-0.64 (m, 27H, C(C*H*$_3$)$_3$), 0.14-(-0.21) (m, 18H, Si-C*H*$_3$)$_2$); Due to two pronounced rotameric populations (**100**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed leading to somewhat poor resolution of the ^{1}H NMR spectrum.

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 155.8 (C=O), 137.0 (C_q), 128.6-127.8 (Cbz), 75.0 (C-3), 71.3 (C-2, C-4), 71.3 (*C*H$_2$Cbz), 46.7 (C-1, C-5), 26.9 (C_q-TBDMS), 26.1, 26.0, 25.9 (C(*C*H$_3$)$_3$-TBDMS), -4.3 ((*C*H$_3$)$_2$-TBDMS); Due to two pronounced rotameric populations (**100**) of the *N*-Cbz group, signal splitting in the respective NMR spectra has been observed in the ^{13}C NMR spectrum.

2,3,4-Tri*-O-*TBDMS-1,5-dideoxy-1,5-imino-xylitol (101)

Compound **100** (710 mg, 0.114 mmol, 1 eq) was dissolved in MeOH (10 mL). $Pd/BaSO_4$ was added and the reaction mixture was stirred under hydrogen atmosphere. Upon consumption of the starting material (detected by TLC: CH/EtOAc = 5/1, v/v) the reaction mixture was filtered and concentrated under reduced pressure. Compound **101** (460 mg) was obtained as colourless oil in a yield of 85%.



Product **101**

MS: Calcd. for $[C_{23}H_{53}NO_3Si_3Na]$:m/z 498.3231

MS: Found: -

TLC: Rf = 0.45

(CH/EtOAc = 3/1, v/v)

VAN

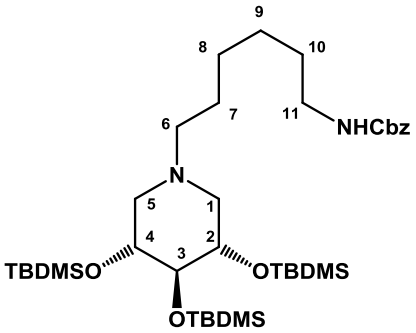
$[\alpha]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 3.55-3.50 (m, 1H, H-3), 3.41-3.35 (m, 2H, H-2, H-4), 2.99-2.89 (m, 2H, H-1e, H-5e), 2.69-2.60 (m, 2H, H-1a, H-5a), 0.84 (s, 27H, C(C*H*$_3$)$_3$), 0.00 (s, 18H, Si-C*H*$_3$)$_2$);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 72.1 (C-3), 70.1 (C-2, C-4), 49.4 (C-1, C-5), 26.1, 25.8 (C(*C*H_3)$_3$-TBDMS), 18.3, 17.8 (C_q-TBDMS), -4.29, -4.70, -4.83 ((*C*H_3)$_2$-TBDMS);

5*-N-*(11*-N-*Cbz-Hexylaminyl)-2,3,4-tri*-O-*TBDSM-1,5-dideoxy-1,5-imino-xylitol (102)

Compound **101** (440 mg, 0.92 mmol, 1 eq) was dissolved in DMF (10 mL). Cbz-NH-iodohexyl (330 mg, 0.92 mmol, 1 eq) and Na_2CO_3 (200 mg, 1.84 mmol, 2 eq) were added and the reaction mixture was stirred at 60°C until no starting material was detected on TLC (CH/EtOAc = 3/1, v/v). The reaction mixture was concentrated under reduced pressure, taken up in CH_2Cl_2 and extracted with 2N HCl and satd. $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and purified utilizing silica gel chromatography (CH/EtOAc = 12/1, v/v) to give compound **102** (600 mg) as colorless oil with a yield of 92%.



Product **102**

MS: Calcd. for $[C_{37}H_{72}N_2O_5Si_3Na]$:m/z 731.4647

MS: Found: -

TLC: Rf = 0.55

(CH/EtOAc = 3/1, v/v)

CAM

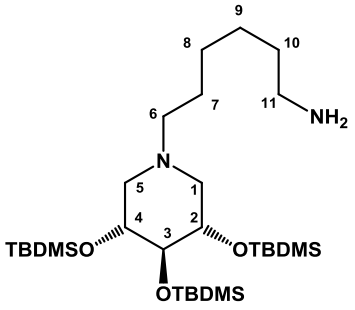
$[a]_D^{20}$ = -

^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.35-7.20 (m, 5H, Cbz), 5.09-4.95 (m, 2H, C*H*$_2$Cbz), 3.48 (ddd, $J_{4,3=2,3}$ 8.1 Hz, $J_{4,5e=2,1e}$ 4.1 Hz, $J_{4,5a=2,1a}$ 9.1 Hz, H-2, H-4), 3.16 (dd, 1H, H-3), 3.10 (t, 2H, H-10), 2.78 (dd, $J_{5e,5a}$ 10.7 Hz, H-5e, H-1e), 2.22 (t, 2H, H-6), 1.79 (dd, 2H, H-5a, H-1a), 1.46-1.29 (m, 4H, H-7, H-9), 1.28-1.16 (m, 4H, H-8, H-11), 0.82 (s, 27H, C(C*H*$_3$)$_3$), 0.00 (s, 18H, Si-(C*H*$_3$)$_2$);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 156.4 (C=O), 136.7 (C_q-Cbz), 128.5-128.1 (Cbz), 79.9 (C-3), 72.7 (C-2, C-4), 66.6 (*C*H$_2$Cbz), 59.6 (C-5), 57.5 (C-6), 41.1 (C-10), 30.2 (C-9, C-11), 27.0, 26.7 (C-7, C-8), 26.5, 26.3 (C(*C*H$_3$)$_3$-TBDMS), 18.3, 18.0 (C_q-TBDMS), -2.94, -4.48 ((CH$_3$)$_2$-TBDMS);

5*-N-*Hexylaminyl-2,3,4-tri*-O-*TBDSM-1,5-dideoxy-1,5-imino-xylitol (103)

Compound **102** (550 mg, 0.78 mmol, 1 eq) was dissolved in MeOH (10 mL). Pd/$BaSO_4$ was added and the reaction was stirred under hydrogen atmosphere. After consumption of the starting material (detected by TLC: CH/EtOAc = 3/1, v/v) the reaction mixture was filtered and concentrated under reduced pressure. Purification utilizing silica gel chromatography ($CHCl_3$/MeOH/concd. NH_4OH = 3/1/0.01, v/v/v) afforded compound **103** (170 mg) as colorless oil with a yield of 38%.



Product **103**

MS: Calcd. for $[C_{37}H_{72}N_2O_5Si_3Na]$:m/z 731.4647

MS: Found: -

TLC: Rf = 0.2

($CHCl_3$/MeOH/concd. NH_4OH = 3/1/0.01, v/v/v)

CAM

$[a]_D^{20}$ = -

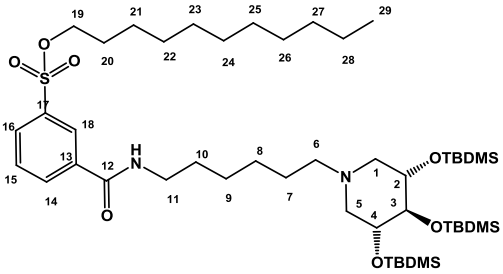
^{1}H NMR (300 MHz, MeOH-d_4) δ = 3.48 (ddd, 2H, $J_{4,3=2,3}$ 1.6 Hz, $J_{4,5e=2,1e}$ 2.9 Hz, $J_{4,5a=2,1a}$ 7.6 Hz, H-2, H-4), 3.20 (dd, 1H, H-3), 2.81(dd, $J_{5e,5a=1e,1a}$ 10.8 Hz, H-5e, H-5a), 2.62 (t, 2H, H-10), 2.29 (t, 2H, H-6), 1.88 (dd, 2H, H-5a, H-1a), 1.48-1.32 (m, 4H, H-7, H-9), 1.31-1.20 (m, 4H, H-8, H-11), 0.81 (s, 27H, Si-C(CH_3)$_3$), 0.00 (s, 18H, Si-(CH_3)$_2$);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 80.6 (C-3), 73.6 (C-2, C-4), 60.2 (C-1, C-5), 58.7 (C-6), 41.7 (C-10), 30.6 (C-7, C-11), 28.3, 27.7 (C-8, C-9), 27.1, 26.9 (C(CH_3)$_3$-TBDMS), 19.2, 18.9 (C_q-TBDMS), -2.68, -4.23 ((CH_3)$_2$-TBDMS);

TBDMS protected* N-*tosyl derived LDPP probe, compound 105

3-(chlorosulfonyl) benzoyl chloride (290 mg, 1.22 mmol, 2 eq) was dissolved in CH_2Cl_2 (10 ml) and stirred at 0°C for 5 minutes. Compound **103** was dissolved in CH_2Cl_2 (5 ml) in a separate flask and Et_3N (188 µl, 1.34 mmol, 2.2 eq) was added. The solution of compound **103** was added dropwise to the 3-(chlorosulfonyl) benzoyl chloride reaction mixture throughout a time of 10 minutes and the reaction mixture was stirred at 0°C for another 40 minutes until it turned yellowish and all starting material vanished on TLC ($CHCl_3$/MeOH/concd.NH_4OH = 3/1/0.01, v/v/v) and a UV active spot appeared in an unpolar region (detected by TLC: CH/EA = 2/1, v/v). The reaction mixture was concentrated under reduced pressure at 40°C and purified utilizing column chromatography (CH/EtOAc = 3/1–2/1, v/v). The intermediate **104** (280 mg) was obtained as yellowish, sticky solid with a yield of 59%.

The intermediate **104** (280 mg, 0.36 mmol, 1 eq) was dissolved in CH_2Cl_2 and stirred at room temperature. Undecanol (75 µl, 0.36 mmol, 1 eq) was dissolved in CH_2Cl_2 (1 ml) in a separate flask, Et_3N (101 µl, 0.72 mmol, 2 eq) and DMAP (10 pellets) were added. Throughout 60 minutes, the undecanol reaction mixture was added in steps of 100 µl, until no intermediate was found on TLC (CH/EtOAc = 2/1, v/v). The reaction mixture was concentrated under reduced pressure at 40°C and purified utilizing silica gel chromatography (CH/EtOAc = 10/1, v/v, removes excess of undecanol, CH/EtOAc = 2/1, v/v, gives pure product.) Compound **105** (40 mg) was obtained as colorless liquid with a yield of 10%.



Product **105**

MS: Calcd. for[$C_{47}H_{92}N_2O_7Si_3SNa$]:m/z 731.4647

MS: Found: -

TLC: Rf = 0.33

(CH/EtOAc = 2/1, v/v)

CAM

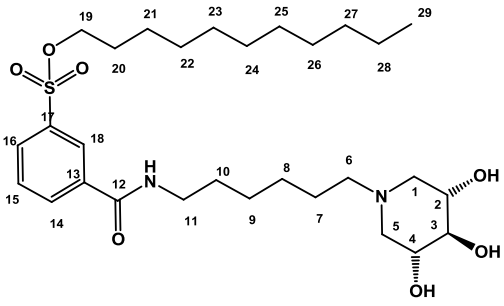
$[a]_D^{20}$ = 0°

^{1}H NMR (300 MHz, $CDCl_3$) δ = 8.22 (s, 1H, H-14), 8.11 (d, 1H, H-15), 8.01 (d, 1H, H-16), 7.64 (t, 1H, H-18), 4.06 (t, 2H, H-19), 3.56 (ddd, 2H, $J_{2,1e=4,5e}$ 4.0 Hz, $J_{2,3=4,3}$ 7.5 Hz, $J_{2,1a=4,5a}$ 9.3 Hz, H-2, H-4), 3.46 (dd, 2H, H-6), 3.25 (dd, 1H, H-3), 2.87 (dd, 2H, $J_{5e,5a=1e,1a}$ 10.6 Hz, H-1e, H-5e), 2.41-2.27 (m, 2H, H-11), 2.00-1.84 (m, 2H, H-1a, H-5a), 1.70-1.16 (m, 26H, H-7, H-8, H-9, H-10, H-20, H-21, H-22, H-23, H-24, H-25, H-26, H-27, H-28), 0.94-0.82 (m, 30H, 3x C(CH_3)$_3$, H-29), 0.12-0.03 (m, 18H, 3x Si(CH_3)$_2$);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ = 165.7 (C-12), 137.0 (C-13), 136.2 (C-17), 132.7 (C-14), 130.4 (C-15), 130.0 (C-16), 126.1 (C-18), 77.6 (C-3), 72.7 (C-2, C-4), 71.7 (C-19), 59.2 (C-1, C-5), 57.5 (C-6), 40.5 (C-11), 32.0, 29.7, 29.7, 29.6, 29.5, 29.4, 29.0, 29.0, 27.2, 27.2, 26.1, 26.0, 25.4, 22.8, 18.4, 18.1, (C-7, C-8, C-9, C-10, C-21, C-22, C-23, C-24, C-25, C-26, C-27, C-28), 26.6, 26.4 (3x C(CH_3)$_3$), 14.2 (C-29), -2.9, -3.0, -4.4 (3x Si(CH_3)$_2$);

N-*Tosyl derived LDPP probe, compound 106*

Compound **105** (40 mg, 0.04 mmol, 1 eq) was dissolved in CH_3CN (2.5 ml) and stirred at room temperature. Para-toluenesulfonic acid (pTSA) was added to the reaction mixture until a pH value of 2 was reached. The reaction mixture was stirred for 48h, until no starting material was detected on TLC (CH/EtOAc = 2/1, v/v). The reaction mixture was concentrated under reduced pressure and purified utilizing silica gel chromatography ($CHCl_3$/MeOH/concd. NH_4OH = 6/1/0.01, v/v/v) to give compound **106** (20 mg) as a 5/1 mixture of product and pTSA. It was further purified utilizing preparative TLC (2mm silica gel plate, $CHCl_3$/MeOH/concd. NH_4OH = 6/1/0.01, v/v/v). The UV-active band with an R_f-value of 0.45 was collected in dest. MeOH (1 ml) and extracted overnight. After filtration and concentration under reduced pressure, compound **106** (12 mg) was obtained as colorless solid with a yield of 48%.



Product **106**

MS: Calcd. for [$C_{29}H_{50}N_2O_7SNa$]: m/z 731.4647

MS: Found: -

TLC: Rf = 0.45

($CHCl_3$/MeOH/concd. NH_4OH = 6/1/0.01, v/v/v)

CAM

$[a]_D^{20}$ = 0°

^{1}H NMR (300 MHz, MeOH-d_4) δ = 8.35 (t, 1H, H-14), 8.17 (d, 1H, H-15), 8.06 (d, 1H, H-16), 7.75 (t, 1H, H-18), 4.10 (t, 2H, H-19), 3.66 (t, 1H, H-6), 3.49 (ddd, 2H, $J_{2,3=4,3}$ 8.6 Hz, $J_{2,1e=4,5e}$ 4.7 Hz, $J_{2,1a=4,5a}$ 5.8 Hz, H-2, H-4), 3.40 (t, 2H, H-11), 3.09 (dd, 1H, H-3), 2.97 (dd, $J_{5e,5a=1e,1a}$ 10.6 Hz, H-1e, H-5e), 2.45-2.36 (m, 3H, H-6', H-20), 1.96-1.86 (m, 2H, H-1a, H-5a), 1.70-1.17 (m, 24H, H-7, H-8, H-9, H-10, H-21, H-22, H-23, H-24, H-25, H-26, H-27, H-28), 0.90 (t, 3H, H-29);

^{13}C NMR (75.5 MHz, MeOH-d_4) δ = 167.9 (C-12), 138.3 (C-17), 137.2 (C-13), 133.5 (C-14), 131.5 (C-15), 131.0 (C-16), 127.7 (C-18), 80.4 (C-3), 72.7 (C-19), 71.4 (C-2, C-4), 60.5 (C-6), 59.5 (C-1, C-5), 58.9 (C-20), 41.2 (C-11), 33.6, 30.7, 30.6, 30.5, 30.4, 30.3, 30.0, 29.9, 28.2, 28.0, 27.7, 26.4, 23.7 (C-7, C-8, C-9, C-10, C-21, C-22, C-23, C-24, C-25, C-26, C-27, C-28), 14.4 (C-29);

6.6. Spin off VMI

Electrolyte and electrochemical cell preparations were carried out in an Ar-filled glove box. The obtained compound **109** was dried under Vacuum at 75°C overnight and DMF was dried over molecular sieve (4 A). Graphite based anodes were prepared from 90w% natural graphite (Qingdao Nanshu Graphite Co., Ltd., average size 20µm) with 7 w% polyvinylidene fluoride (PVDF, Solvay) and 3 w% carbon black (Super-P, Timcal Ltd.) Typical masses of the obtained electrodes were 1.8-2.1 $mg*cm^{-2}$. Electrodes were prepared according to literature.[126]
Electrochemical experiments were recorded on a Bio-Logic MPG-2 battery test station with a scan rate of 0.1 $mV*s^{-1}$ for CV and a C-rate of 0.1 C for CCC.

4,5-Dicyano-2-methyl*-1H-*imidazole (108)

Diaminomaleonitrile (**107**, 17.53 g, 162.25 mmol) was dissolved in CH_3CN (40 mL) and triethylorthoacetate (TOA, 32.40 mL, 177.23 mmol, 1.1 eq) was added to the reaction mixture. It was stirred at reflux for 24h. The reaction mixture was concentrated under reduced pressure, taken up in xylene and stirred at reflux for 24h. After removing the solvent under reduced pressure, the obtained syrup was taken up in EtOH, charcoal (2 g) was added and the reaction mixture was stirred at reflux for 24h. After filtration and concentration under reduced pressure, compound **108** (18.11 g) was obtained as colorless solid with a yield of 85%.

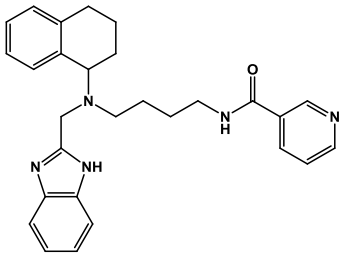
Bis-(4,5-dicyano-2-methylimidazoyl)magnesium (109)

Compound **108** (3.00 g, 22.7 mmol, 1 eq) was dissolved in THF (45 mL). A solution of magnesium-bis-(diisopropylamide) (0.7 M in THF, 13.17 mL, 9.21 mmol, 0.4 eq) in THF (25 mL) was added dropwise to the reaction mixture and stirred at reflux under nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure, washed with CH_3CN several times and dried at 75°C *en vacou*. Compound **109** (1.83 g) was obtained as yellowish solid with a yield of 85%.

6.7. Spin off Center for Medical Research, Graz

N-*(4-(((1H-benzo[d]imidazol-2-yl)methyl)(1,2,3,4-tetrahydronaphthalen-1-yl)amino)butyl)nicotinamide (111)*

Compound **110** (40 mg, 0.12 mmol, 1 eq) was dissolved in MeOH (800 µL). Nicotinyl chloride hydrochloride (20.4 mg, 0.12 mmol, 1 eq) and Et_3N (32 µL, 0.23 mmol, 2 eq) were added to the reaction mixture and it was stirred at ambient temperature. After consumption of the starting material (detected by TLC: $CHCl_3$/MeOH/concd. NH_4OH = 6/1/0.01, v/v/v) the reaction mixture was concentrated under reduced pressure and purified utilizing silica gel chromatography ($CHCl_3$/MeOH/concd. NH_4OH = 20/1/0.01, v/v/v). Compound **111** (20 mg) was obtained as colorless solid with a yield of 36%.



Product **111**

MS: Calcd. for [$C_{28}H_{31}N_5ONa$]: m/z 246.2426

MS: Found: -

TLC: Rf = 0.75

($CHCl_3$/MeOH/concd. NH_4OH = 6/1/0.01, v/v/v)

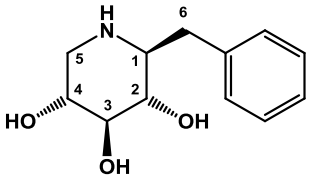
CAM

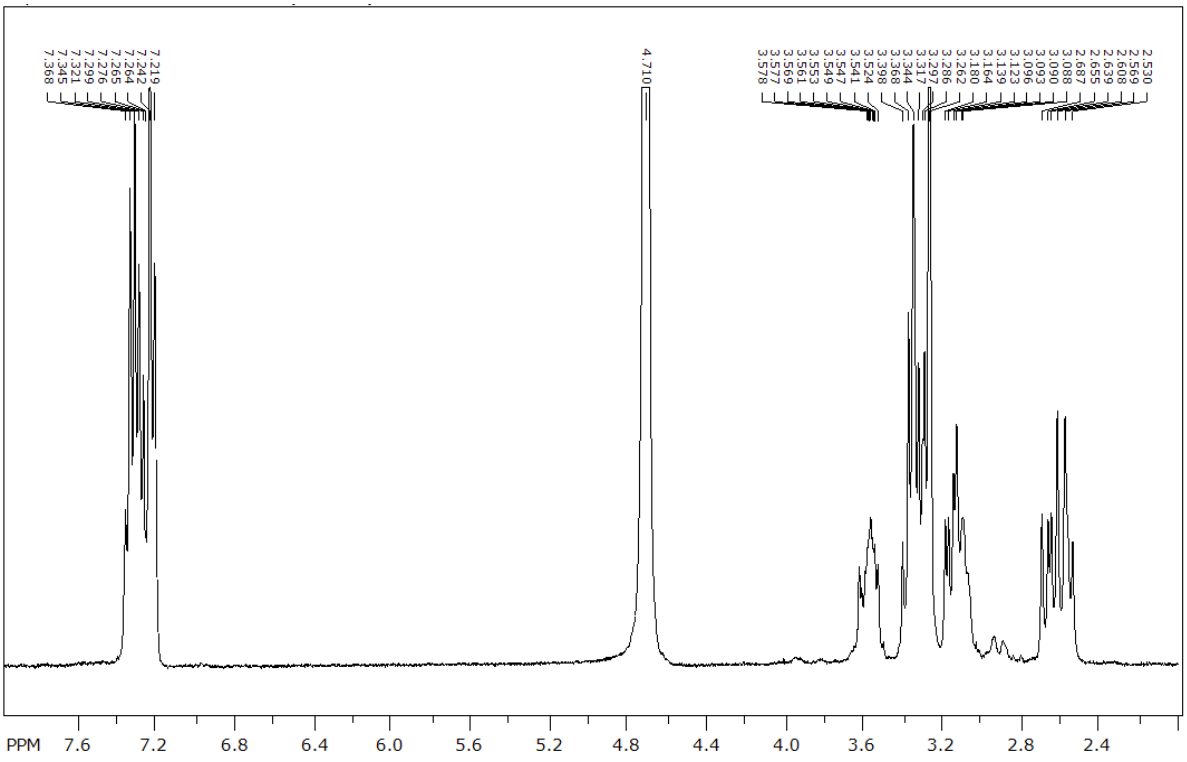
$[a]_D^{20}$ = -

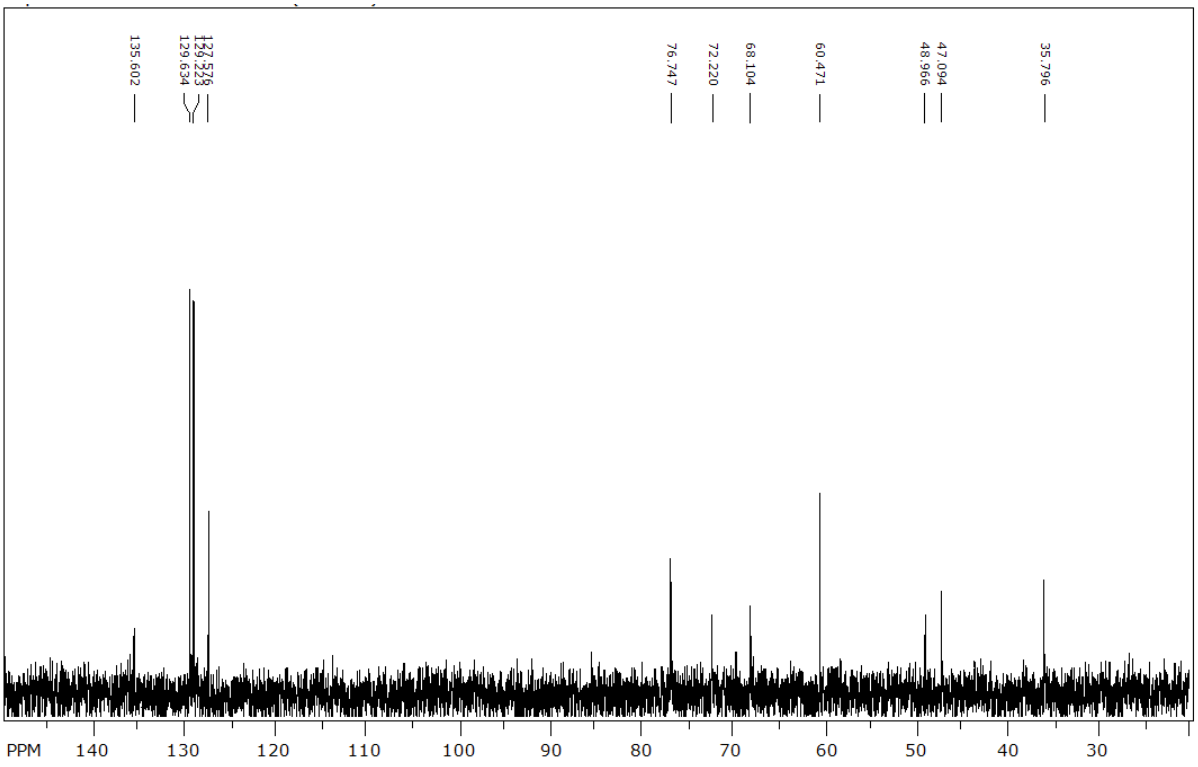
The NMR spectra of compound **111** are shown in chapter 7.

7. Appendix

Product Nr.: **32**







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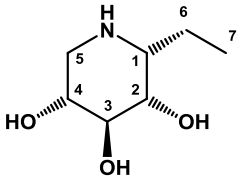

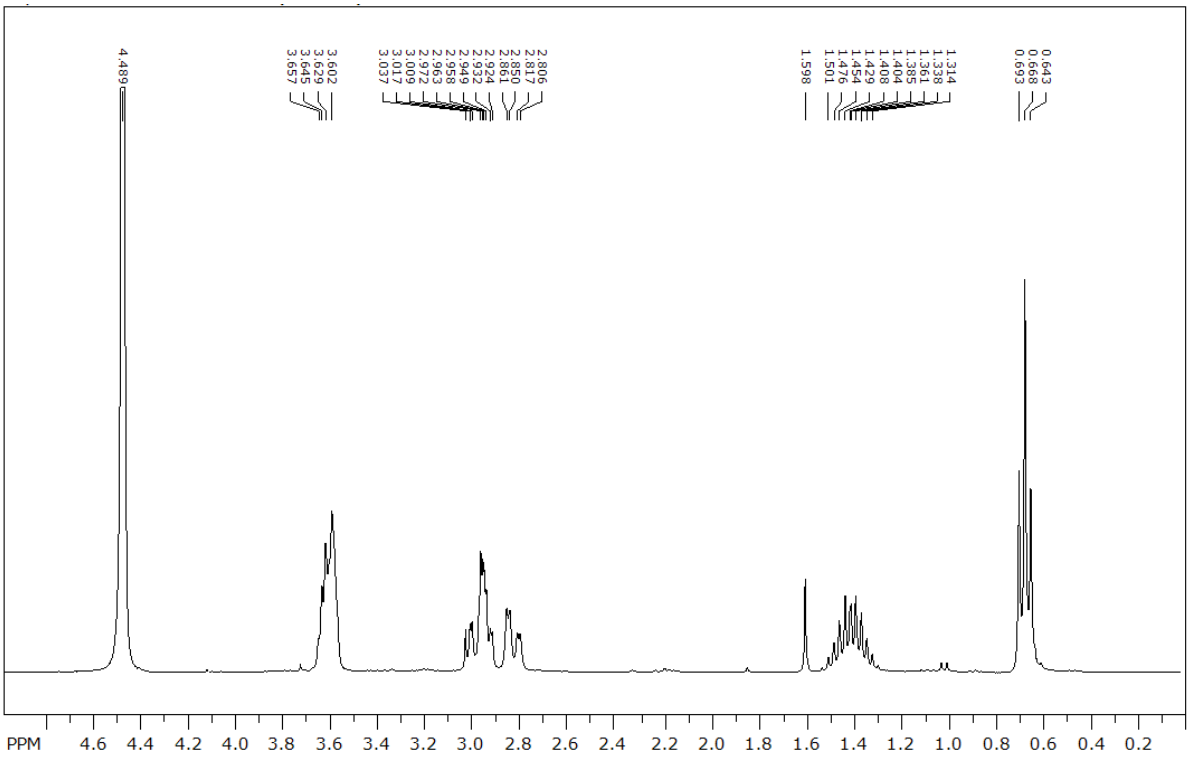

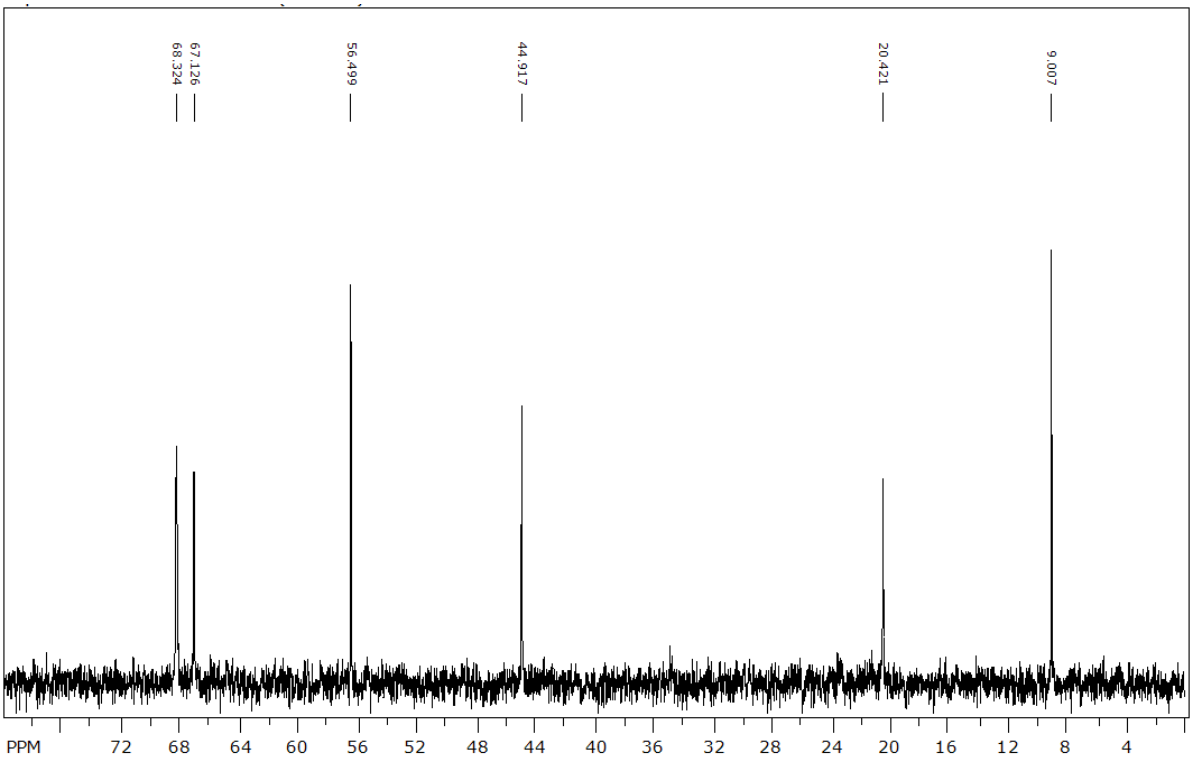

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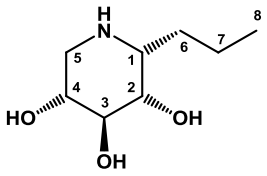

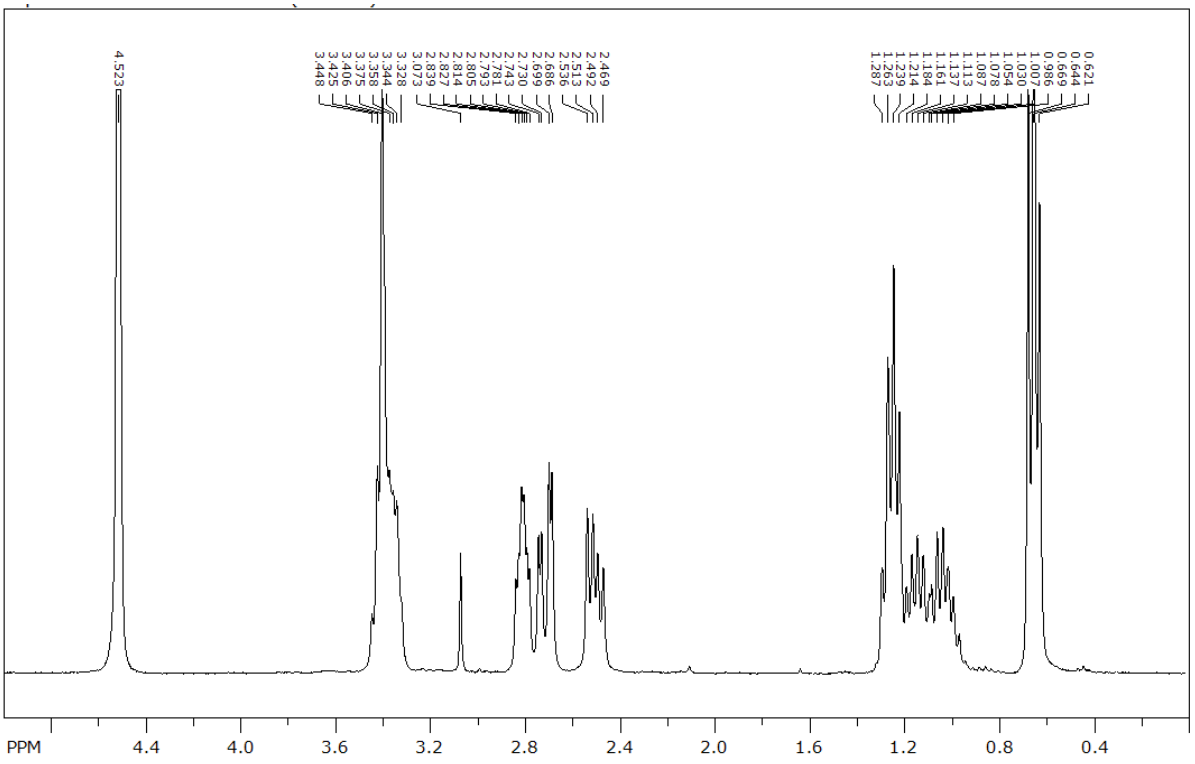

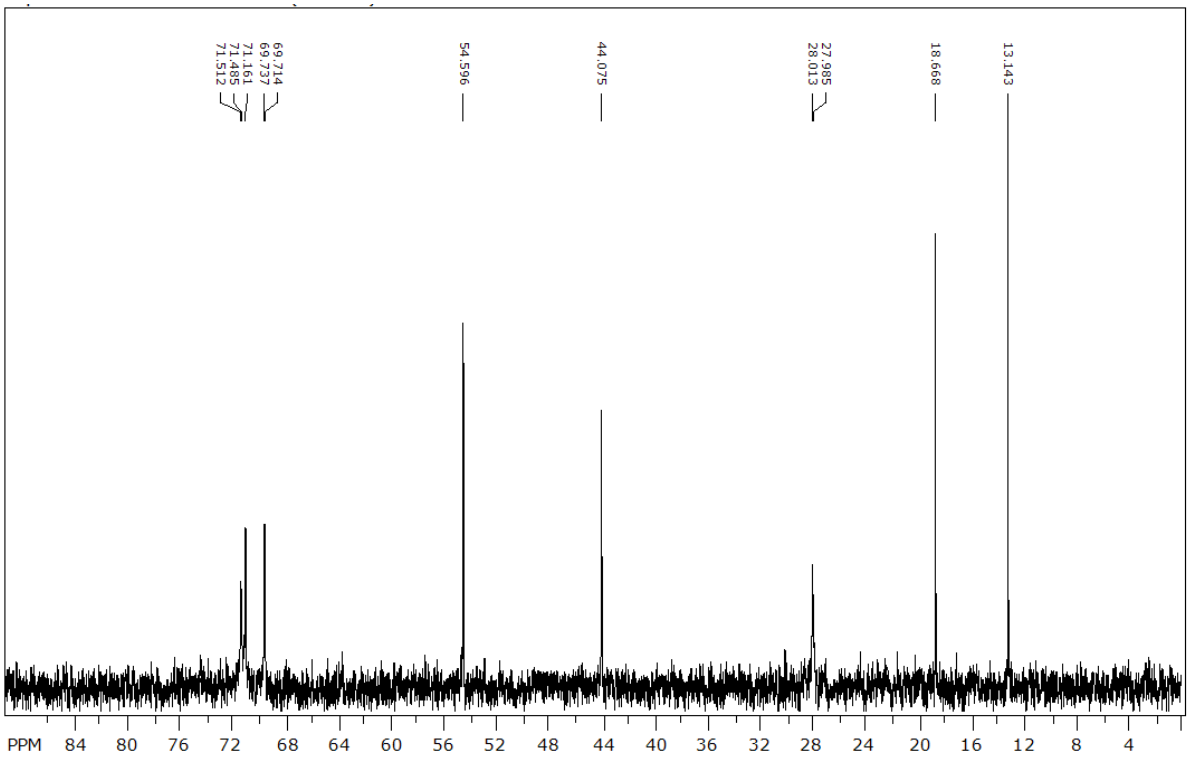

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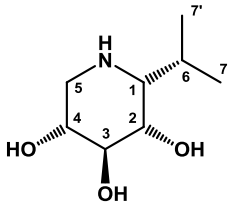

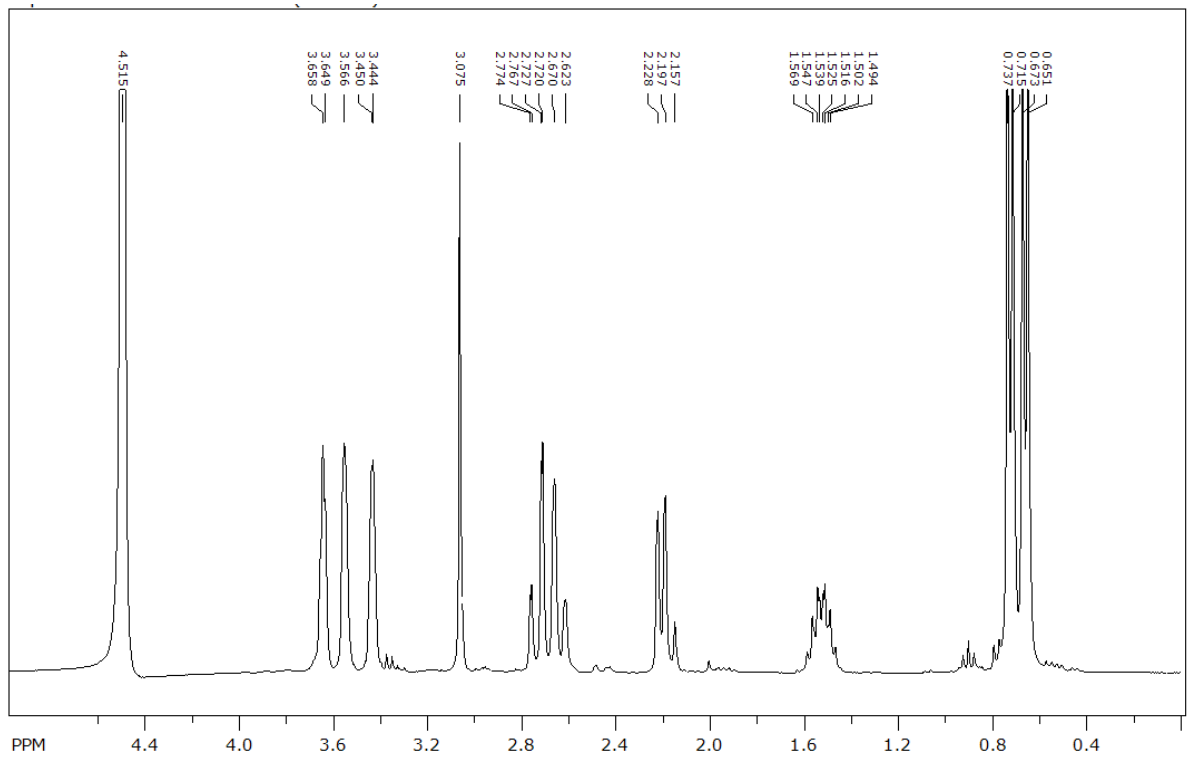

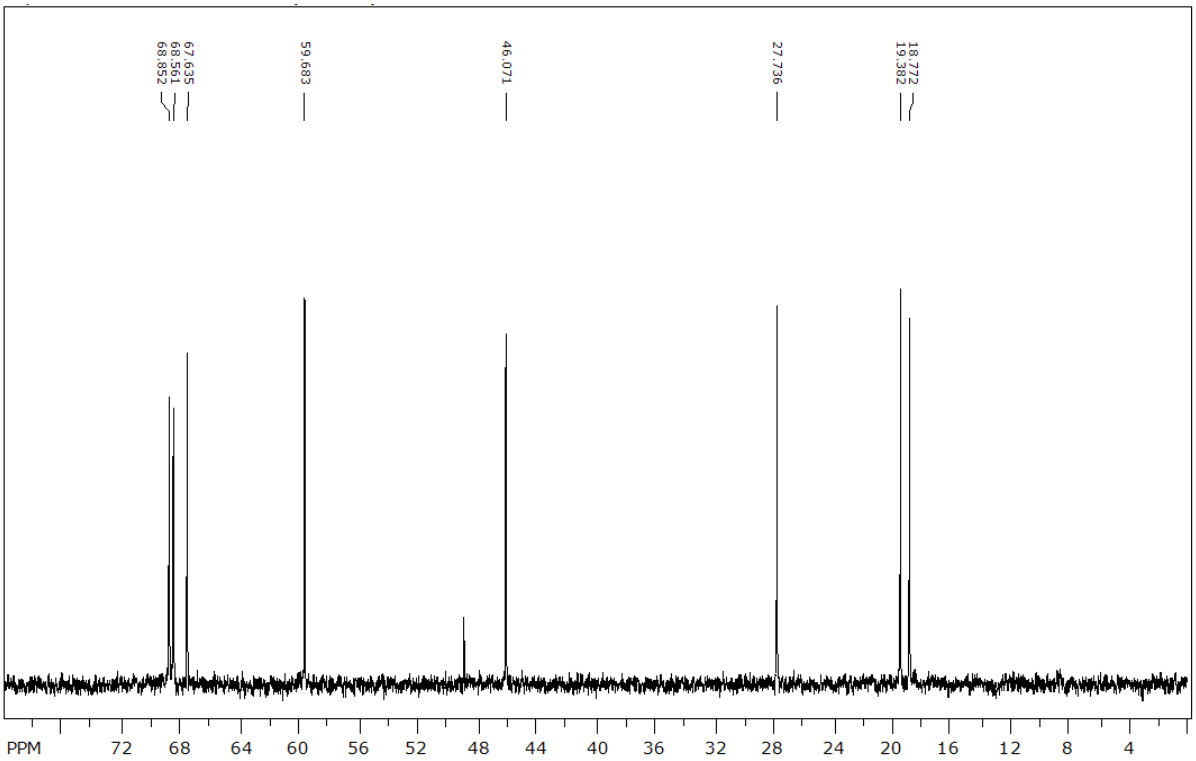

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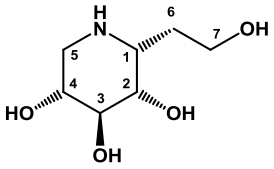

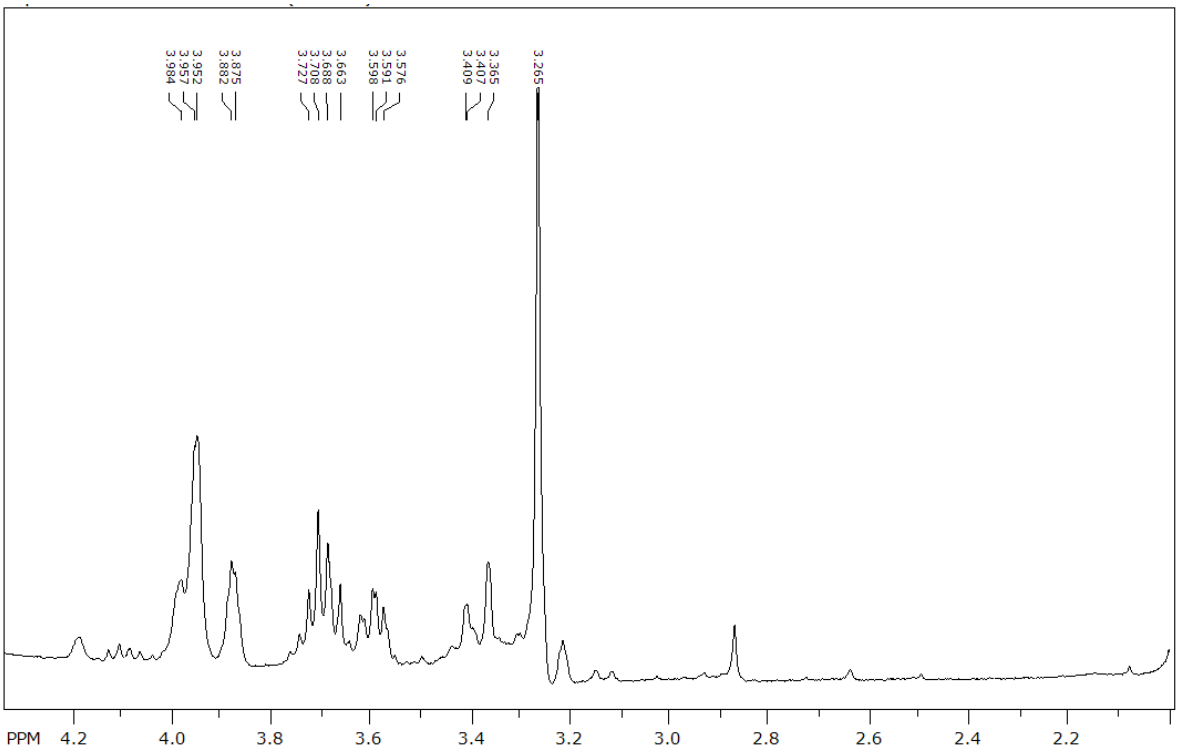

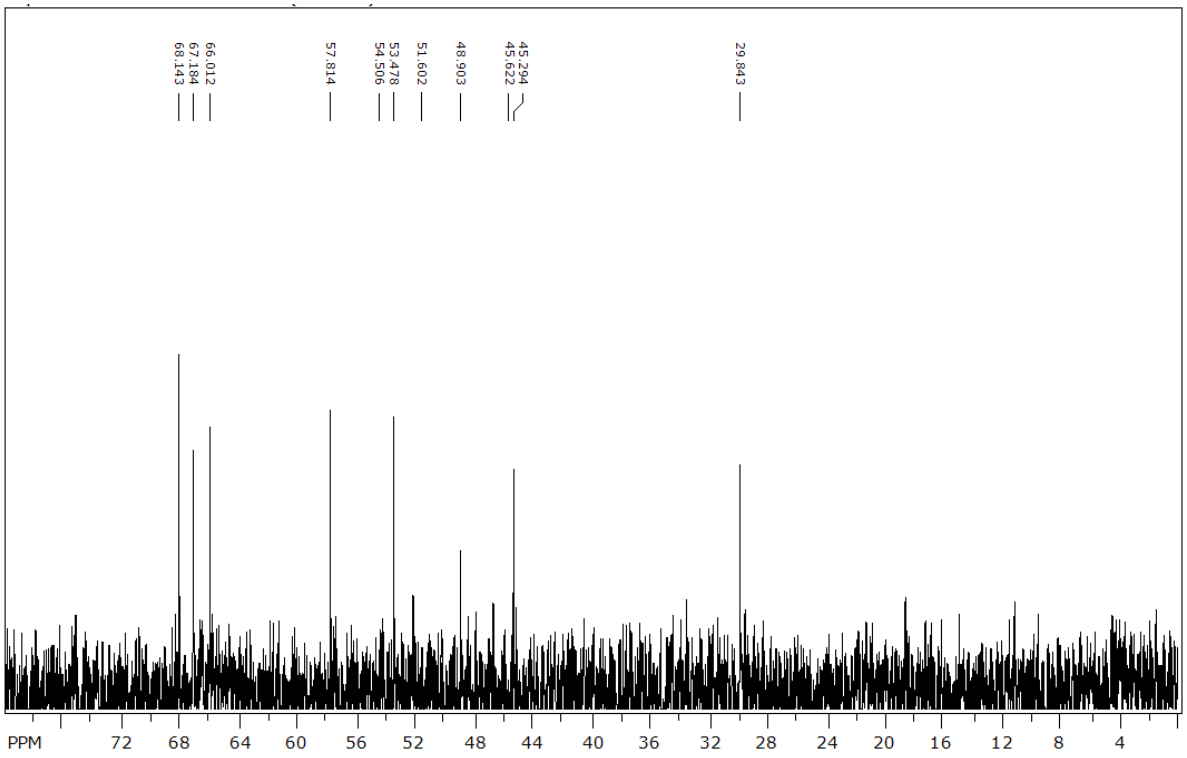

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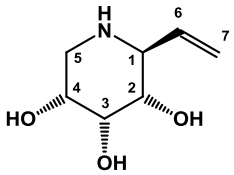

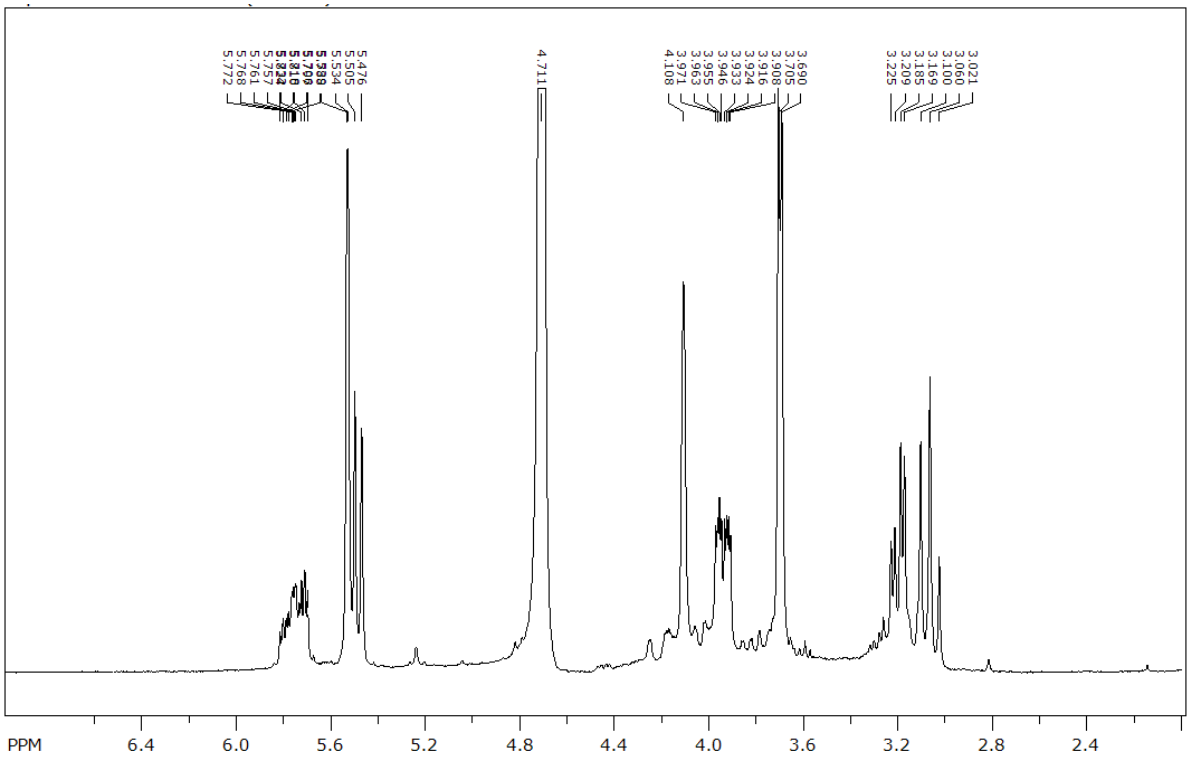

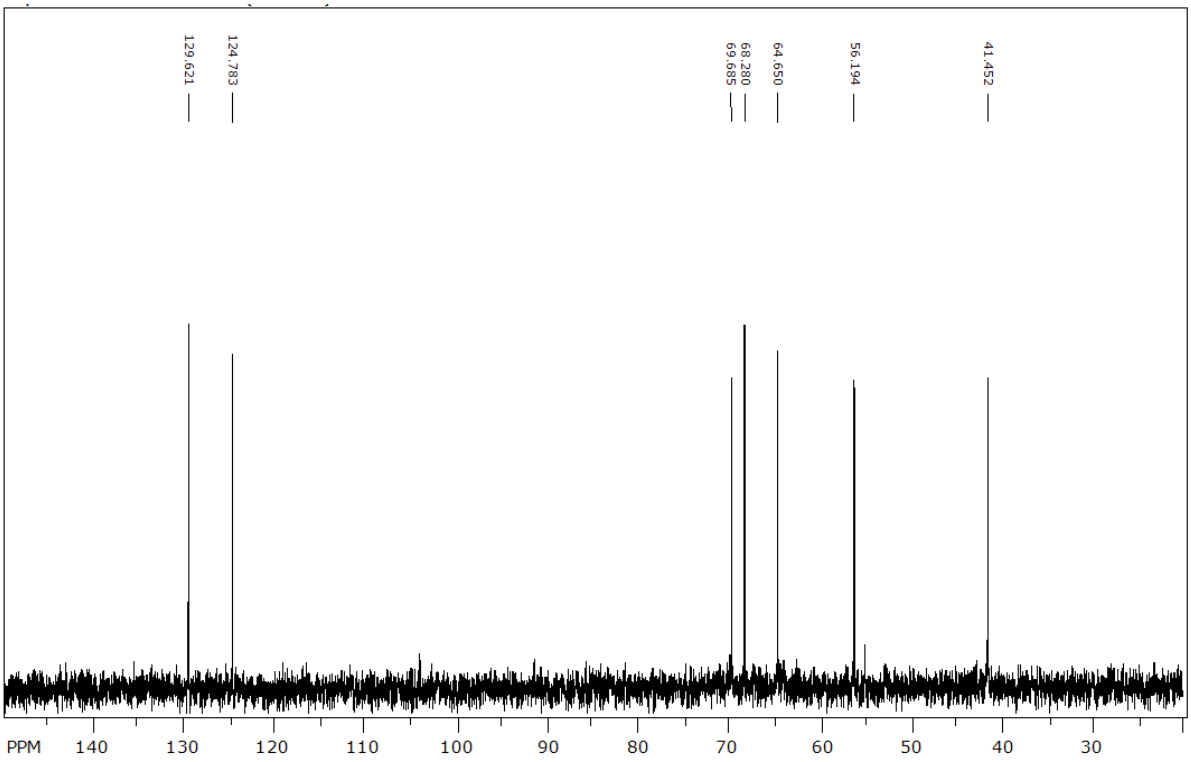

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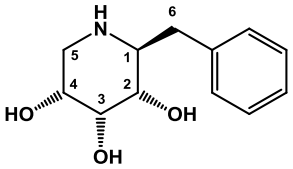

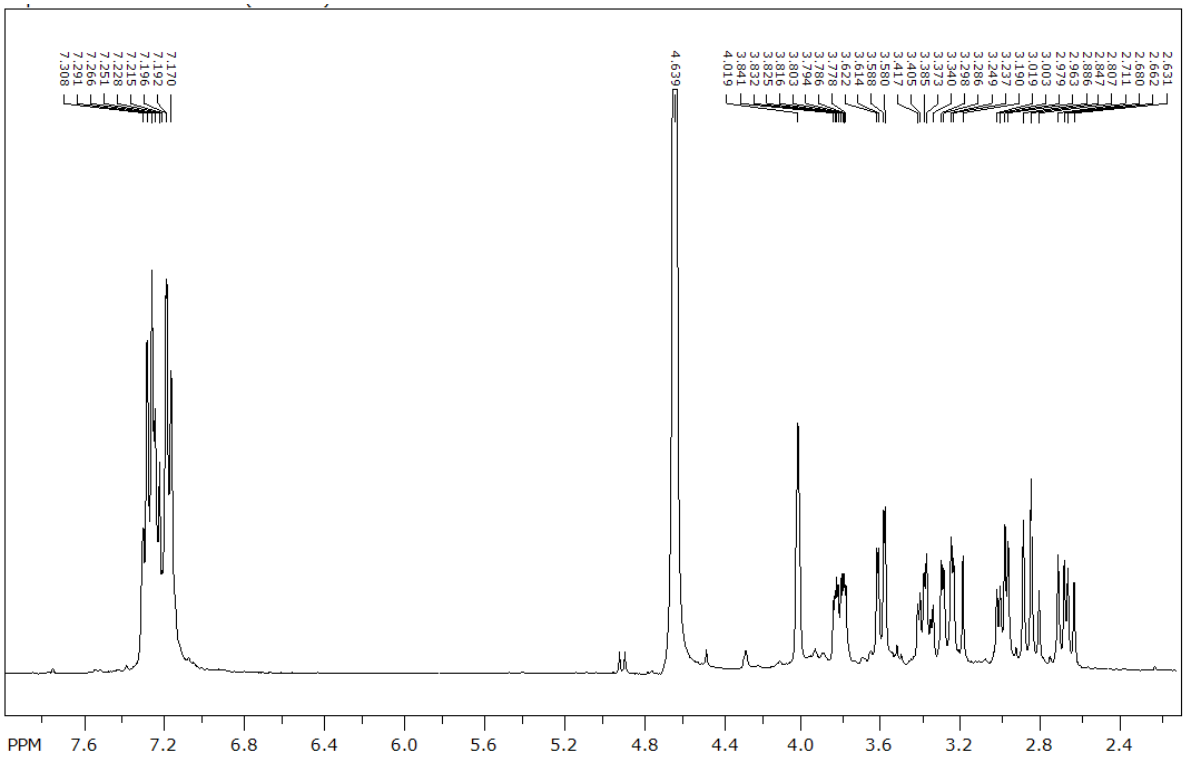

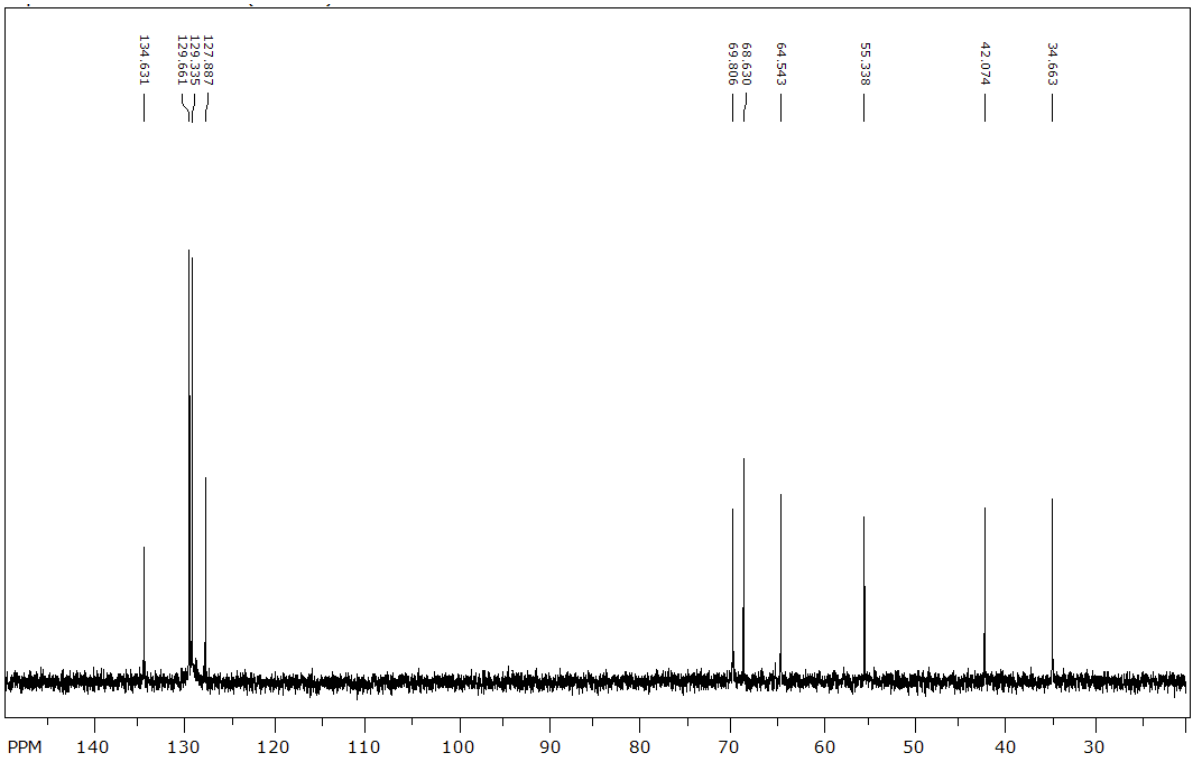

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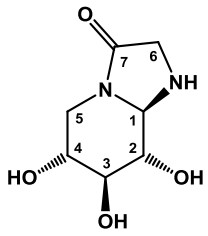

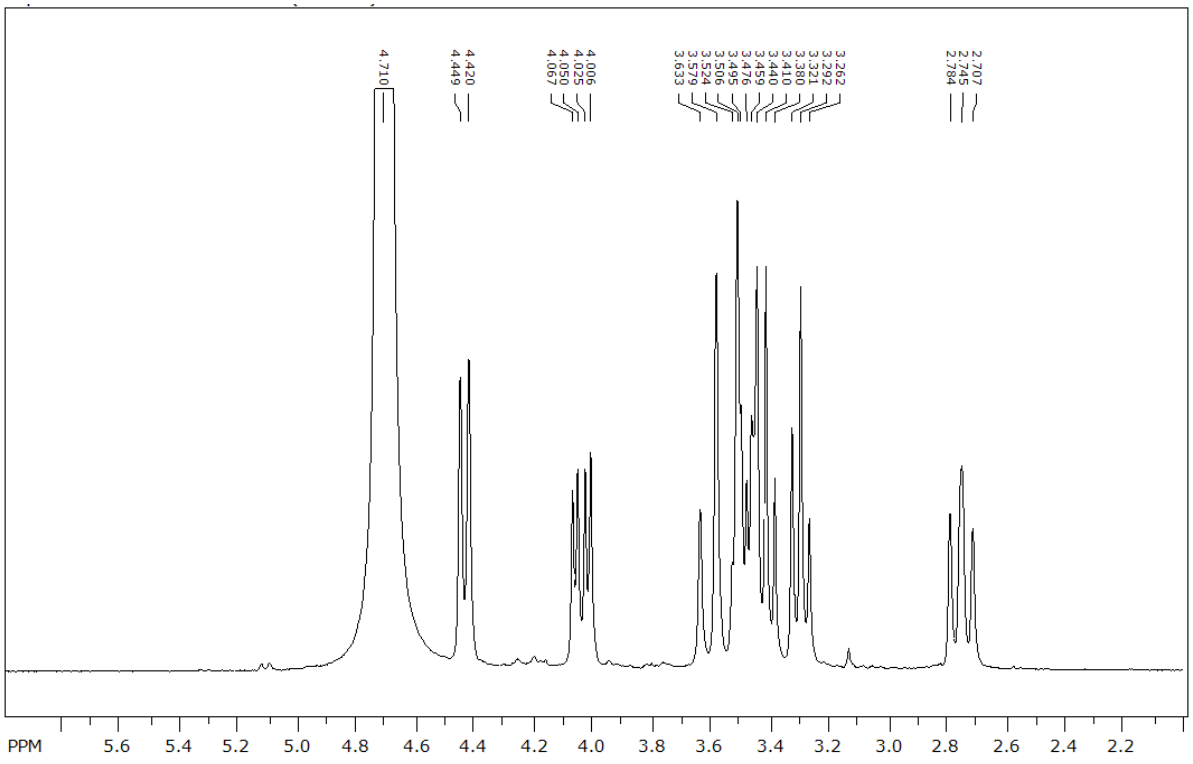

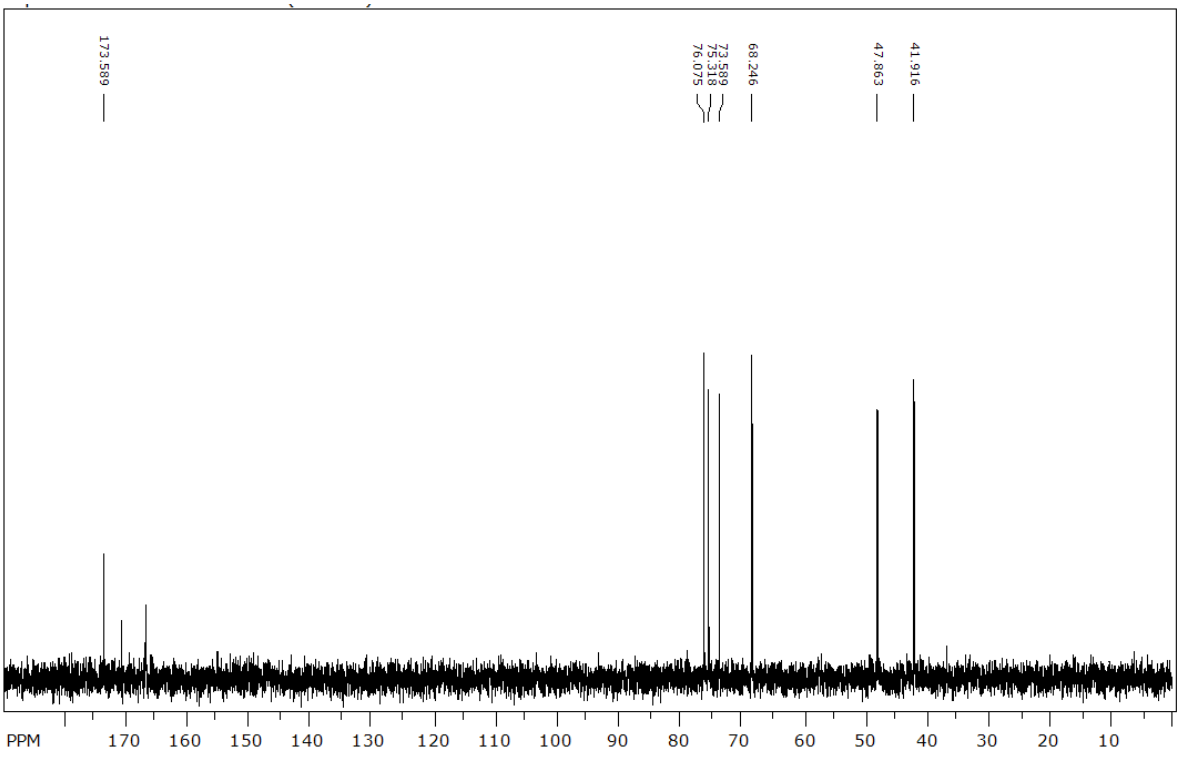

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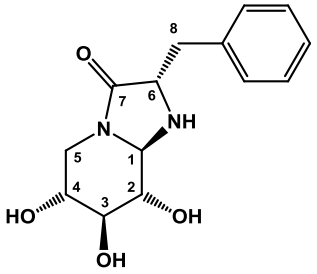

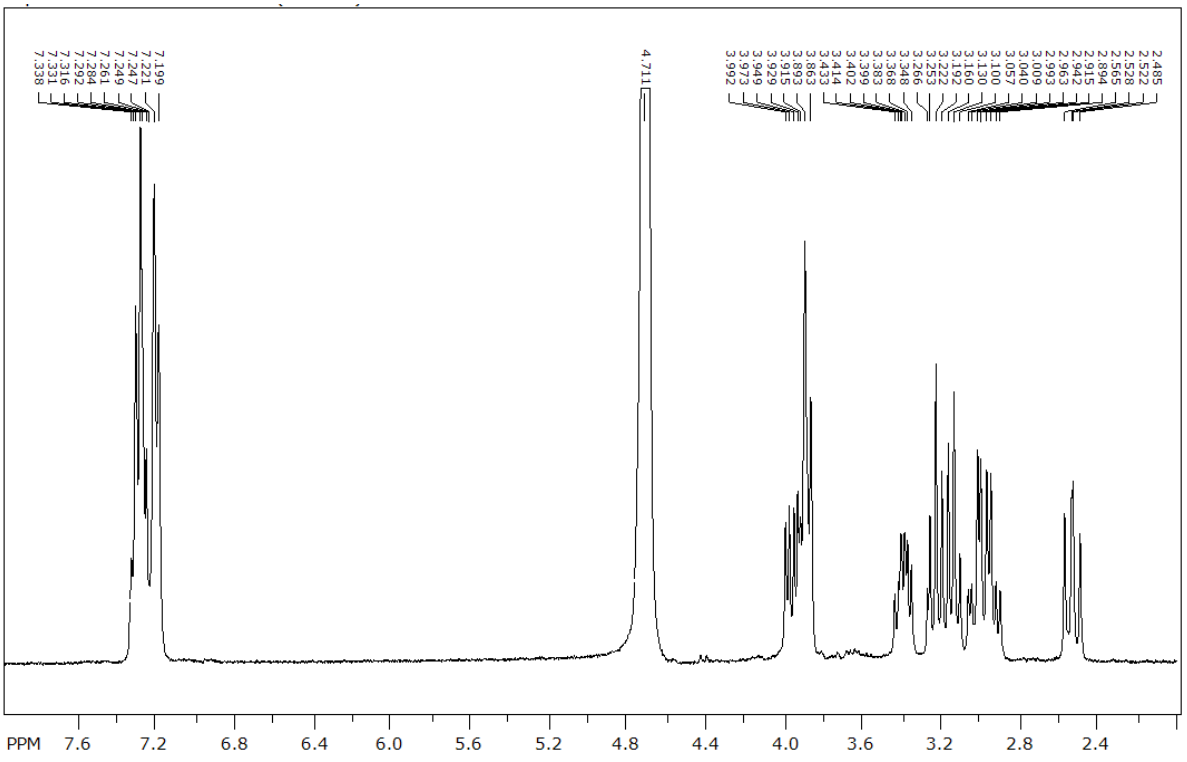

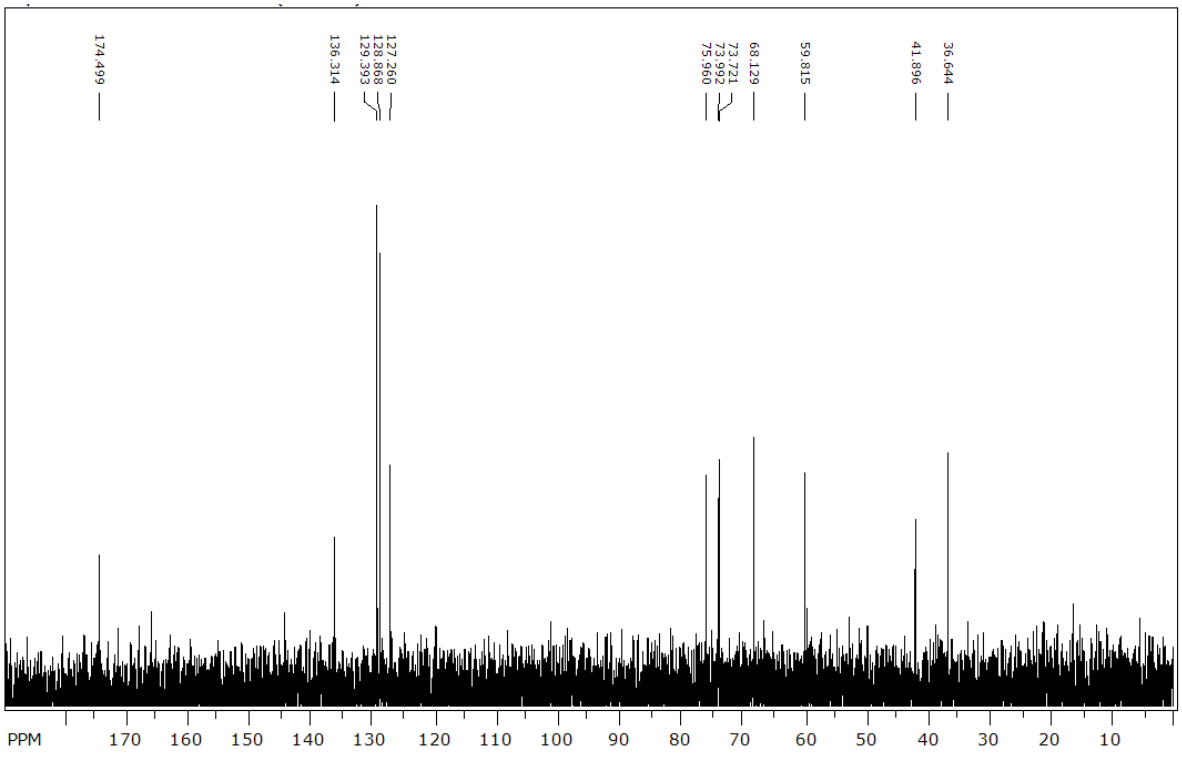

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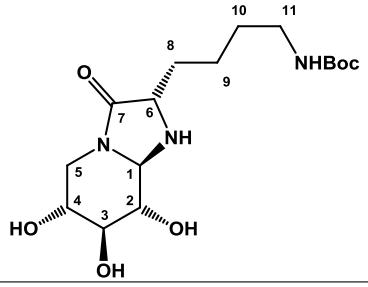

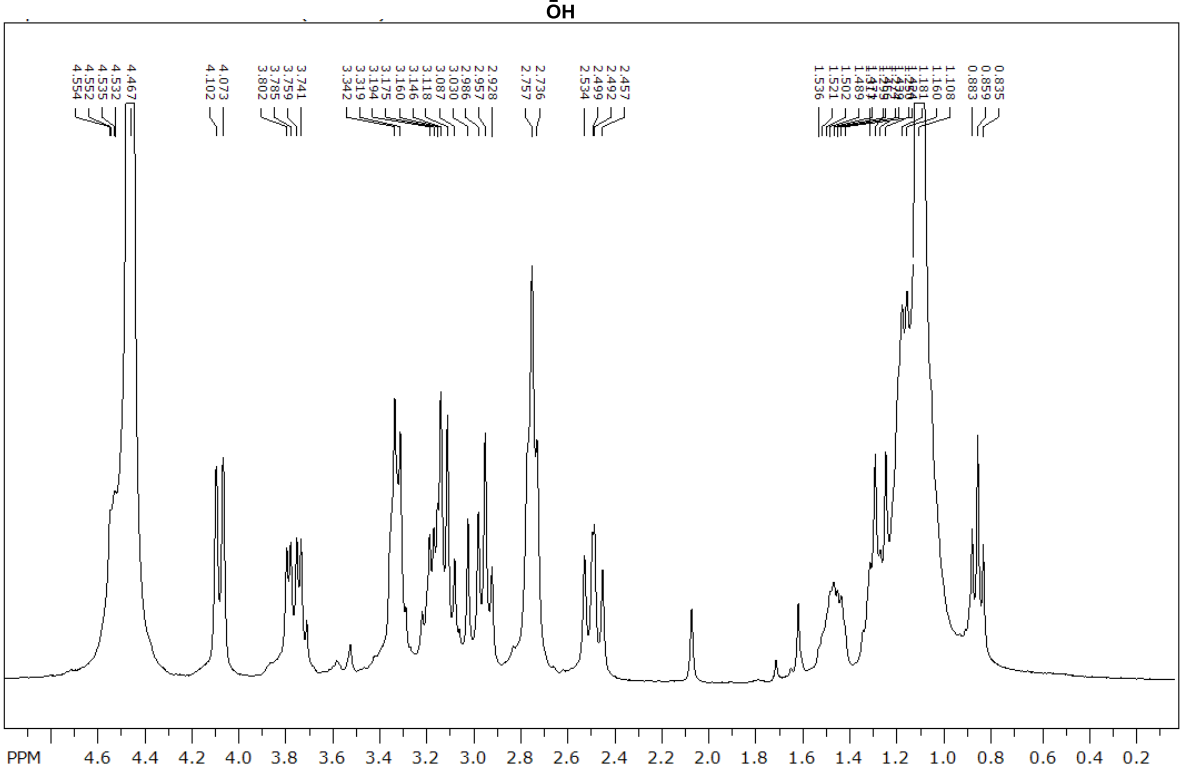

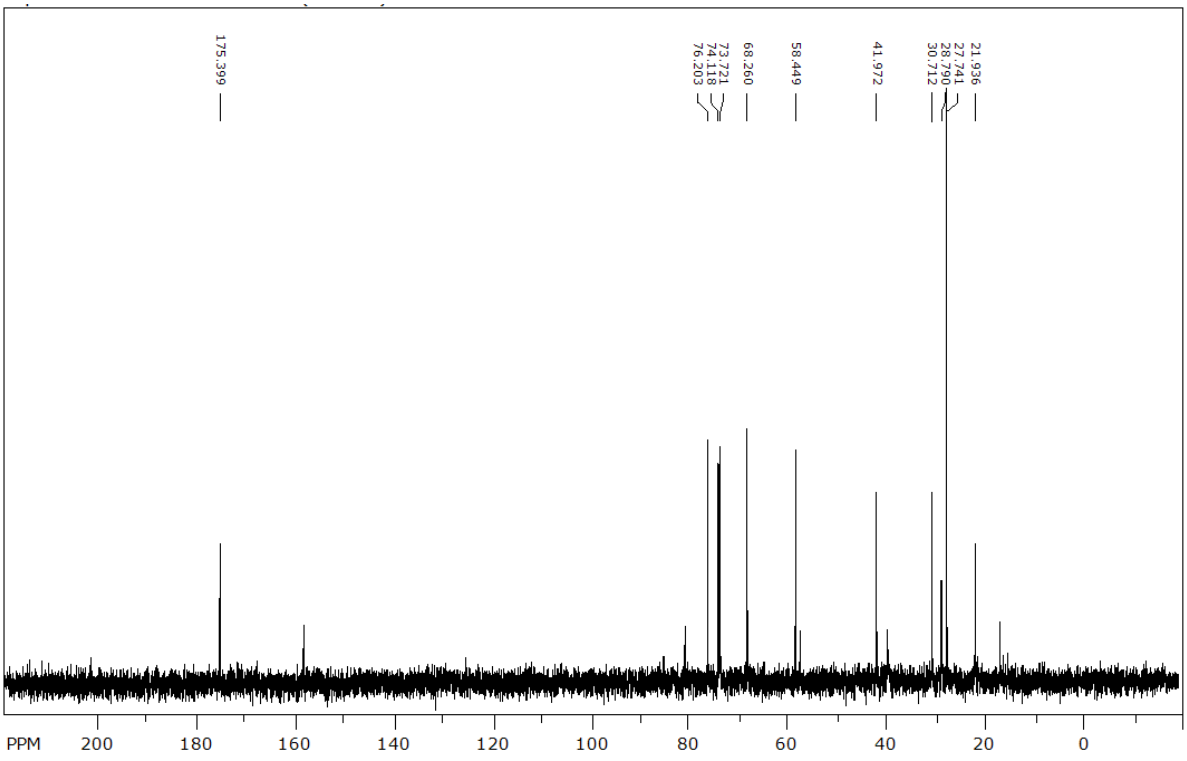

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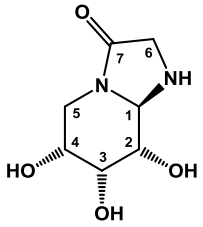

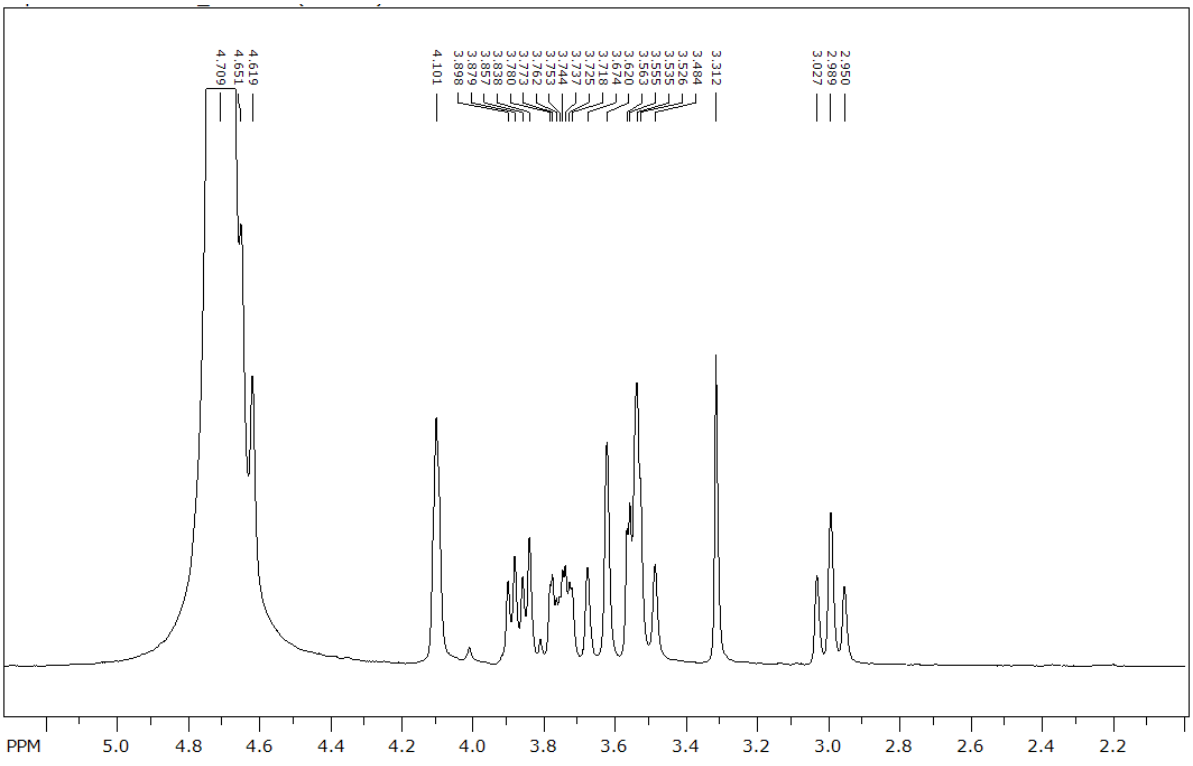

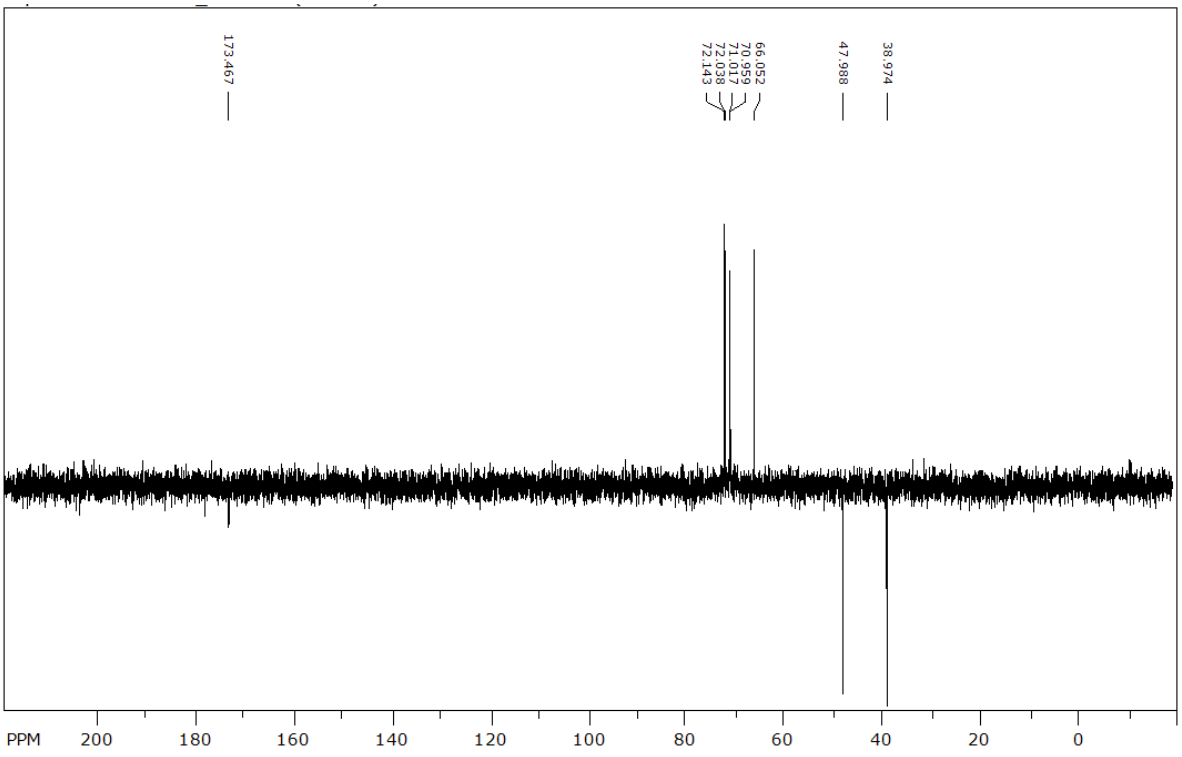

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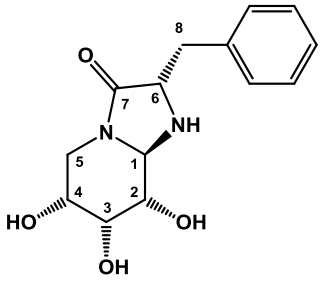

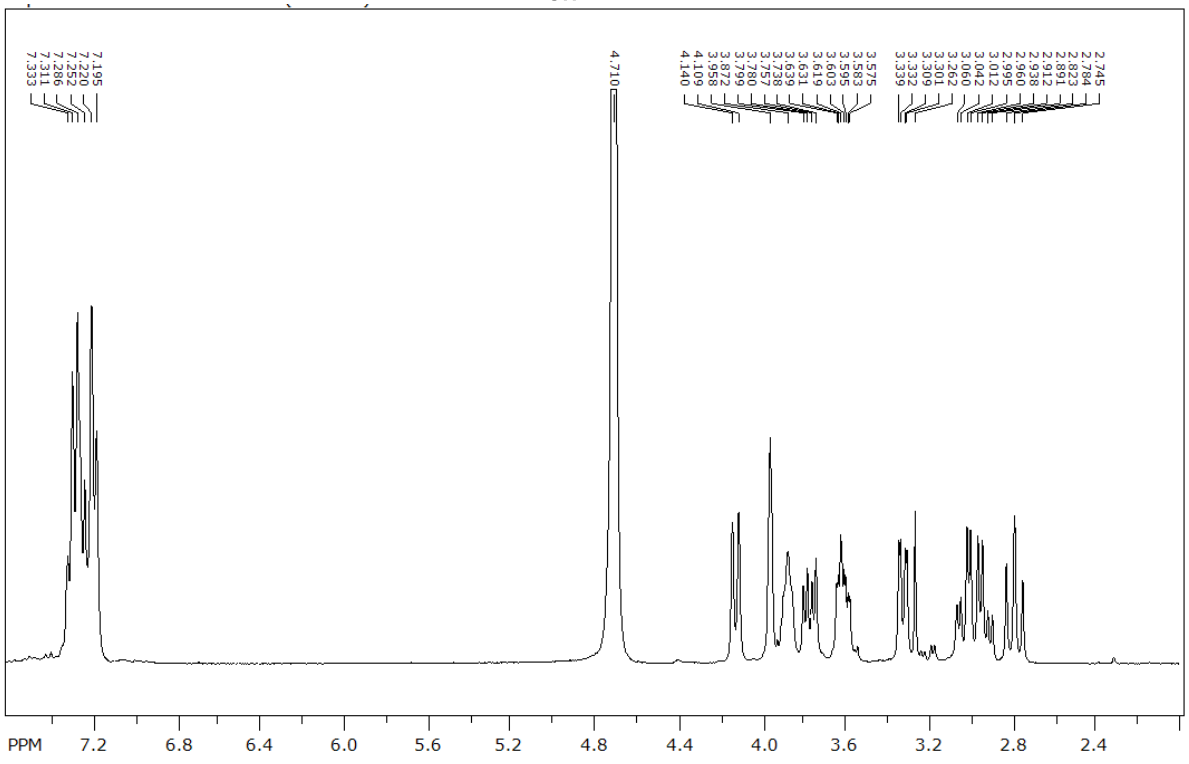

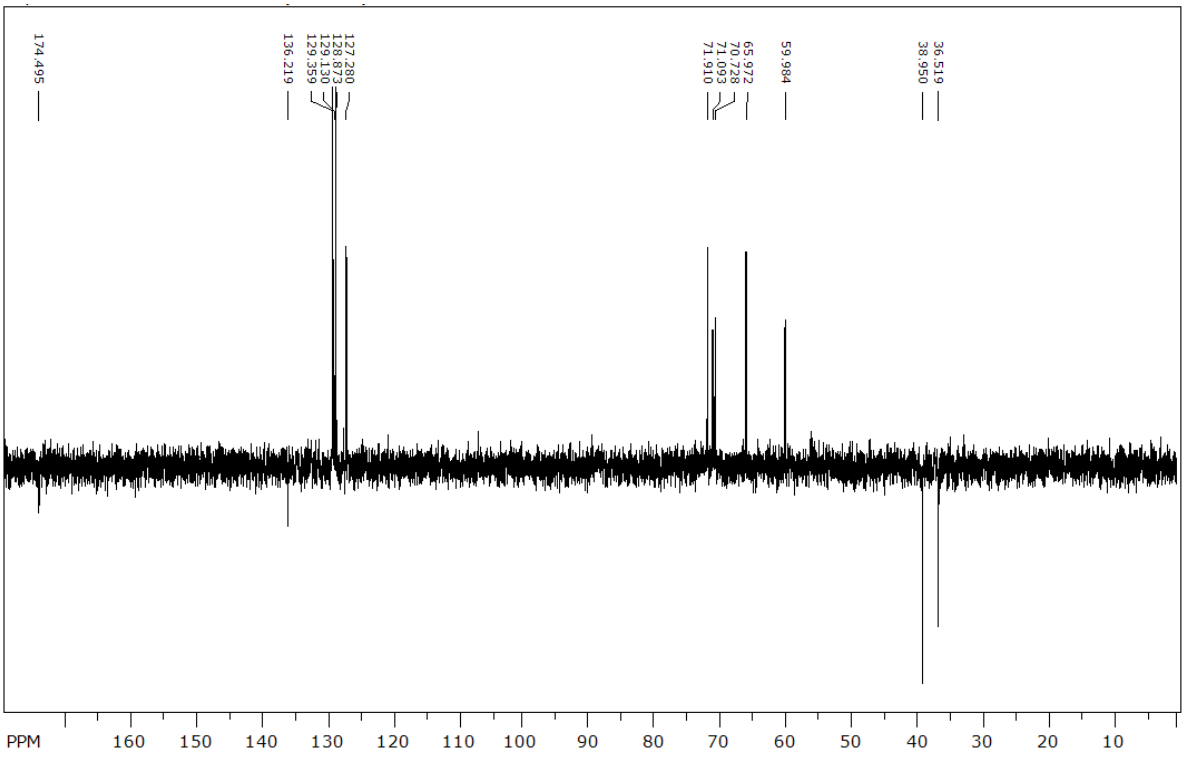

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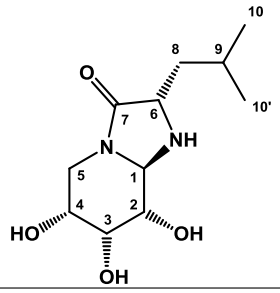

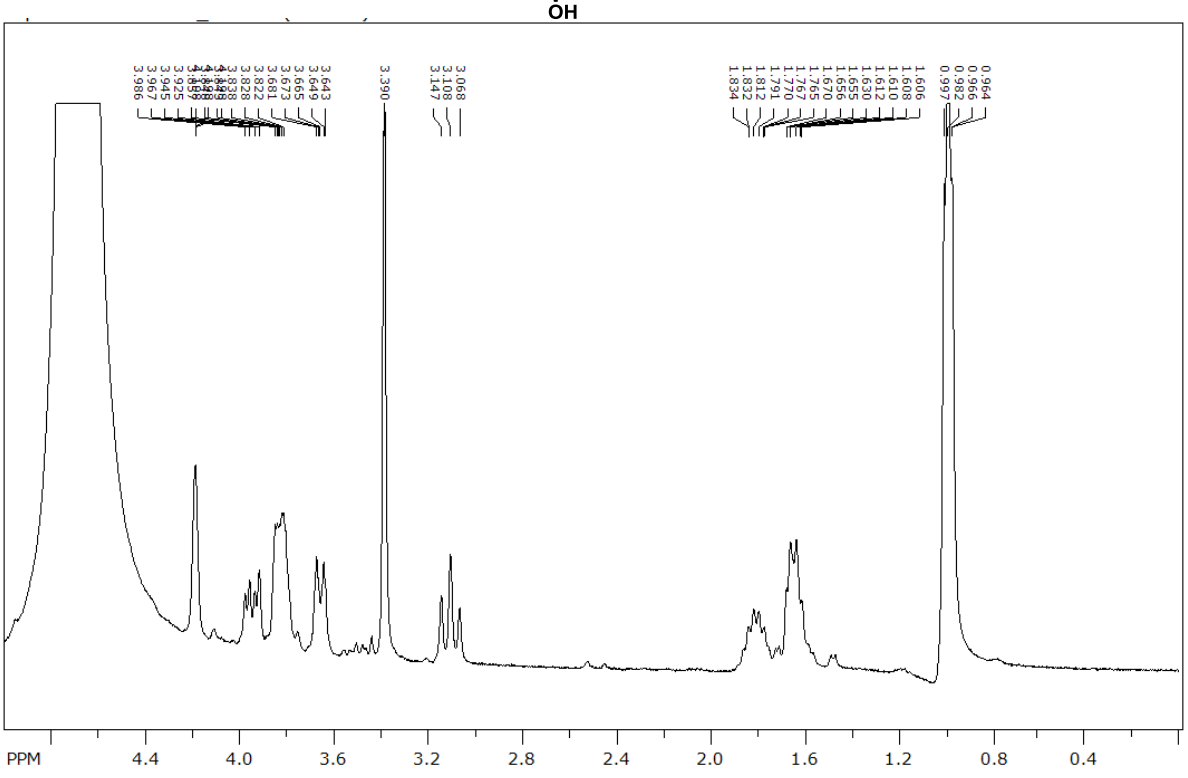

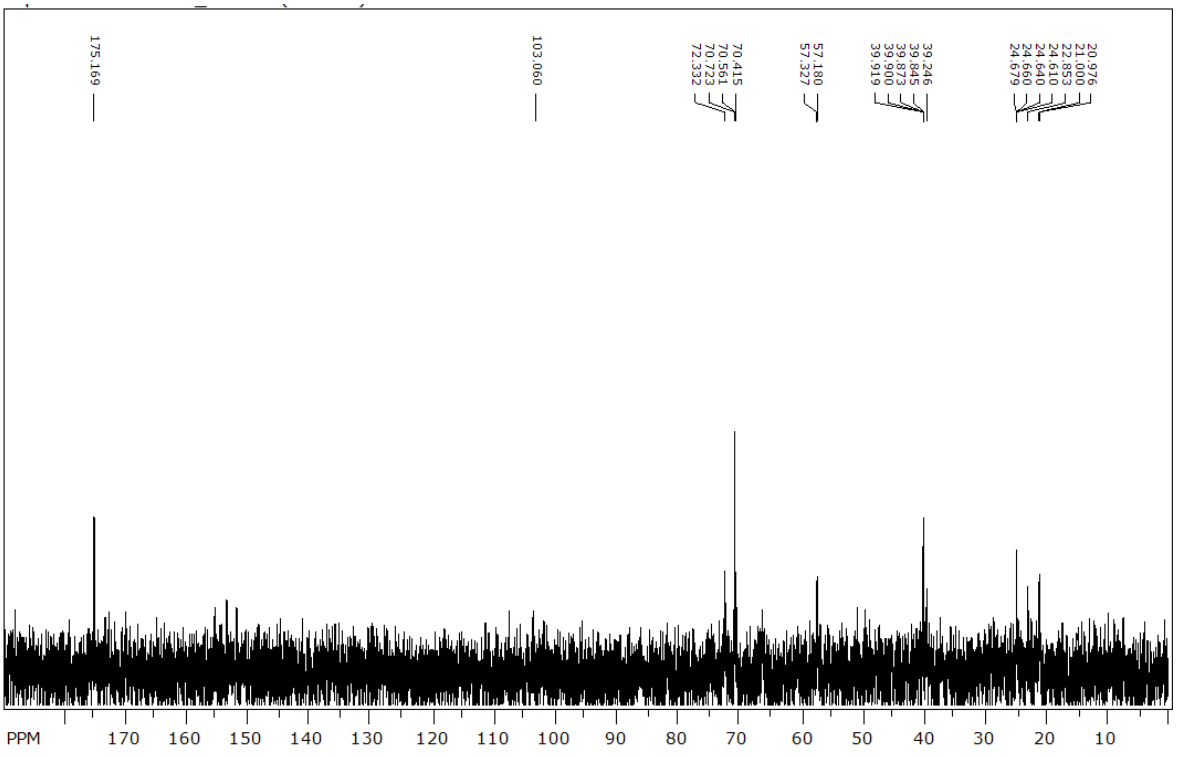

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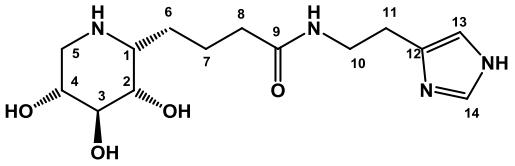

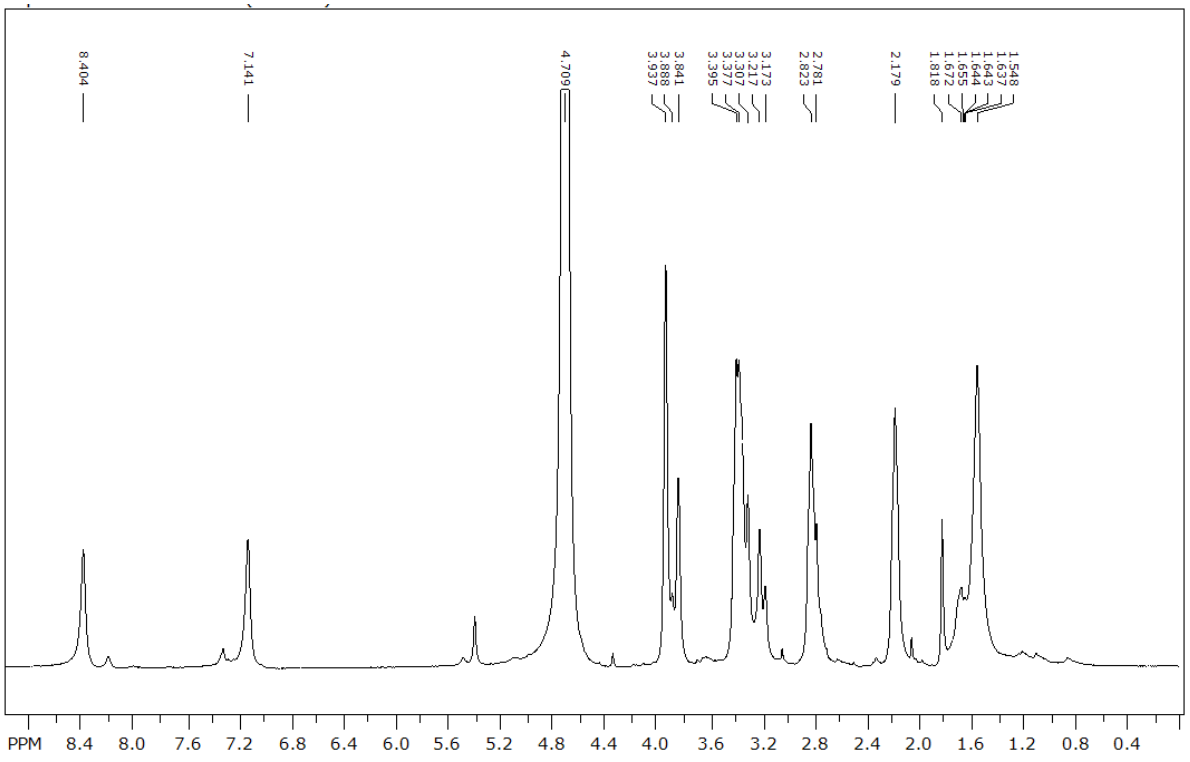

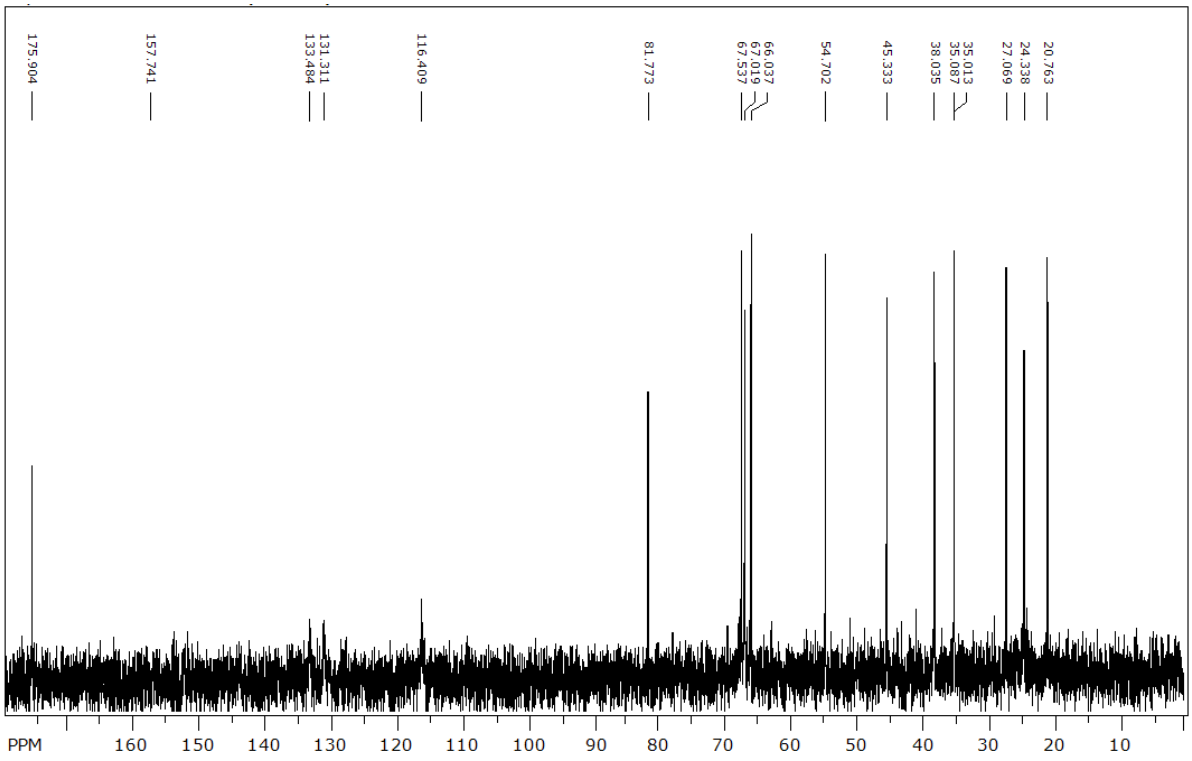

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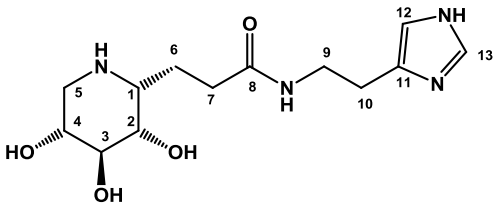
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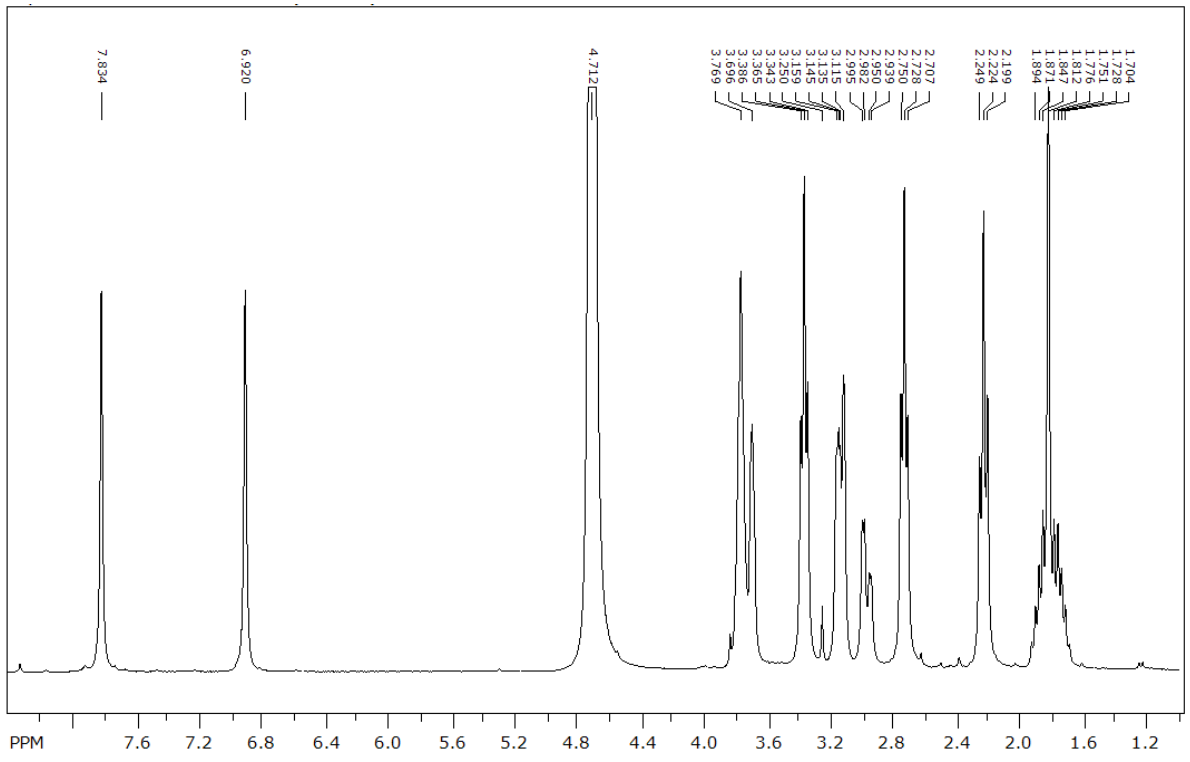
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3.159
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2.995
2.982
2.950
2.939
2.750
2.728
2.707
2.249
2.224
2.199
1.894
1.871
1.847
1.812
1.776
1.751
1.728
1.704
PPM
7.6
7.2
6.8
6.4
6.0
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4.4
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2.8
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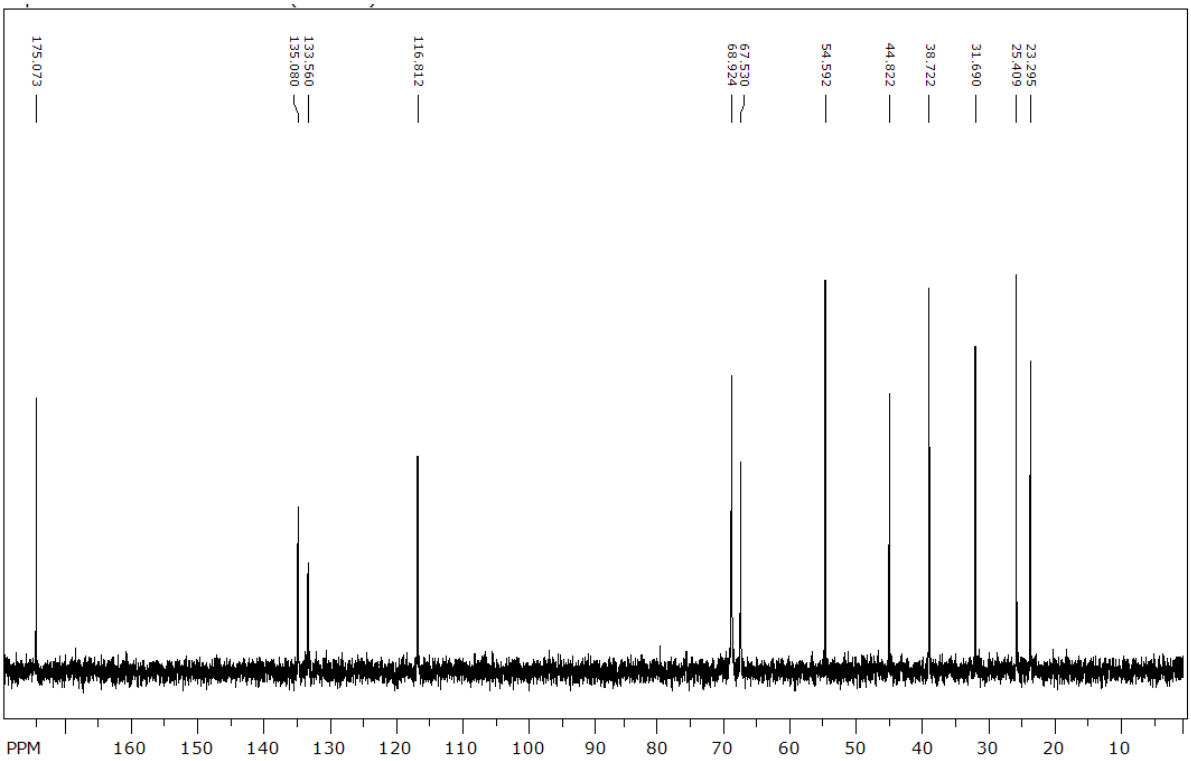
1.6
1.2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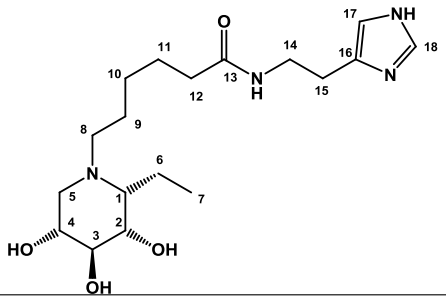

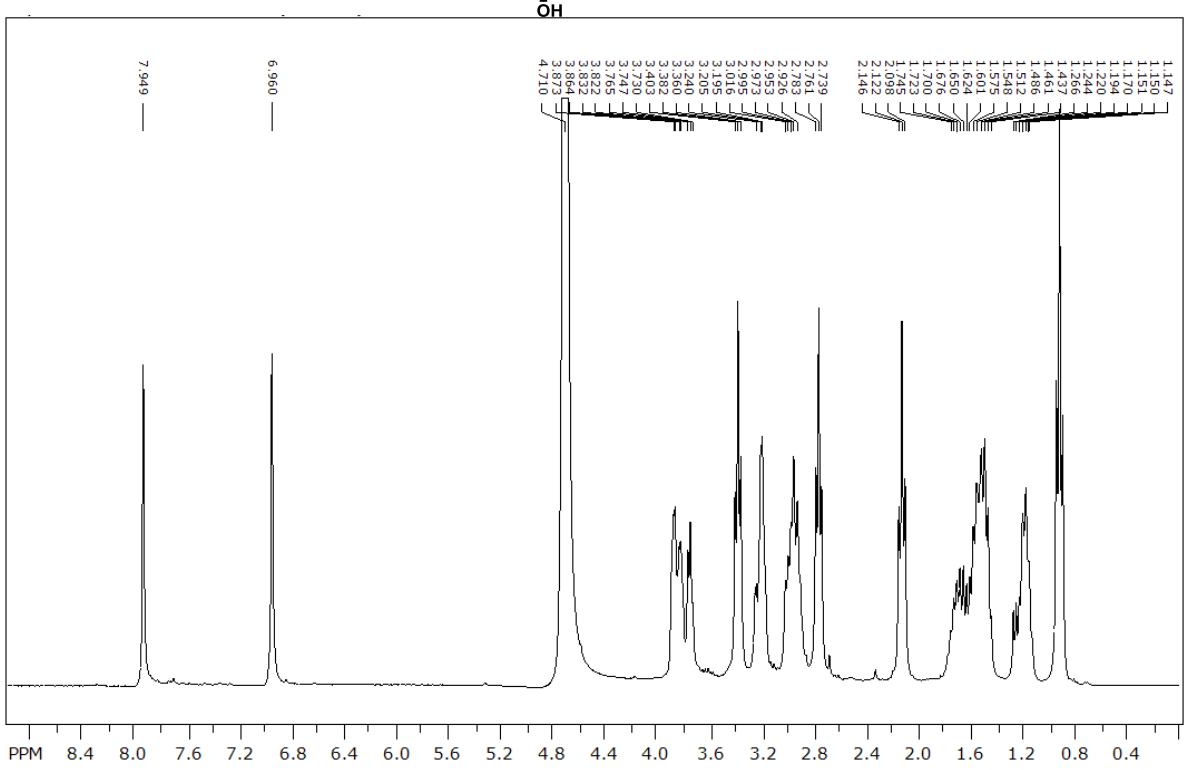

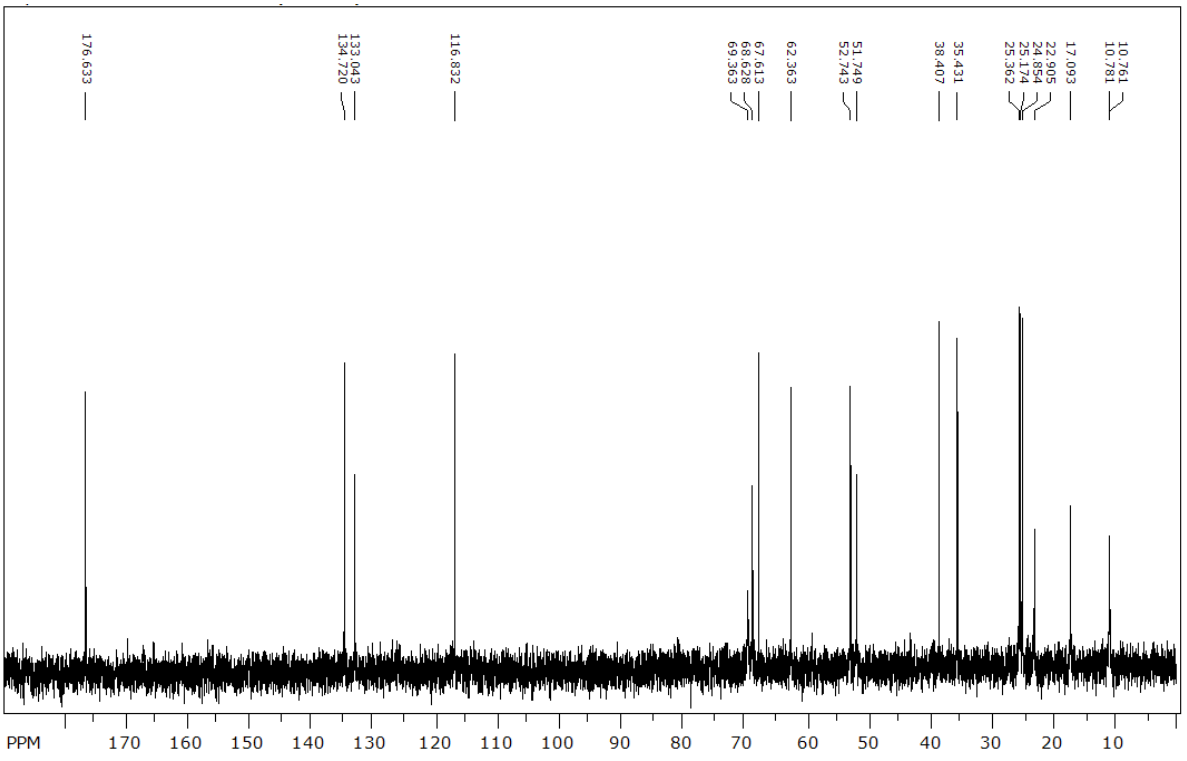

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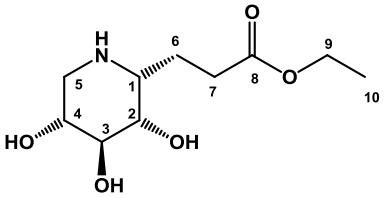

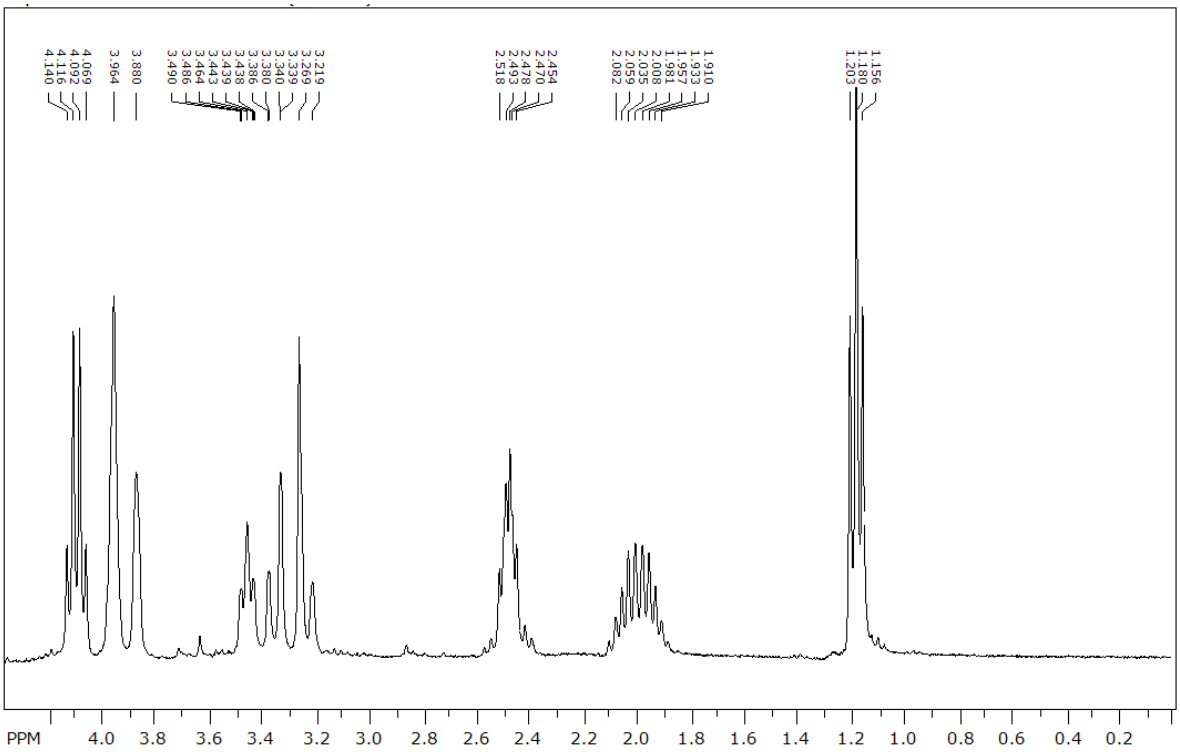

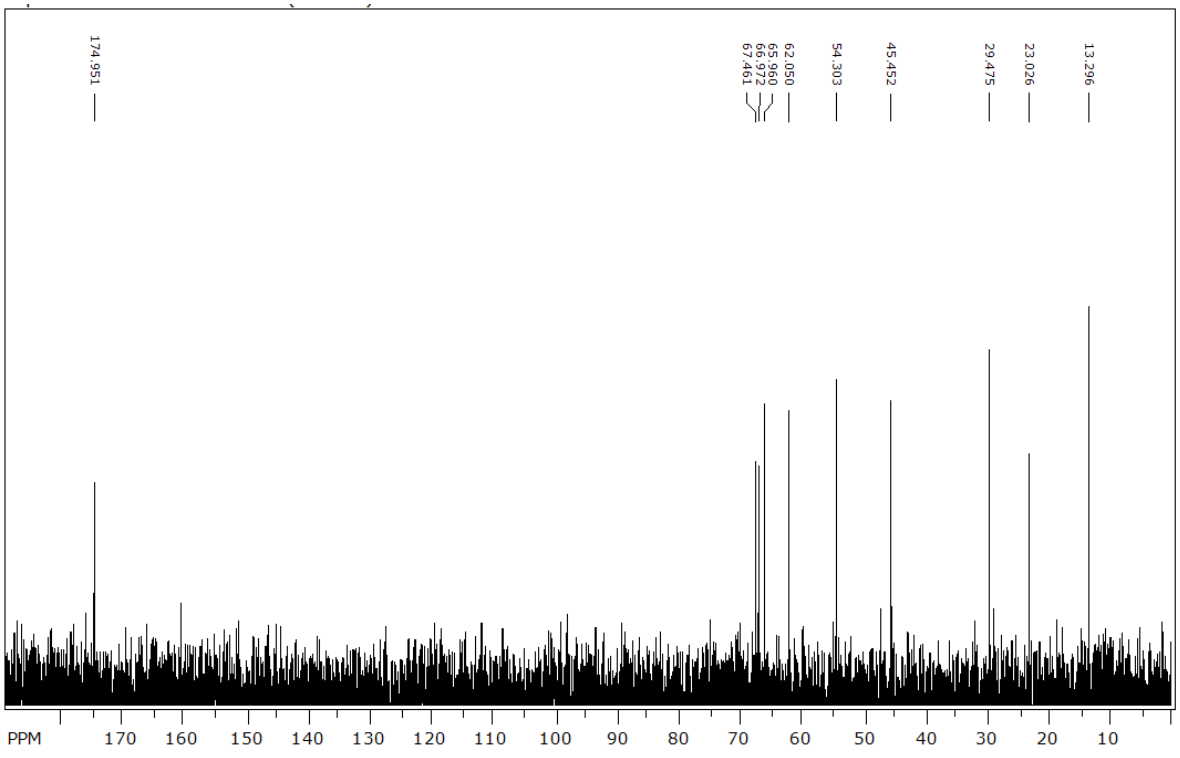

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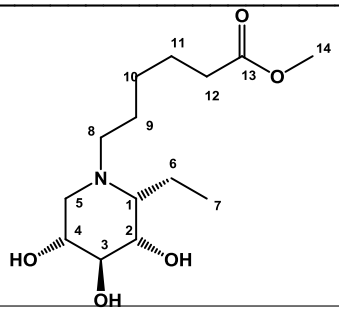

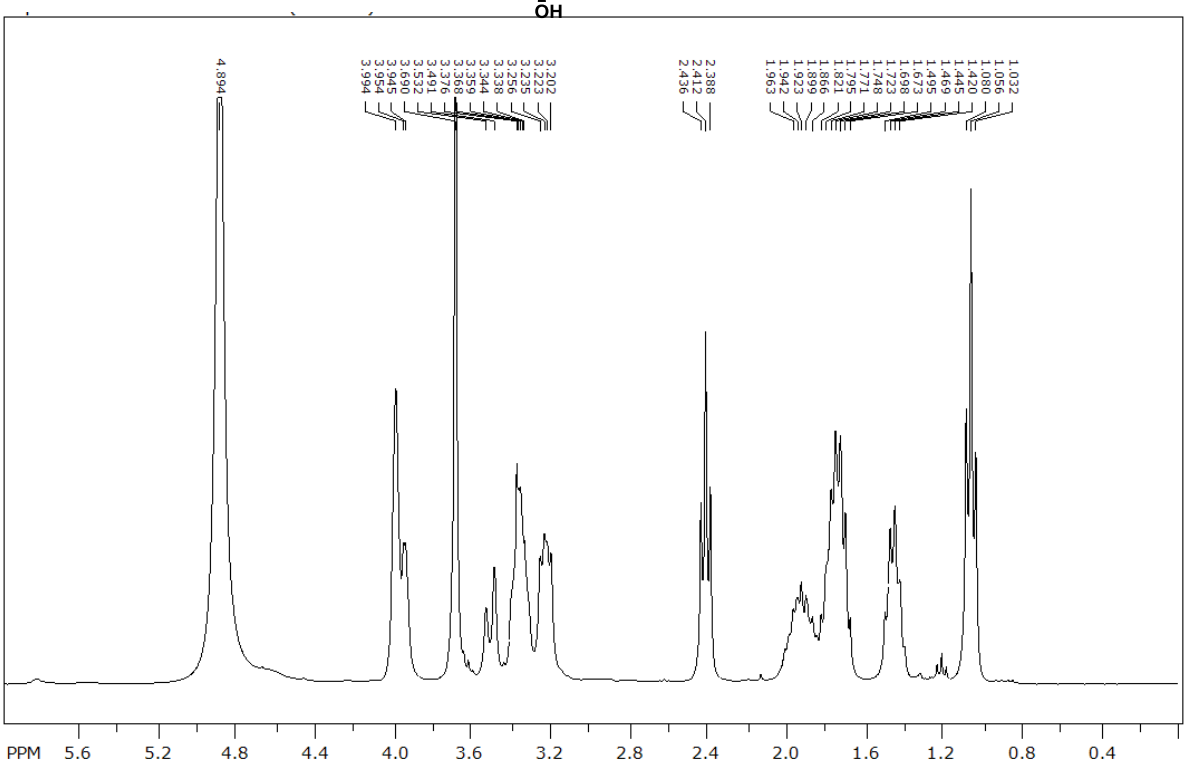

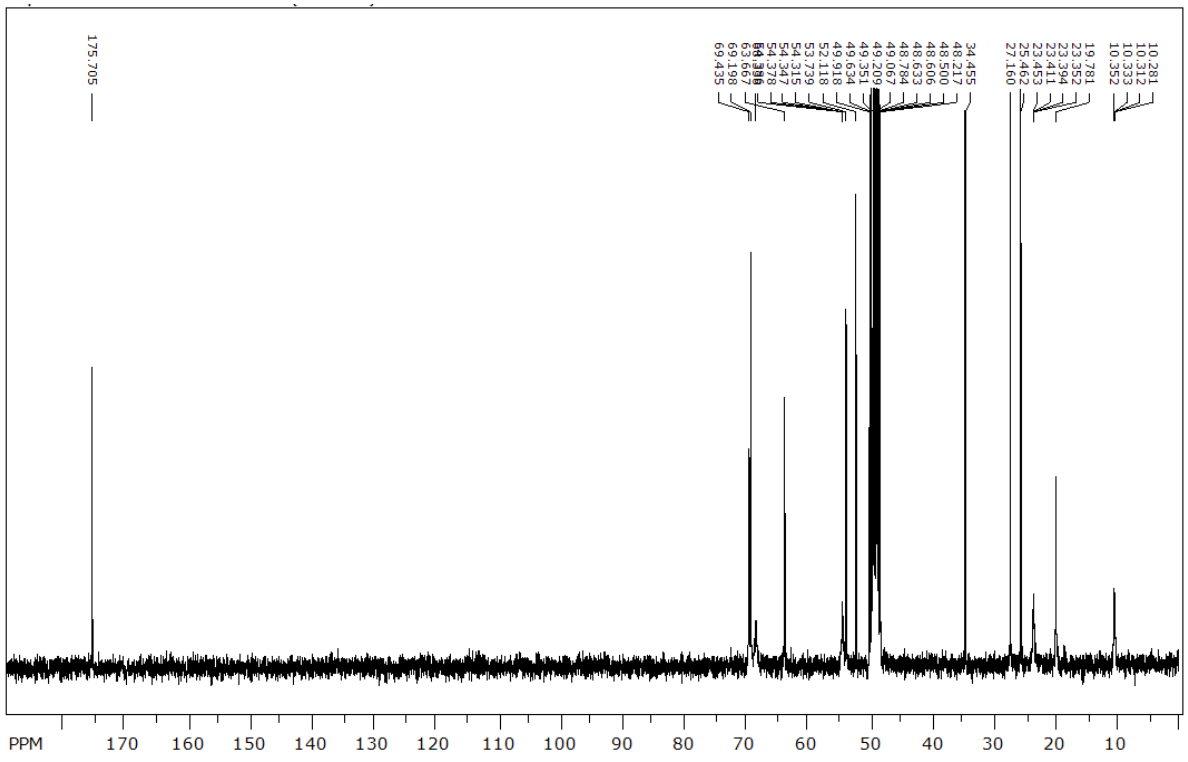

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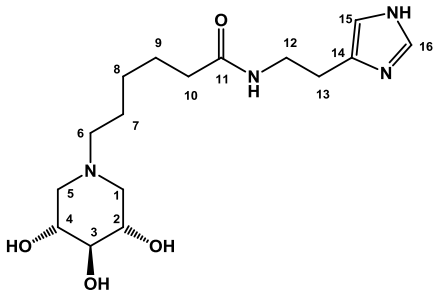

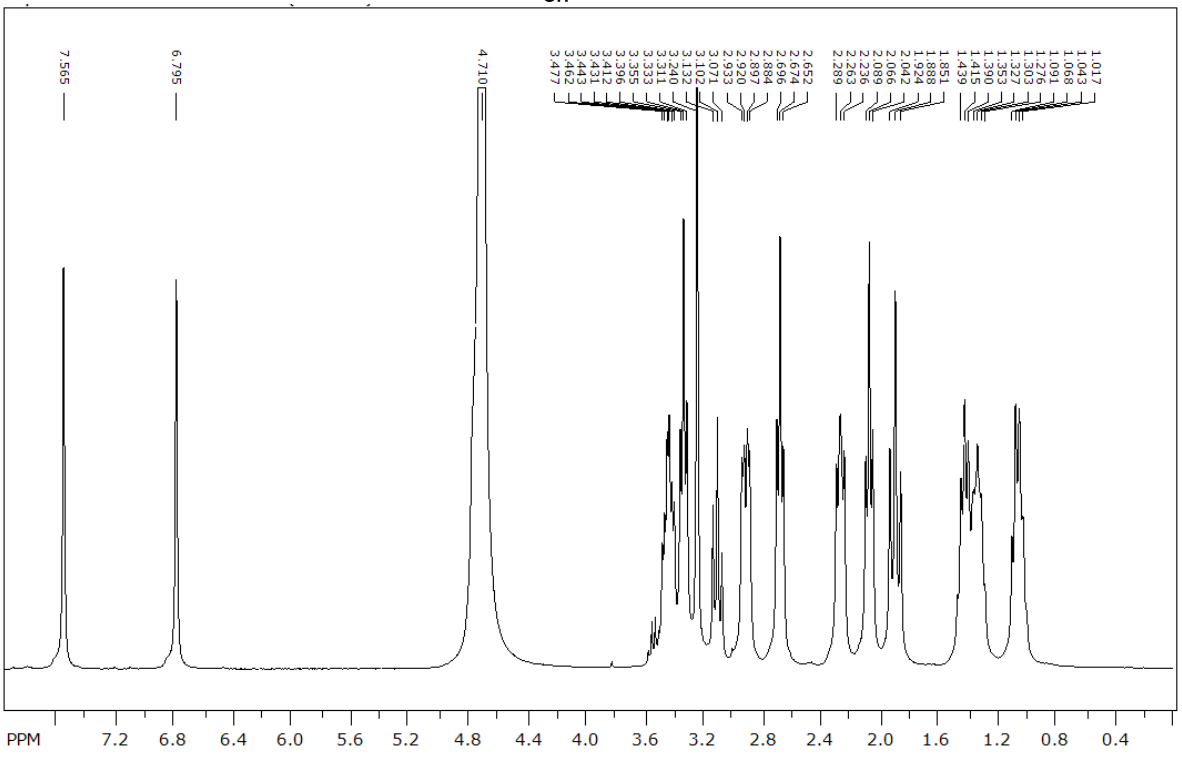

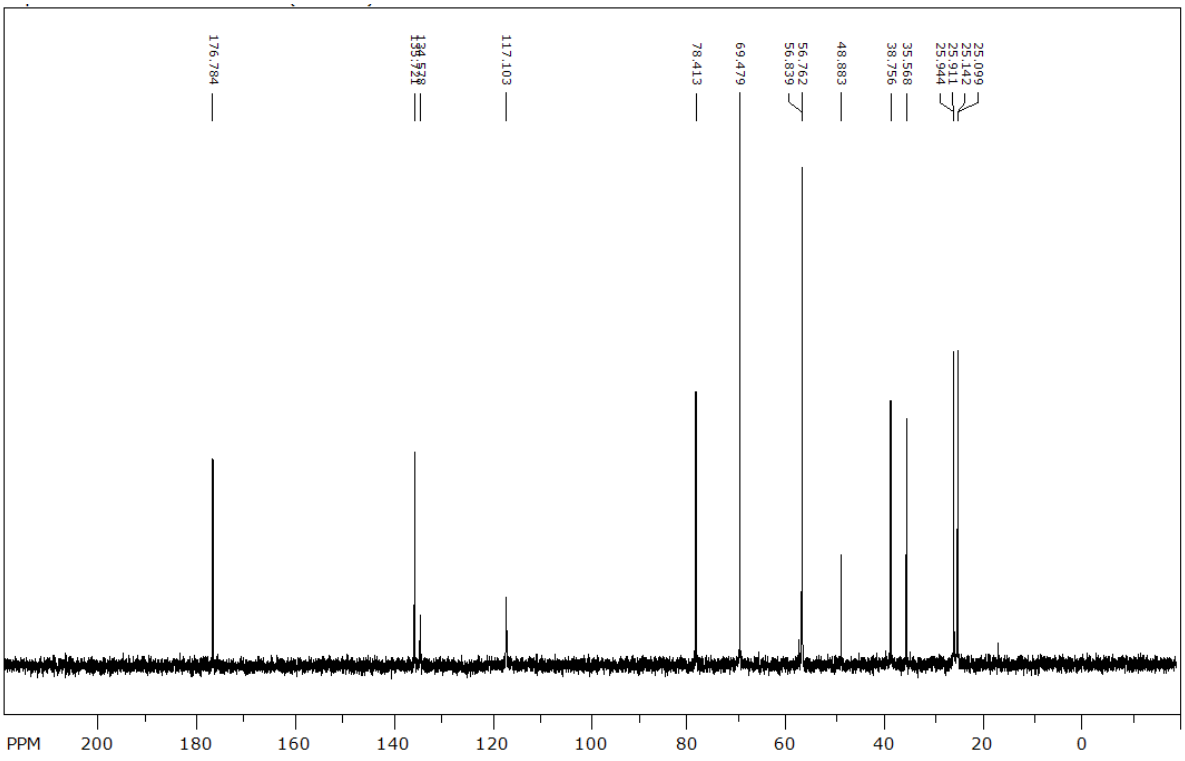

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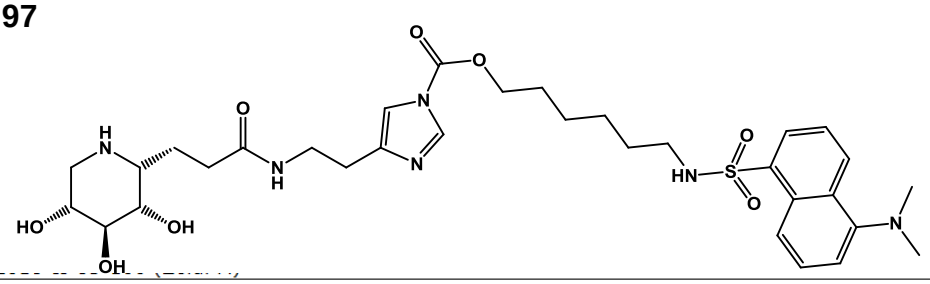

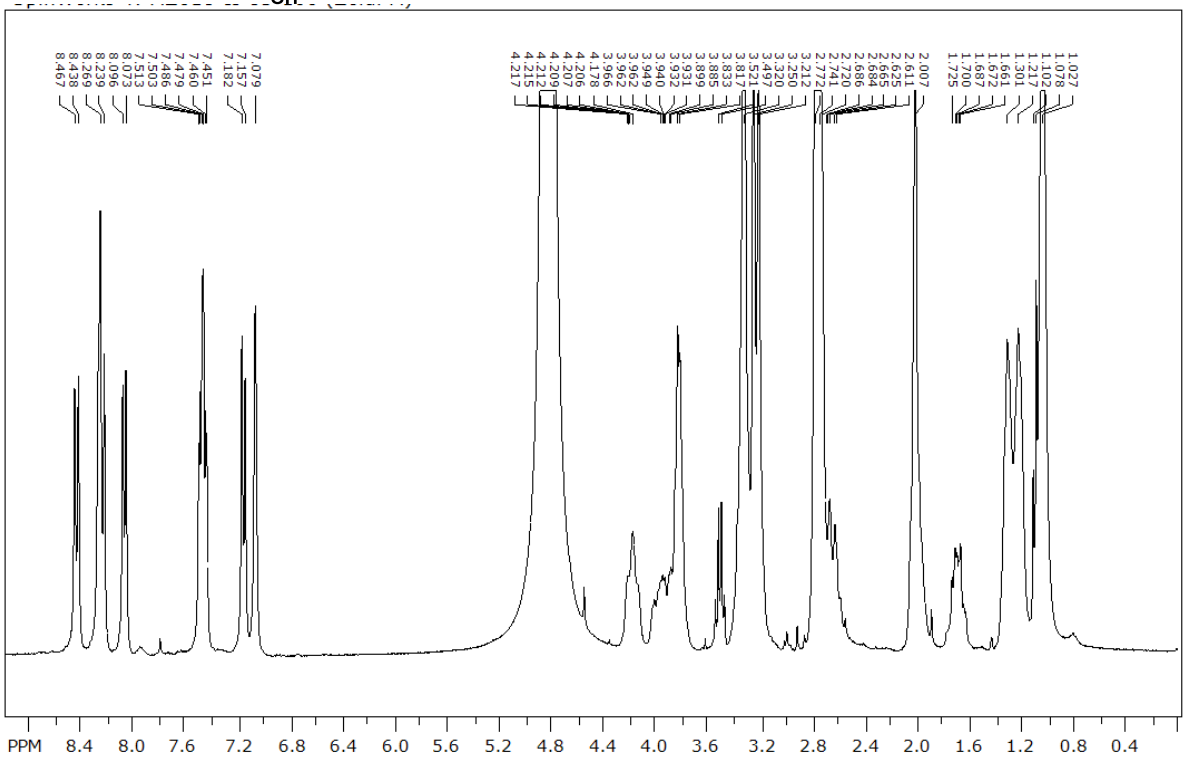

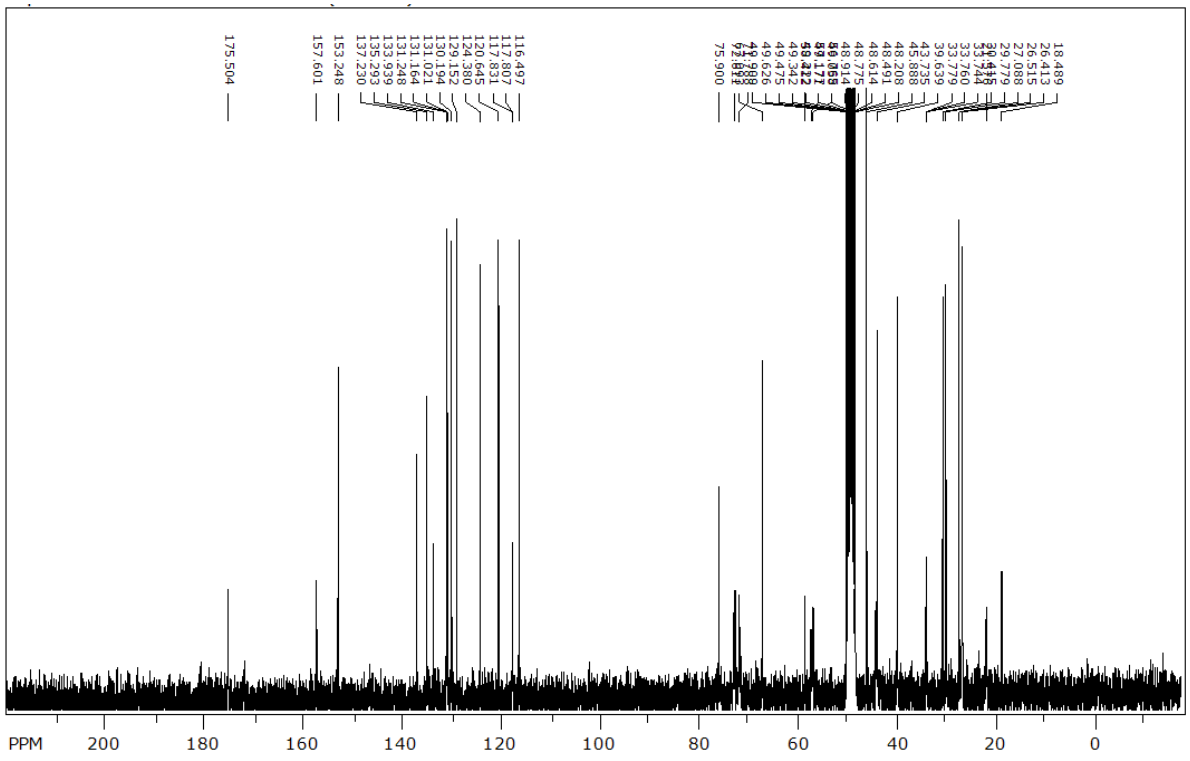

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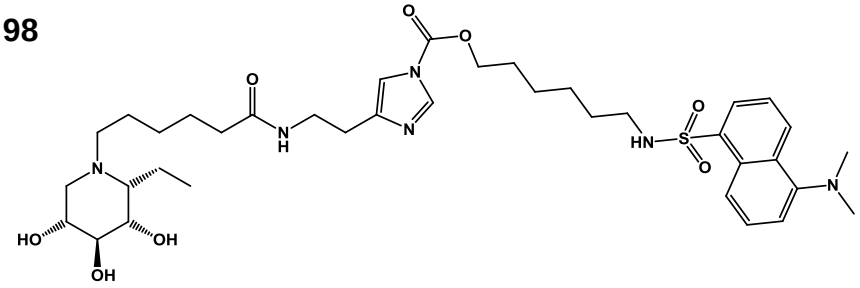

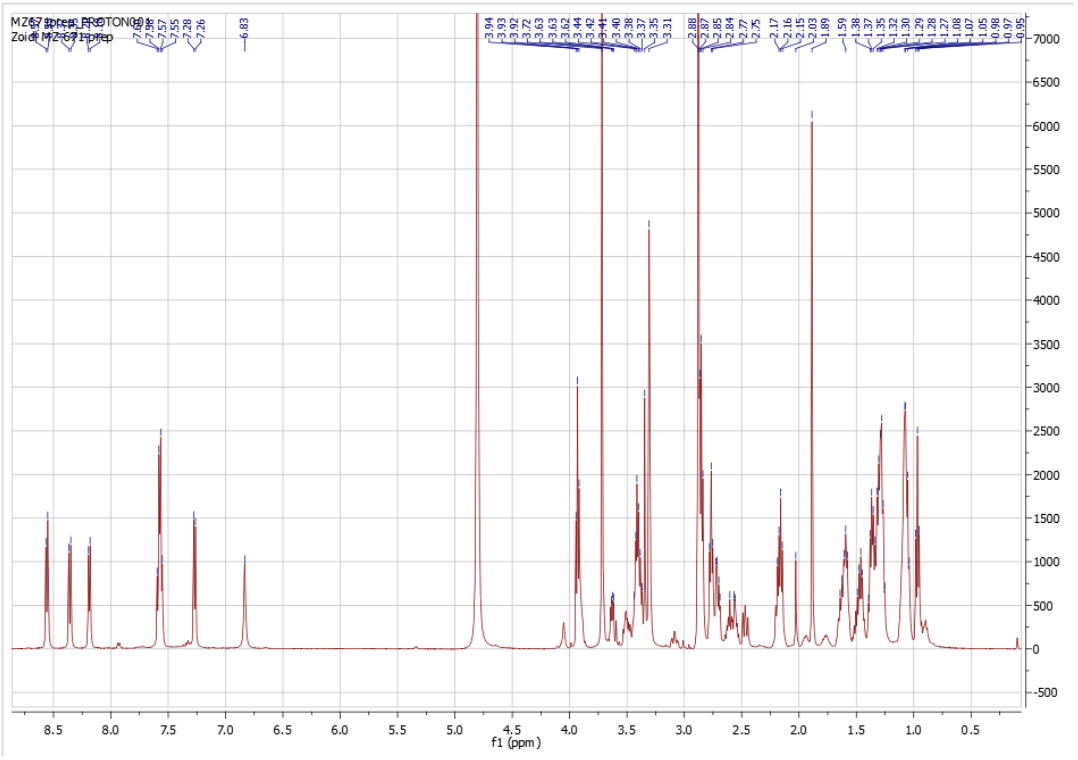

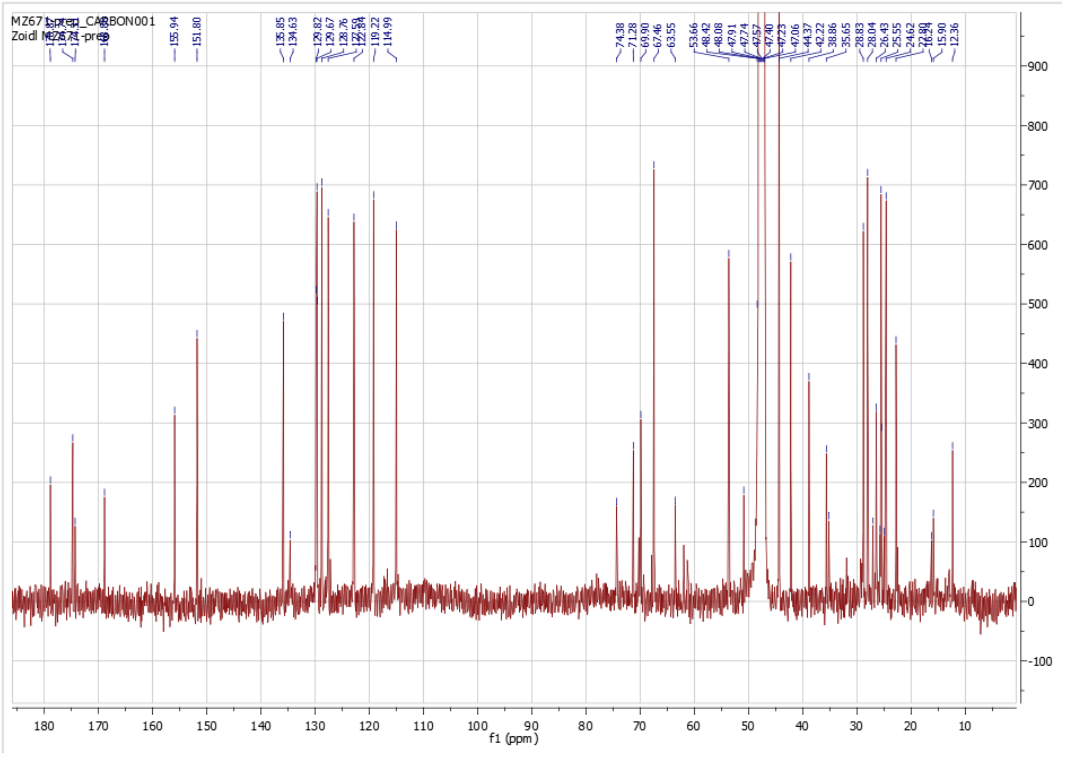

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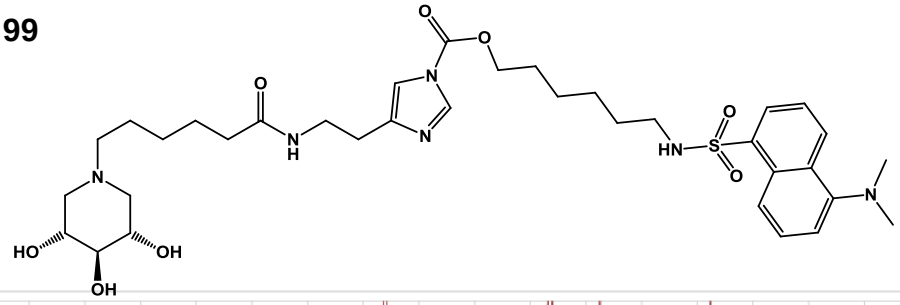

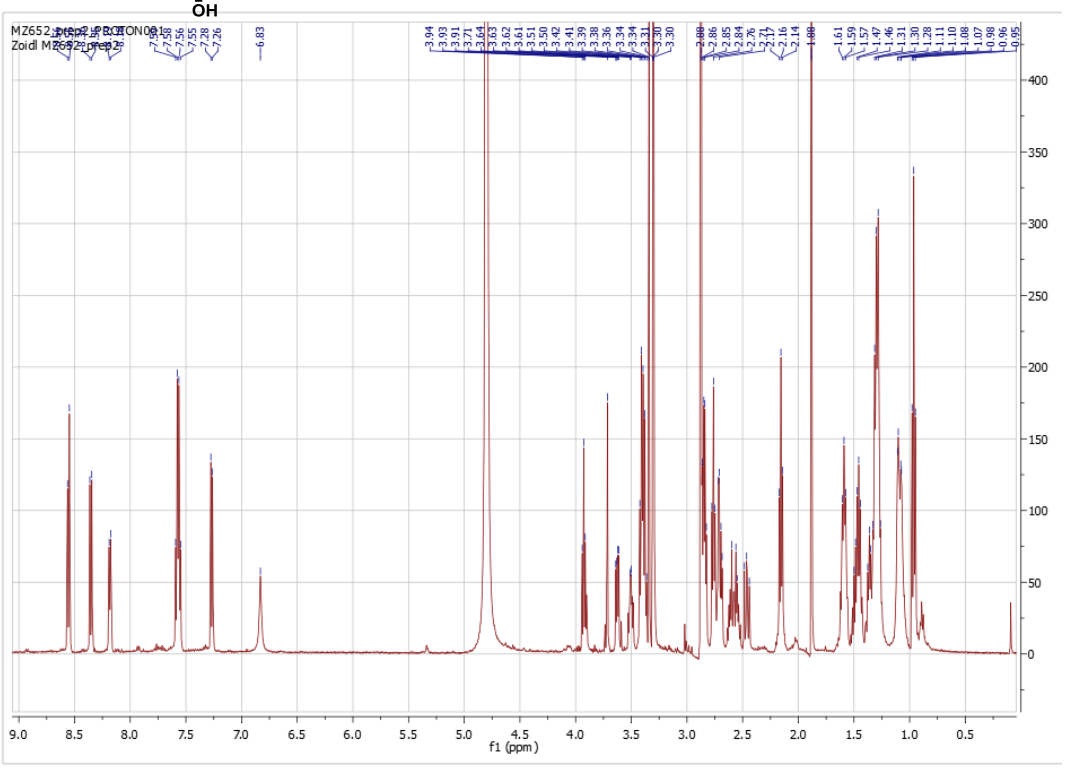

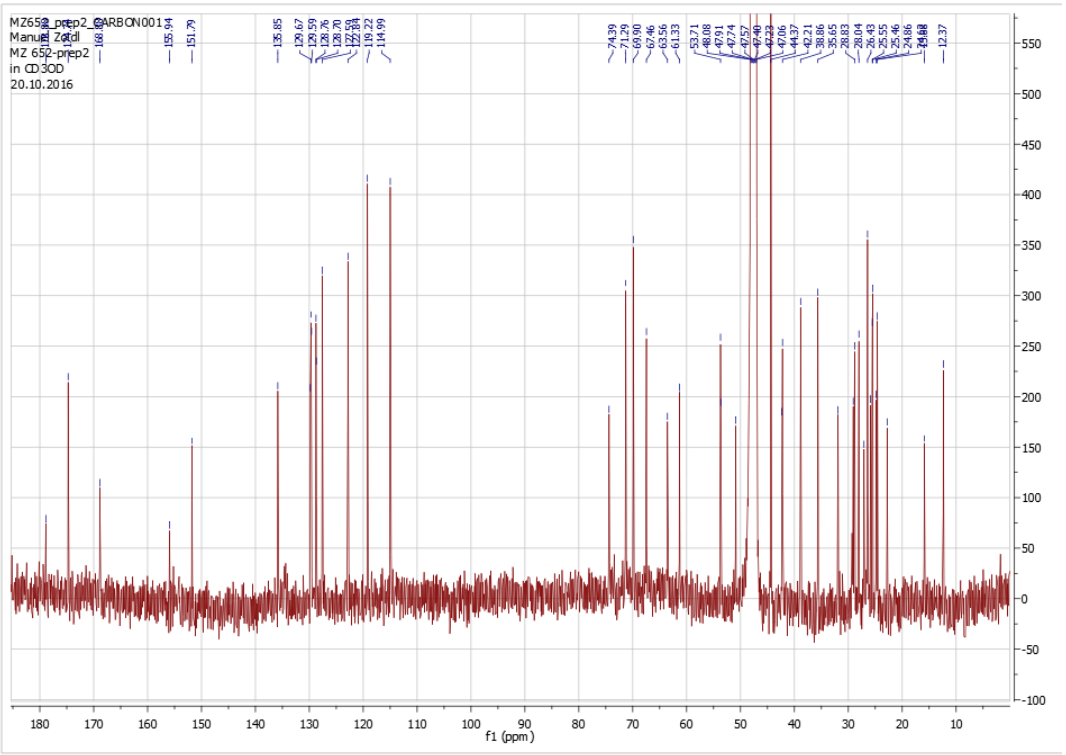

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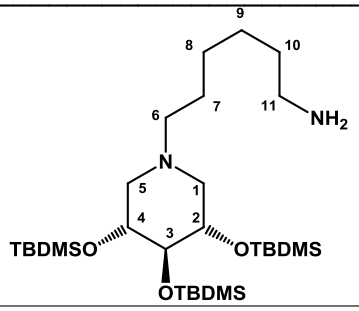

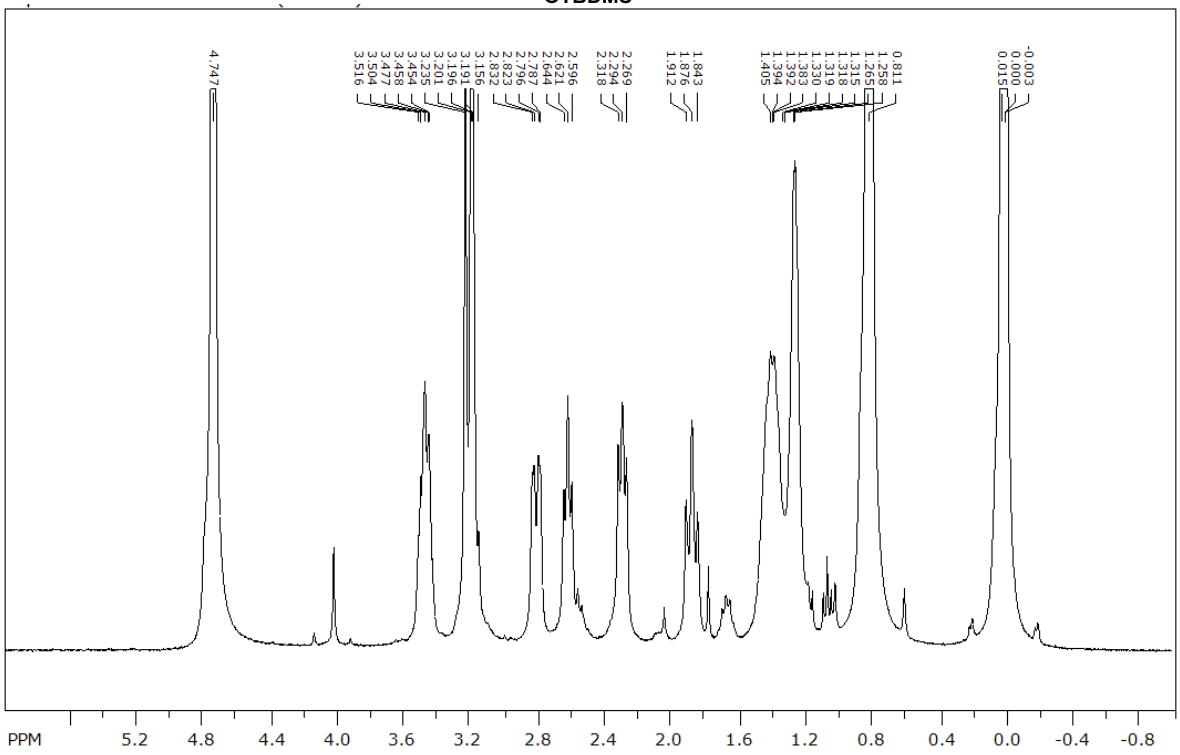

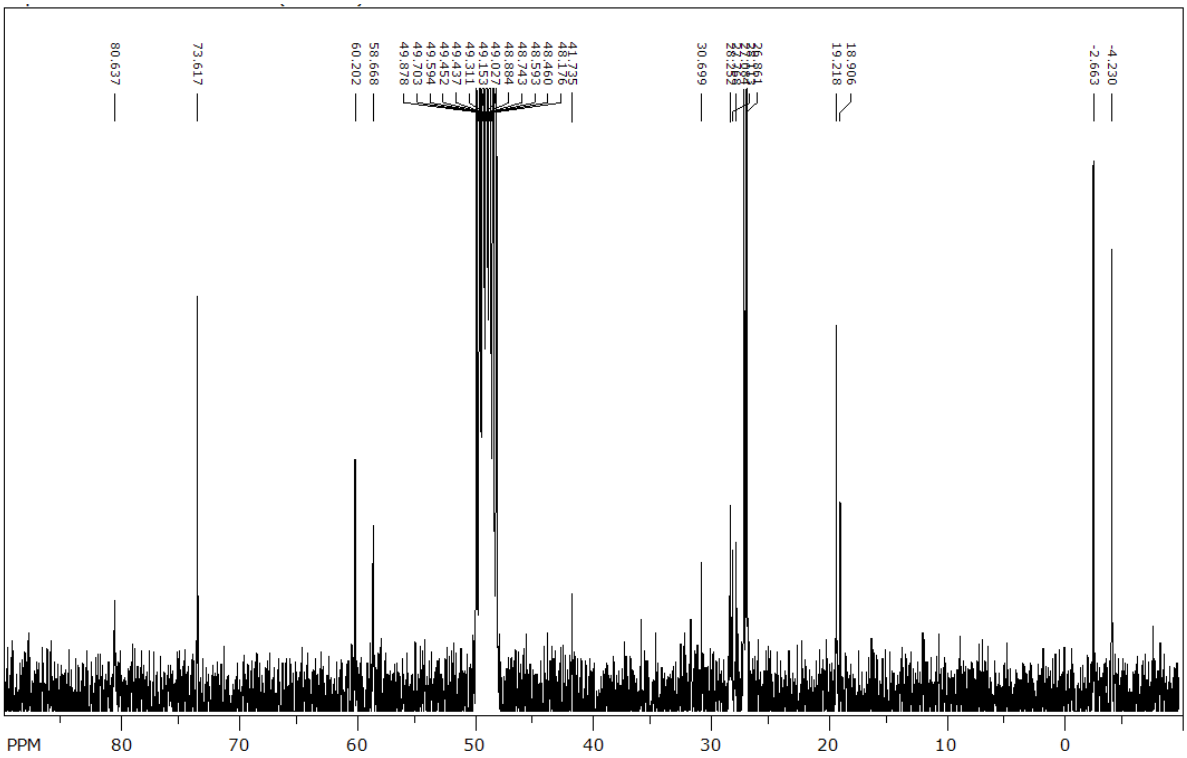

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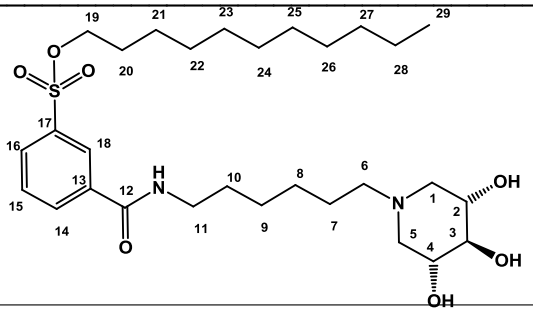

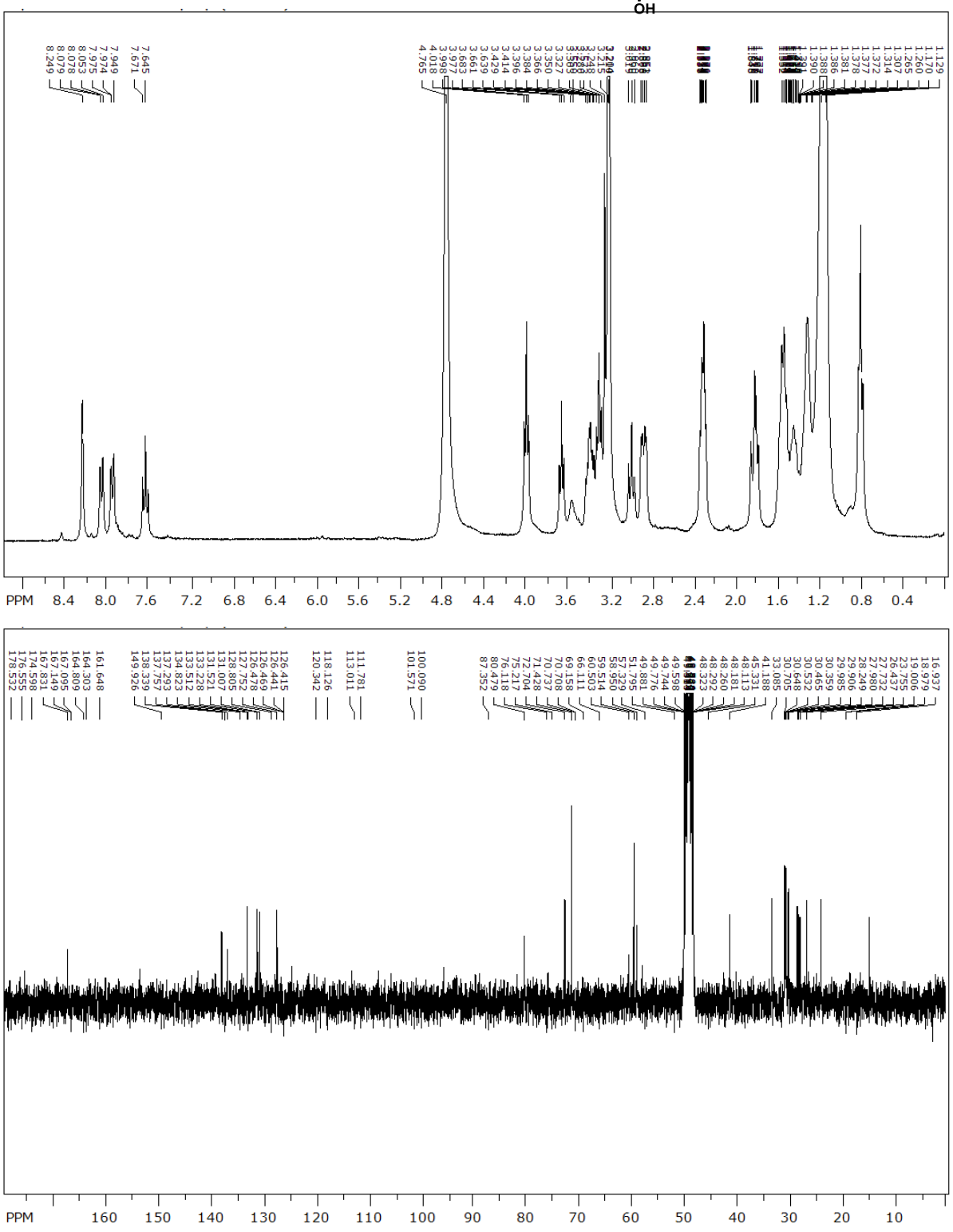

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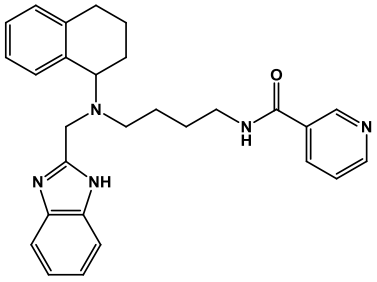

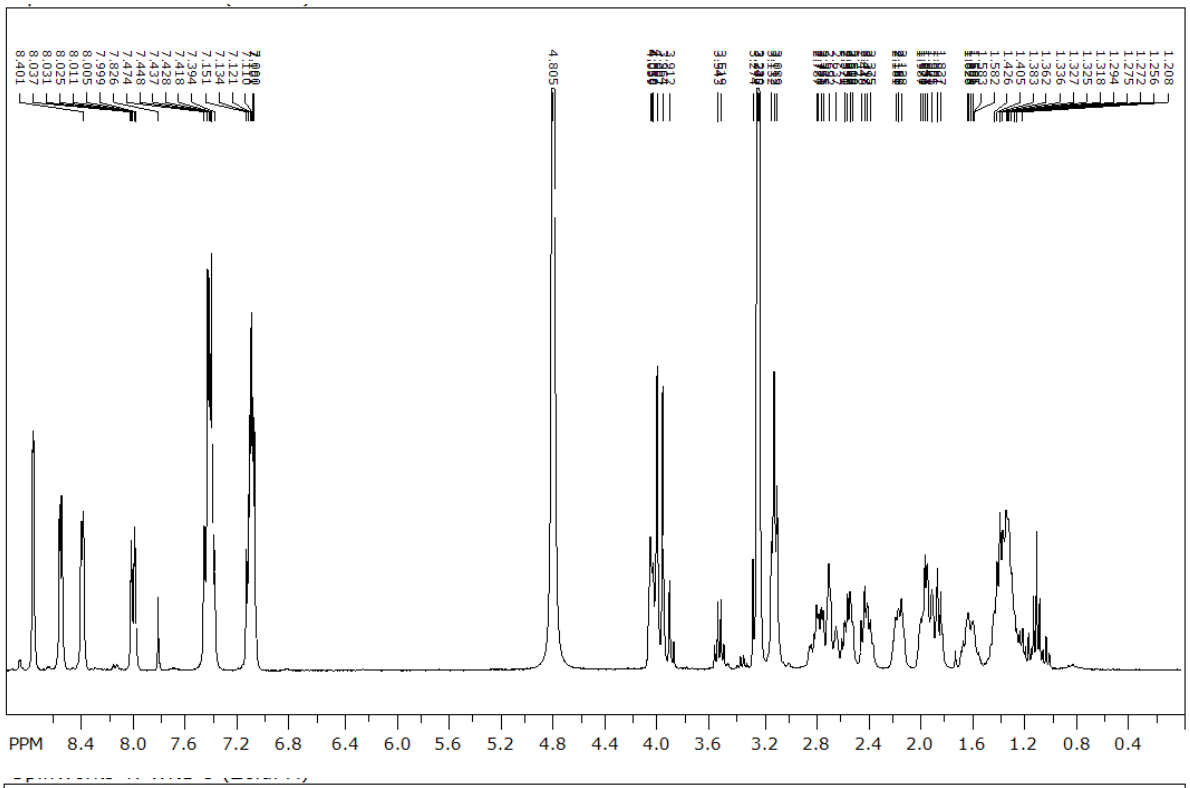

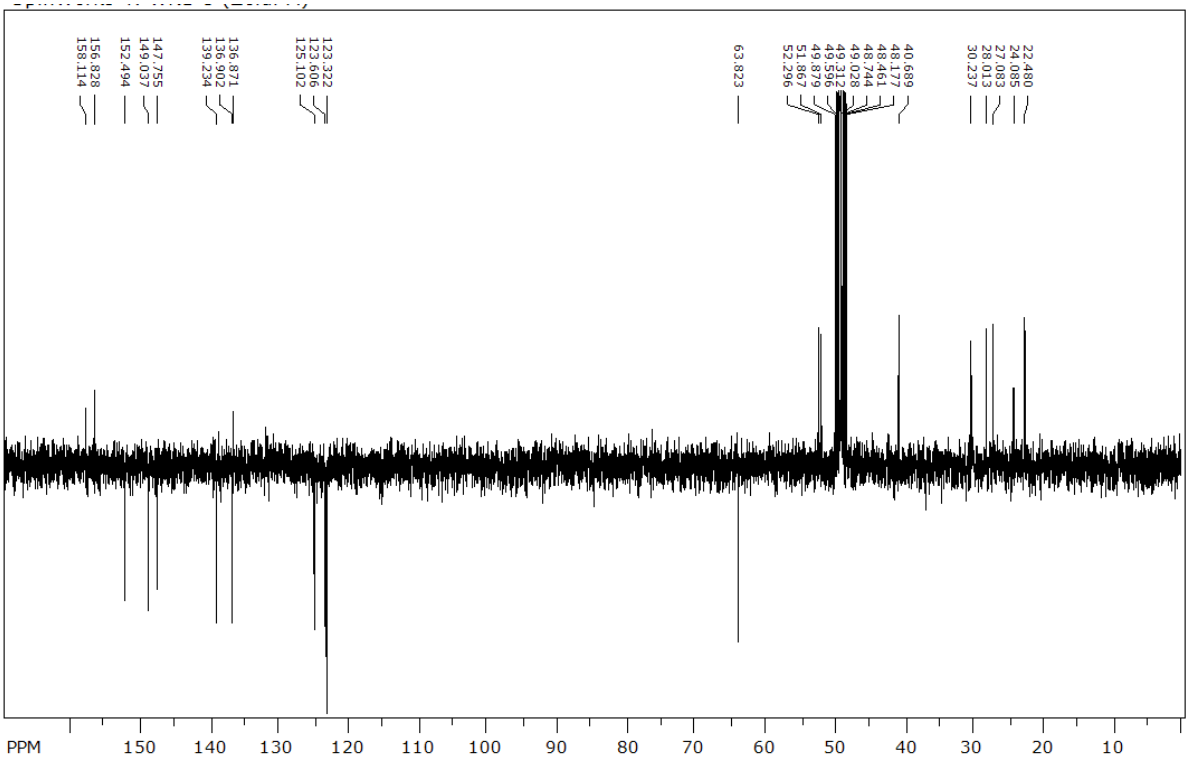

8. Literature, CV, List of Publications

[1] Pang, P-C.; Chiu, P.C.N.; Lee, C-L.; Chang, L-Y.; Panico, M.; Morris, H.R.; Haslam, S.M.; Khoo, K-H.; Clark, G.F.; Yeung, W.S.B.; Delli, A. *Science* **2011**, *333*, 1761-1764.

[2] Flitsch, S.L.; Ulijn, R.V. *Nature* **2003**, *421*, 219-220.

[3] Ganem, B. *Acc. Chem. Res.* **1996**, *29*, 340-347.

[4] Bertozzi, C.R.; Kiessling, L.L. *Science* **2001**, *291*, 2357-2364.

[5] Osborn, H.M.I.; Evans, P.G.; Gemmel, N.; Osborne, S.D. *J. Pharm. Pharmacol*. **2004** *56*, 691-702.

[6] Cox, T.; Lachmann, R.; Hollak, C.; Aerts, J.; van Wealy, S.; Hrebicek, M.; Platt, F.; Butters, T.; Dwek, R.; Moyses, C.; Gow, I.; Elstein, D.; Zimran, A. *Lancet* **2000**, *355*, 1481-1485.

[7] Asano, N.; Nash, R.J.; Molyneux, R.J.; Fleet, G.W.J. *Tetrahedron: Asymm*. **2000**, *11*, 1645-1680.

[8] http://www.cazy.org/Welcome-to-the-Carbohydrate-Active.html; 09.08.2016

[9] Legler,G. *Adv. Carbohydr. Chem. Biochem*. **1990**, *48*, 319-384.

[10] Larkin, A.; Imperiali, B. *Biochemistry* **2011**, *50*, 4411-4426.

[11] Zechel, D.L.; Withers, S.G. *Acc. Chem. Res.* **2000**, *33*, 11-18.

[12] Koshland, D.E. *Biol. Rev.* **1953**, *28*, 416-426.

[13] Murray, R.K.; Granner, D.K.; Mayes, P.A.; Rodwell, V.W. *Harper: Bioquímica; Atheneu:* São Paulo, **1994**; p 628-646.

[14] Stütz, A.E. In: *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*; Stütz, A.E.; Wiley-VCH, Weinheim; **1999**, 9-10.

[15] Hollander, P.; Pi-Sunyer, X.; Coniff, R.E. *Diabetes Care* **1997**, *20*, 248-253.

[16] Kordik, c.P.; Reitz, A.B. *J. Med. Chem*. **1999**, *42*, 181-201.

[17] Papandreou, M.J.; Barbouche, R.; Guieu, R.; Kieny, M.P.; Fenouillet, E. *Mol. Pharmacol.* **2002**, *61*, 186-193.

[18] Asano, N.; Kato, A.; Watson, A.A. *Mini Rev. Med. Chem.* **2001**, *1*, 145-154.

[19] Platt, F.M.; Butters, T.D. *Exp. Rev. Mol. Med*. **2000**, 1-18.

[20] Rempel, B.P.; Withers, S.G. *Glycobiology* **2008**, *18*, 570-586.

[21] Seo, W.D.; Kim, J.H.; Kang, J.E.; Ryu, H.W.; Curtis-Long, M.J.; Lea, H.S.; Yang, M.S.; Park, K.H. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 5514-5516.

[22] Leaback, D.H. *Biochem. Biophys. Res. Commun.* **1968**, *32*, 1023-1025.

[23] Yagi, M.; Kouno, T.; Aoyagi, Y.; Murai, H. *Nippon Nogei Kagaku Kaishi* **1976**, *50*, 571–572.

[24] Jespersen, T.M.; Bols, M. *Tetrahedron* **1994**, *50*, 13449-13460.

[25] Szczepina, M.G.; Johnston, B.D.; Yuan, Y.; Svensson, B.; Pinto, B.M. *J. Am. Chem. Soc.* **2004**, *126*, 12458-12469.

[26] Lillelund, V.H.; Jensen, H.H.; Liang, X.; Bols, M. *Chem. Rev*. **2002**, *102*, 515-553.

[27] Hughes, A.B.; Rudge, A.J. *Nat. Prod. Rep.* **1994**, *11*, 135-162.

[28] Stütz, A.E. In: *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond*; Stütz, A.E.; Wiley-VCH, Weinheim; **1999**, 216-217.

[29] Wrodnigg, T.M.; Stütz, A.E. *Curr. Enz. Inhib.* **2012***, 8,* 1-53.

[30] Martin, O.R.; Compain, P. In: *Iminosugars: From synthesis to therapeutic applications;* Eds. Compain, P.; Martin, O.R.; John Wiley & Sons, Ltd: Chichester, **2007**; pp 63-86.

[31] Compain, P.; Martin, O.R.; Boucheron, C.; Godin, G.; Yu, L.; Ikeda K.; Asano, N. *ChemBioChem*, **2006**, *7*, 1356-1359.

[32] Godin, G.; Compain, P.; Martin, O.R.; Ikeda, K.; Yu, L.; Asano, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5991-5995.

[33] Meikle, P.J.; Hopwood, J.J.; Clague, A.E.; Carey, W.F. *J. Am. Med. Assoc*. **1999**, *281*, 249-254.

[34] Kolter, T.; Sandhoff, K. *BBA-Biomembranes.* **2006**, *1758*, 2057–2079.

[35] Wennekes, T.; van den Berg, R.J.B.H.N.; Boot, R.G.; van der Marel, G.A.; Overkleeft, H.S.; Aerts, J.M.F.G. *Angew. Chem. Int. Ed*. **2009**, *48*, 8848-8869.

[36] Schiffmann, R.; Brady, R.O. *Drugs* **2002**, *62*, 733-742.

[37] M.S. Sands, Gene therapy, in: F.M. Platt, S.U.Walkley (Eds.), Lysosomal Disorders of the Brain, Oxford Univ. Press, Inc., New York, **2004**, pp. 409–430, Chap. 16.

[38] Fan,j J.Q. *Trends Pharmacol. Sci*. **2003**, *24*, 355-360.

[39] Asano, N.; Ishii, S.; Kizu, H.; Ikeda, K.; Yasuda, K.; Kato, A.; Martin, O.R.; Fan, J-Q. *Eur. J. Biochem.* **2000**, *267*, 4179-4186.

[40] Zhu, X.; Sheth, K.A.; Li, S.; Chang, H-H.; Fan, J-Q. *Angew. Chem. Int. Ed*. **2005**, *44*, 7450-7453.

[41] Thonhofer, M.; Weber, P.; Santana, A.G.; Tysoe, C.; Fischer, R.; Pabst, B.M.; Paschke, E.; Schalli, M.; Stuetz, A.E. *Carb. Res.* **2016**, *429*, 71-80.

[42] Biela-Banas, A.; Oulaidi, F.; Front, S.; Gallienne, E.; Ikeda-Obatake, K.; Wenger, D.A.; Martin, O.R. *Chembiochem* **2014**, *9*, 2647-2652.

[43] Sletten, E.M.; Bertozzi, C.R. *Angew. Chem. Int. Ed.* **2009**, *48*, 6974-6998.

[44] Fujishima, S.; Yasui, r.; Miki, T.; Ojida, A.; Hamachi, I. *J. Am. Chem. Soc.* **2012**, *134*, 3961-3964.

[45] Takaoka, Y.; Ojida, A.; Hamachi, I. *Angew. Chem. Int. Ed*. **2013**, *52*, 4088-4106.

[46] Link, A.J.; Mock, M.L.; Tirrell, D.A. *Curr. Opin. Biotechnol*. **2003**, *14*, 603-609.

[47] Wang, L.; Schultz, P.G. *Angew. Chem*. **2005**, *117*, 34-68.

[48] Glazer, A.N. *Annu. Rev. Biochem.* **1970**, *39*, 101-130.

[49] Johnsson, I.; Spence, M.T.Z. *The Molecular Probes Handbook*, 11th ed., **2010**, Part I.

[50] McFarland, J.M.; Francis, M.B. *J. Am. Chem. Soc*. **2005**, *127*, 13490-13491.

[51] Tanaka, K.; Masuyama, T.; Hasegawa, K.; Tahara, T.; Mizuma, H.; Wada, Y.; Watanabe, Y.; Fukase, K. *Angew. Chem*. **2008**, *120*, 108-111.

[52] Bernardes, G.J.L.; Chalker, J.M.; Errey, J.C.; Davis, B.G. *J. Am. Chem. Soc*. **2008**, *130*, 5052-5053.

[53] Tilley, S.D.; Francis, M.B. *J. Am. Chem. Soc*. **2006**, *128*, 1080-1081.

[54] Antos, J.M.; Francis, M.B. *J. Am. Chem. Soc*. **2004**, *126*, 10256-10257.

[55] Keppler, A.; Gendreizig, S.; Gronemeyer, T.; Pick, H.; Vogel, H.; Johnsson, K. *Nat. Biotechnol*. **2003**, *131*, 15769-15776.

[56] Los, G.V.; Encell, L.P.; McDougall, M.G.; Hartzell, D.D.; Karassina, N.; Zimprich, C.; Wood, M.G.; Learish, R.; Ohana, R.F.; Urh, M.; Simpson, D.; Mendez, J.; Zimmerman, K.; Otto, P.; Vidugiris, G.; Zhu, J.; Darzins, A.; Klaubert, D.H.; Bulleit, R.F.; Wood, K.V. *ACS Chem. Biol*. **2008**, *3*, 373-382.

[57] Griffin, B.A.; Adams, S.R.; Tsien, R.Y. *Science* **1998**, *281*, 269-272.

[58] Halo, T.L.; Appelbaum, J.; Hobert, E.M.; Balkin, D.M.; Schepartz, A. *J. Am. Chem. Soc*. **2009**, *131*, 438-439.

[59] Terpe, K.; *Appl. Microbiol. Biotechnol*. **2003**, *60*, 523-533.

[60] Guignet, E.G.; Hovius, R.; Vogel, H. *Nat. Biotechnol*. **2004**, *22*, 440-444.

[61] Hauser, C.T.; Tsien, R.Y. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 3693-3697.

[62] Ojida, A.; Honda, K.; Shinmi, d.; Kiyonaka, S.; Mori, Y.; Hamachi, I. *J. Am. Chem. Soc*. **2006**, *128*, 10452-10459.

[63] Chen, I.; Howarth, M.; Lin, W.Y.; Ting, A.Y. *Nat. Methods* **2005**, *2*, 99-104.

[64] Lin, C.W.; Ting, A.Y. *J. Am. Chem. Soc*. **2006**, *128*, 4542-4543.

[65] Uttamchandani, M.; Li, J.; Sun, H.; Yao, S.Q. *ChemBioChem* **2008**, *9*, 667-675.

[66] Patricelli, M.P.; Szardenings, A.K.; Liyanage, M.; Nomanbhoy, T.K.; Wu, M.; Weissig, H.; Aban, A.; Chun, D.; Tanner, S.; Kozarich, J.W. *Biochemistry* **2007**, *46*, 350-358.

[67] Lo, L.C.; Pang, T.L.; Kuo, C.H.; Chiang, Y.L.; Wang, H.Y.; Lin, J.J. *J. Proteome Res.* **2002**, *1*, 35-40.

[68] Vocadlo, D.J.; Hang, H.C.; Kim, E.J.; Hanover, J.A.; Bertozzi, C.R. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9116-9121.

[69] Witte, M.D.; van der Marel, G.A.; Aerts, J.M.F.G.; Overkleeft, H.S. *Org. Biomol. Chem.* **2011**, *9*, 5908-5926.

[70] Evans, M.J.; Cravatt, B.F. *Chem. Rev.* **2006**, *106*, 3279-3301.

[71] Willems, L.I.; Overkleeft, H.S.; Kasteren, S.I. *Bioconjugate Chem*. **2014**, *25*, 1181-1191.

[72] Misaghi, S.; Pacold, M.E.; Blom, D.; Ploegh, H.L.; Korbel, G.A. *Chem. Biol.* **2004**, *11*, 1677-1687.

[73] Witte, D.M.; Horst, D.; Wiertz, E.J.; van der Marel, G.A.; Overkleeft, H.S. *J. Org. Chem.* **2009**, *74*, 605-616.

[74] Miyazaki, A.; Matsuo, I.; Hagihara, S.; Kagegawa, A.; Suzuki, T.; Ito, Y. *Glycoconj. J*. **2009**, *26*, 133-140.

[75] Withers, S.G.; Street, I.P.; Bird, P.; Dolphin, D.H. *J. Am. Chem. Soc.* **1987**, *109*, 7530-7531.

[76] Vocadlo, D.J.; Bertozzi, C.R. *Angew. Chem. Int. Ed.* **2004**, *43*, 5338-5342.

[77] Williams, S.J.; Hekmat, O.; Withers, S.G. *ChemBioChem* **2006**, *7*, 116-124.

[78] Hekmat, O.; Kim, Y.-W.; Williams, S.J.; He, S.; Withers, S.G. *J. Biol. Chem.* **2005**, *280*, 35126-35135.

[79] Hekmat, O.; Florizone, C.; Kim, Y.-W.; Eltis, L.D.; Warren, R.A.J.; Withers, S.G. *ChemBioChem* **2007**, *8*, 2125-2132.

[80] Hekmat, O.; He, S.; Warren, R.A.; Withers, S.G. *J. Proteome Res*. **2008**, *7*, 3282-3292.

[81] Legler, G.; *Z. Physiol. Chem*. **1966**, *345*, 197-214.

[82] Legler, G.; *Biochim. Biophys. Acta.* **1968**, *151*, 728-729.

[83] (a) Thomas, E.W.; McKelvy, J.F.; Sharon, N., *Nature,* **1969**, *222*, 485-486. (b) Legler, G.; Bause, E., *Carbohydr. Res.* **1973**, *28*, 45-52

[84] Kallemeijn, W.W.; Li, K-Y.; Witte, M.D.; Marques, A.R.A.; Aten, J.; Scheij, S.; Jiang, J.; Willems, L.I.; Voorn-Brouwer, T.M.; van Roomen, C.P.A.A.; Ottenhoff, R.; Boot, R.G.; Verhoek, M.; Donker-

Koopman, W.E.; Ferraz, M.J.; van Rijssel, E.R.; Florea, B.I.; Codée, J.D.C.; van der Marel, G.A.; Aerts, J.M.F.G.; Overkleeft, H.S. *Angew. Chem*. **2012**, *124*, 12697-12701.

[85] Romaniouk, A.V.; Silva, A.; Feng, J.; Vijay, I.K. *Glycobiology* **2004**, *14*, 301-310.

[86] Van Scherpenzeel, M.; Van der Berg, R.J.H.B.N.; Donker-Koopman, W.E.; Liskamp, R.M.J.; Aerts, J.M.F.G.; Overkleeft, H.S.; Pieters, R.J. *Bioorg. Med. Chem.* **2010**, *18*, 267-273.

[87] Gandy, M.N.; Debowski, A.W.; Stubbs, K.A. *Chem. Commun*. **2011**, *47*, 5037-5039.

[88] Halazy, S.; Berges, V.; Ehrhard A.; Danzin, C. *Bioorg. Chem*. **1990**, *18*, 330-344.

[89] Janda, K.D.; Lo, L.-C.; Lo, C.-H.L.; Sim, M.-M.; Wang, R.; Wong, C.H.; Lerner, R.A. *Science* **1997**, *275*, 945-948.

[90] Ichikawa, M.; Ichikawa, Y. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 1769-1773.

[91] Tsai, C.-S.; Li, Y.-K.; Lo, L.-C. *Org. Lett.* **2002**, *4*, 3607-3610.

[92] (a) Kurogochi, M.; Nishimura, S.-I.; Lee, Y.C. *J. Biol. Chem*. **2004**, *279*, 44704-44712. (b) Lu, C.P.; Ren, C.-T.; Lai, Y.-N.; Wu, S.-H.; Wang, W.-M.; Chen, J.-Y. Lo, L.-C. *Angew. Chem. Int. Ed*., **2005**, *44*, 6888-6892.

[93] Greenbaum, D.C.; Arnold, W.D.; Lu, F.; Hayrapetian, L.; Baruch, A.; Krumine, J.; Toba, S.; Chehade, K.; Brömme, D.; Kuntz, I.D.; Bogyo, M. *Chem. Biol.*, **2002**, *9*, 1085-1894.

[94] vanSwieten, P.F.; Maehr, R.; van den Nieuwendijk, A.M.C.H.; Kessler, B.M.; Reich, M.; Wong, C.-S.; Kalbacher, H.; Leeuwenburgh, M.A.; Driessen, C.; van der Marel, G.A.; Ploegh, H.L.; Overkleeft, H.S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3131-3134.

[95] Jessani, N.; Niessen, S.; Wei, B.Q.; Nicolau, M.; Humphrey, M.; Ji, Y.; Han, W.; Noh, D.-Y.; Yates III, J.R.; Jeffrey, S.S.; Cravatt, B.F. *Nat. Methods*, **2005**, *2*, 691.

[96] Griffin, R.J. *Prog. Med. Chem*. **1994**, *31*, 121-232.

[97] Saxon, E.; Bertozzi, C.R. *Science* **2000**, *287*, 2007-2010.

[98] Rostovtsev, V.V.; Green, L.G.; Fokin, V.V.; Sharpless, K.B. *Angew. Chem. Int. Ed*. **2002**, *41*, 2596-2599.

[99] Agard, N.J.; Prescher, J.A.; Bertozzi, C.R. *J. Am. Chem. Soc*. **2004**, *126*, 15046-15047.

[100] Mahal, L.K.; Yarema, K.J.; Bertozzi, C.R. *Science* **1997**, *276*, 1125-1128.

[101] Devarah, N.K.; Weissleder, R.; Hilderbrand, S.A. *Bioconjugate Chem.* **2008**, *19*, 2297-2299.

[102] Wang, Y.; Hu, W.J.; Song, W.; Lim, R.K.V.; Lin, Q. *Org. Lett.* **2008**, *10*, 3725-3728.

[103] Lin, Y.A.; Chalker, J.M.; Floyd, N.; Bernardes, G.J.L.; Davis, B.G.I. *J. Am. Chem. Soc*. **2008**, *130*, 9642-9643.

[104] Kwan, D.H.; Chen, H-M.; Ratananikom, K.; Hancock, S.M.; Watanabe, Y.; Kongsaeree, P.T.; Samuels, A.L.; Withers, S.G. *Angew. Chem. Int. Ed*. **2011**, *50*, 300-303.

[105] Hamachi, I.; Nagase, T.; Shinkai, S. *J. Am. Chem. Soc*. **2000**, *122*, 12065-12066.

[106] Takaoka, Y.; Tsutsumi, H.; Kasagi, N.; Nakata, E.; Hamachi, I. *J. Am. Chem. Soc*. **2006**, *128*, 3273-3280.

[107] Tsukiji, S.; Miyagawa, M.; Takaoka, Y.; Tomonori, T.; Hamachi, I. *Nat. Chem. Biol.* **2009**, *5*, 341-343.

[108] Hughes, C.C.; Yang, Y-L.; Liu, W-T.; Dorrestein, P.C.; Clair, J.L.; Fenical W. *J. Am. Chem. Soc*. **2009**, *131*, 12094-12096.

[109] Compain, P.; Chagnault, V.; Martin, O.R. *Tetrahedron: Asymmetry* **2009**, *20*, 672-711.

[110] Böshagen, H.; Geiger, W.; Junge, B. *Angew. Chem. Int. Ed. Engl.* **1981**, 20, 800-801.

[111] Dransfield, P.J.; Gore, P.M.; Shipman, M.; Slawin, A.M.Z. *Chem. Commun.* **2002**, 150-151.

[112] Maughan, M.A.T.; Davies, I.G.; Claridge, T.D.W.; Courtney, S.; Hay, P.; Davis, B.G. *Angew. Chem. Int. Ed.* **2003**, *42*, 3788-3792.

[113] Szceśniak, P.; Stecko, S.; Staszewska-Krajewska, O.; Furman, B. *Tetrahedron* **2014**, *70*, 1880-1888.

[114] Palacios, F.; Alonso, C.; Aparicio, D.; Rubiales, G.; de los Santos, J. *Tetrahedron* **2007***, 63,* 523-575

[115] Zoidl, M.; Müller, B., Torvisco, A.; Tysoe, C.; Benazza, M.; Siriwardena, A.; Withers, S.; Wrodnigg, T. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 2777-2780.

[116] Timmer, M.S.M.; Risseeuw, M.D.P.; Verdoes, M.; Filippov, D.V.; Plaisier, J.R.; Van der Marel, G.A.; Overkleeft, H.S.; Van Boom, J.H. *Tetrahedron: Asymmetry* **2005**, *16*, 177-185.

[117] Smith, M.B.; J.M., Addition to Carbon-Hetero Multiple Bonds. In March's Advanced Organic Chemistry, 6th ed., **2007**; pp 1251-1476.

[118] Ayala, L.; Lucero, C.; Romero, J.; Tabacco, S.; Woerpel, K. *J. Am. Chem. Soc.* **2003**, *125*, 15521-15528.

[119] Zoidl, M. Diplomarbeit, University of Technology, Graz, **2013**

[120] Li, X.; Qin, Z.; Yang, T.;Zhang, H.; Wei, S.; Li, C.; Chen, H.; Meng, M. *Bioorg. Med. Chem. Lett*. **2012**, *22*, 2712-2716.

[121] The reactions starting from compound **23** were performed by Lukas Tribuser in the course of a project laboratory.

[122] X-Ray analysis of the synthesized compounds is performed in house by Ana Torvisco, Institute of Inorganic Chemistry, Technical University Graz.

[123] Crawford, J.B.; Chen, G.; Gauthier, D.; Wilson, T.; Carpenter, B.; Baird, I.R.; McEachern, E.; Kaller, A.; Harwig, C.; Atsma, B.; Skerlj, R.T.; Bridger, G.J. *Org. Process Res. Dev.* **2008**, *12*, 823-830.

[124] Pursche, B. *Master's thesis*, University of Technology Graz, **2016**.

[125] Zoidl, M.; Santana, A.G.; Torvisco, A.; Tysoe, C.; Siriwardena, A.; Withers, S.G.; Wrodnigg, T.M. *Carb. Res*. **2016**, *429*, 62-70.

[126] Handel, P.; Stang., C.; God, C.; Filzwieder, F.; Uhlig, F., Schroettner, H.; Koller, S. *ECS Transactions* **2015**, *66*, 127-137.

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Education

01/2014 - present **PhD candidate** in Technical Chemistry at Graz University of Technology, Topic: *Synthesis of C-glycosyl type iminosugar building blocks via a Multicomponent Reaction Strategy.*
Supervisor: Prof. Tanja Wrodnigg

03/2013 - 09/2013 **Master program** in Technical Chemistry at Graz University of Technology and Karl-Franzens University Graz (NAWI Project). Thesis: *Synthesis of Cyano-Iminosugar Building Blocks for Glycoprobes*
Supervisor: Prof. Tanja Wrodnigg

10/2008 - 11/2011 **Bachelor program** in Chemistry; Graz University of Technology and Karl-Franzens University Graz (NAWI Project), Thesis written at: Institute of Organic Chemistry and Bioorganic Chemistry, Biocatalysis
Supervisor: Prof. Wolfgang Kroutil

Relevant work experience

05/2012 – 11/2012 **Research assistant** at Varta Micro Innovation, Graz, Austria

07/2009 – 08/2009 **Trainee** at Borealis Polyolefine GmbH, Austria

Research Stays

04/2013 **Scientific exchange student**, Amiens, France, with Dr. Aloysius Siriwardena (Amadée, FR 18/2011)

Soft skills

- Competence training for teaching assistants: leadership, communication, teamwork and motivation
- Patent law
- Project management in the industrial practice
- Founding of an enterprise

Teaching Experience

10/2010 - present **Teaching assistant** in various laboratory courses (General Chemistry, Organic Chemistry)
Teaching assistant in various practical courses (Seminar for General Chemistry)
Supervisor in various project laboratories and bachelor theses in Glycogroup, University of Technology Graz

Additional Information

Languages	German (native speaker), English (fluent), Spanish (basic)
Computer Skills	Microsoft Office, chemical softwares (Scifinder, ChemDraw, MestReNova, SpinWorks, MassLynx)
Other skills	Qualified paramedic with 8 years of experience at the Austrian Red Cross

Awards and Fellowships

11/2015	Inventors award from the University of Technology, Graz
01/2014	Doctoral Fellowship of the Austrian Academy of Science
11/2014	Award for the Diploma thesis from the Austrian Chemical Society

Participation in National and International Conferences and Congresses

07/2017	19th European Carbohydrate Symposium, Barcelona, Spain,
02/2017	21st Austrian Carbohydrate Workshop, Graz, Austria
02/2016	20th Austrian Carbohydrate Workshop, Vienna, Austria
09/2015	23rd International Symposium on Glycoconjugates. Split, Croatia
02/2015	19th Austrian Carbohydrate Workshop, Graz, Austria
09/2014	Mini Symposium on Glycomics, Glycochemistry and Glycobiology. Vienna, Austria
06/2014	13th Bratislava Symposium on Saccharides, Bratislava, Slovakia
06/2014	97th Canadian Chemistry Conference and Exhibition, Vancouver, Canada
02/2014	18th Austrian Carbohydrate Workshop. Vienna, Austria

List of Publications

Publications in peer-reviewed journals:

2016

1. *"The Staudinger/aza-Wittig/Grignard Reaction as Key Step for the Concise Synthesis of 1-C-Alkyl-iminoalditol Glycomimetics"*

   Zoidl, M.; Gonzalez, S. A.; Torvisco, A.; Tysoe, C.; Siriwardena, A.; Withers, S. G.; Wrodnigg, T. M. *Carb. Res.* **2016**, *429*, 62-70.

2. *"Imidazole Based Magnesium Salt as Conductive Salt for Rechargeable Magnesium-Ion Batteries"*

   Zoidl, M.; God, C.; Fischer, R.; Lenardt, C.; Schmuck, M.; Wrodnigg, T.M. *J. Electrochem. Soc.* **2016**, *10*, A2461-A2463

2014

3. *"Concise Synthesis of C-Cyano-Iminosugar Building Blocks via the Staudinger/aza Wittig/CN Reaction Sequence"*

Zoidl, M.; Müller, B.; Torvisco, A.; Tysoe, C.; Benazza, M.; Siriwardena, A.; Withers, S. G.; Wrodnigg, T. M. *Bioorg. Med. Chem. Lett.*, **2014**, *24,* 2777-2780.

Patent

2017

"Electrolyte system and anode materials for secondary magnesium batteries"

God, Colin; Handel, Patricia; Zoidl, Manuel; Wrodnigg, Tanja

Eur. Pat. Appl. (2017), EP 3113275 A1 20170104.

Oral presentations

2017

1. *"Synthesis of C-Glycosyl Iminosugar Based Gycomimetics for Different Applications"*

   Zoidl, M.; Lebl, R.; Santana-Gonzales, A.; Schalli, M.; Tysoe, C.; Stütz, A.E.; Weber, P.; Withers, S.G.; Wrodnigg, T.M.

   21st Austrian Carbohydrate Workshop, Graz, Austria, 16.02.2017-17.02.2017

2. *"Novel Furanoid Carbasugars as Hexosaminidase Inhibitors and Potential Pharmalogical Chaparones for Tay Sachs, Sanfilippo B and Schindler/Kanzaki Diseases"*

   Weber, P.; Schalli, M.; Tysoe, C.; Pabst, B.; Lebl, R.; Müller, S.; Paschke, E.; Stütz, A.E.; Tschernutter, M.; Windischhofer, W.; Withers, S.G.; Wrodnigg, T.M.; Zoidl, M.

   21st Austrian Carbohydrate Workshop, Graz, Austria, 16.02.2017-17.02.2017

3. *"A Morita-Baylis-Hillman Based de novo Synthesis of 5a-C-Pentyl-4-epi-Isofagomine - A Powerful Pharmacological Chaperone for G_{M1}-Gangliosidosis"*

   Lebl, R.; Schalli, M.; Tysoe, C.; Pabst, B.; Paschke, E.; Stütz, A.E.; Tschnernutter, M.; Weber, P.; Windischhofer, W.; Withers, S.G.; Wrodnigg, T.M.; Zoidl, M.

   21st Austrian Carbohydrate Workshop, Graz, Austria, 16.02.2017-17.02.2017

2016

4. "The Amadori Rearrangement as Conjugation Method: Scope and Limitations"

   Hojnik, C.; Gloe, T.; Lebl, R.; Lindhorst, T.; Schalli, M.; Stütz, A.E.; Torvisco Gomez, A.; Thonhofer, M.; Weber, P.; Wrodnigg, T.M.; Zoidl, M.

   20th Austrian Carbohydrate Workshop, Vienna, Austria, 11.02.2016-12.02.2016

5. *"The Staudinger/aza-Wittig - Grignard Reaction Cascade: Stereochemical Considerations and Biological Evaluation"*

   Zoidl, M.; Hojnik, C.; Lebl, R.; Schalli, M.; Stütz, A.E.; Thonhofer, M.; Torvisco Gomez, A.; Weber, P.; Wrodnigg, T.M.

   20th Austrian Carbohydrate Workshop, Vienna, Austria, 11.02.2016-12.02.2016

6. *"The Staudinger/aza-Wittig Nucleophile Reaction Cascade: Scope and Limitations"*

Lebl, R.; Hojnik, C.; Schalli, M.; Stütz, A.E.; Thonhofer, M.; Torvisco Gomez, A.; Weber, P.; Wrodnigg, T.M.; Zoidl, M.

20th Austrian Carbohydrate Workshop, Vienna, Austria 11.02.2016-12.02.2016

7. *"Synthesis and Biological Evaluation of C-5a Extended Derivatives of 4-epi-Isofagomine"*

Thonhofer, M.; Fischer, R.; Gonzales-Santana, A.; Hojnik, C.; Lebl, R.; Papst, B.; Schalli, M.; Stütz, A.E.; Tschernutter, M.; Weber, P.; Windischhofer, W.; Withers, S. G.; Wrodnigg, T.M.; Zoidl, M.

20th Austrian Carbohydrate Workshop, Vienna, Austria, 11.02.2016-12.02.2016

8. "*Synthesis of Carbacyclic Inhibitors for ß-Galactosidases*"

Schalli, M.; Fischer, R.; Hojnik, C.; Lebl, R.; Thonhofer, M.; Stütz, A.E.; Weber, P.; Wrodnigg, T.M.; Zoidl, M.

20th Austrian Carbohydrate Workshop, Vienna, Austria, 11.02.2016-12.02.2016

2015

9. *"Exploration of the SAW-Grignard Reaction for the Synthesis of Iminoalditol Based Glycoprobes."*

Zoidl, M.; Hoff, O.; Hojnik, C.; Schalli, M.; Siriwardena, A.; Stütz, A.E.; Thonhofer, M.; Wrodnigg, T.M.

19th Austrian Carbohydrate Workshop, Graz, Austria, 12.02.2015

10. *"New Synthesis of C-5a-Derivatives of 4-epi-Isofagomine and Analogues."*

Thonhofer, M.; Stütz, A.E.; Wrodnigg, T.M.; Fischer, R.; Hojnik, C.; Zoidl, M.; Weber, P.; Schalli, M.

19th Austrian Carbohydrate Workshop, Graz, Austria, 12.02.2015

11. *"Are D-manno-Configured Amadori Products Ligands of Bacterial Lectin FimH?."*

Hojnik, C.; Gloe, T.-E.; Hoff, O.; Lindhorst, T. K.; Schalli, M.; Stütz, A.E.; Thonhofer, M.; Wrodnigg, T.M; Zoidl, M.

19th Austrian Carbohydrate Workshop, Graz, Austria, 12.02.2015

12. *"Synthesis of D-galacto-Validamine Derivatives."*

Schalli, M.; Hojnik, C.; Thonhofer, M.; Stütz, A.E.; Wrodnigg, T.M; Zoidl, M.

19th Austrian Carbohydrate Workshop, Graz, Austria, 12.02.2015

2014

13. *"Diversity Oriented Synthesis of Iminoalditol Building Blocks for Glycoprobes."*

Zoidl, M.; Gonzalez Santana, A. .; Hojnik, C.; Saf, R.; Schalli, M.; Stütz, A.E.; Thonhofer, M.; Withers, S.G; Wrodnigg, T.M.

Mini Symposium on Glycomics, Glycochemistry and Glycobiology. Vienna, Austria, 05.09.2014

14. *"A new Approach to Fluorinated 4-epi-Isofagomine Derivatives and their D-Galactosidase Inhibitory Properties."*

Thonhofer, M.; Fischer, R.; Gonzalez-Santana, A.; Hojnik, C.; Saf, R.; Schalli, M.; Stütz, A.E.; Torvisco Gomez, A.; Withers, S.G.; Wrodnigg, T.M.; Zoidl, M.

Mini Symposium on Glycomics, Glycochemistry and Glycobiology. Vienna, Austria, 05.09.2014

15. *"Synthesis of Functionalised Iminoalditol Building Blocks for Glycoprobes via a Staudinger/aza-Wittig/Strecker Multi-component Reaction."*

Zoidl, M.; Schalli, M.; Thonhofer, M.; Torvisco Gomez, A.; Saf, R.; Stütz, A.E.; Siriwardena, A.; Wrodnigg, T.M.

18th. Austrian Carbohydrate Workshop. Vienna, Austria, 13.02.2014.

16. *"The Ferrier II Reaction and Selected Examples of Follow-up Chemistry."*
Schalli, M.; Thonhofer, M.; Zoidl, M.; Wrodnigg, T.M.; Fischer, R.; Torvisco Gomez, A.; Stütz, A.E.

18th. Austrian Carbohydrate Workshop. Vienna, Austria, 13.02.2014.

17. *"New Synthesis of 4-epi-Isofagomine and Fluoro Derivatives."*
Thonhofer, M.; Schalli, M.; Torvisco Gomez, A.; Zoidl, M.; Wrodnigg, T.M.; Stütz, A.E.
18th. Austrian Carbohydrate Workshop. Vienna, Austria, 13.02.2014.

Poster presentations

2017

1. *"Synthesis of Iminoalditol Based Glycomimetics for Different Applications"*

Zoidl, M.; Lebl, R.; Schalli, M.; Stütz, A.E.; Thonhofer, M.; Tysoe, C.; Weber, P.; Withers, S.G.; Wrodnigg, T.M.

19th European Carbohydrate Symposium, Barcelona, Spain, 02.07.2017-06.07.2017 – *accepted.*

2. *"Furanoid Carbasugars as Hexosaminidase Inhibitors and Potential Pharmacological Chaperones"*

Weber, P.; Schalli, M.; Tysoe, C.; Pabst, B.; Lebl, R.; Müller, S.; Paschke, E.; Stütz, A.E.; Tschernutter, M.; Windischhofer, W.; Withers, S.G.; Wrodnigg, T.M.; Zoidl, M.

19th European Carbohydrate Symposium, Barcelona, Spain, 02.07.2017-06.07.2017 – *accepted.*

3. *"Synthesis of 5a-C-Extended 4-epi-Isofagomine Derivatives from D-Glyceraldehyde – Powerful Pharmacological Chaperones for G_{M1}-Gangliosidosis"*

Lebl, R.; Thonhofer, M.; Tysoe, C.; Pabst, B.; Paschke, E.; Schalli, M.; Stütz, A.E.; Tschernutter, M.; Weber, P.; Windischhofer, W.; Withers, S.G.; Zoidl, M.; Wrodnigg, T.M.

19th European Carbohydrate Symposium, Barcelona, Spain, 02.07.2017-06.07.2017 – *accepted.*

4. *"New Dansylated Derivates of 4-epi-Isofagomine"*

   Thonhofer, M.; Schalli, M.; Tysoe, C.; Pabst, B.; Lebl, R.; Paschke, E.; Stütz, A.E.; Tschernutter, M.; Weber, P.; Windischhofer, W.; Withers, S.G.; Zoidl, M.; Wrodnigg, T.M.

   19th European Carbohydrate Symposium, Barcelona, Spain, 02.07.2017-06.07.2017 *accepted.*

5. *"Experimental Pharmacological Chaperones for Lysosomal Glycosidase Mutants"*

   Schalli, M.; Thonhofer, M.; Lebl, R.; Weber, P.; Tysoe, C.; Pabst, B.; Paschke, E.; Stütz, A.E.; Tschernutter, M.; Windischhofer, W.; Withers, S.G.; Wrodnigg, T.M.; Zoidl M.

   19th European Carbohydrate Symposium, Barcelona, Spain, 02.07.2017-06.07.2017 *accepted.*

6. *"N-Substituted 5-amino-1-hydroxymethyl-Cyclopentanetriols: A new Family of Promotors for G_{M1}-Gangliosidosis Related Human Lysosomal β-Galactosidase Mutants"*

   Schalli, M.; Weber, P.; Tysoe, C.; Pabst, B.; Lebl, R.; Paschke, E.; Stütz, A.E.; Tschernutter, M.; Windischhofer, W.; Withers, S.G.; Wrodnigg, T.M.; Zoidl, M.

   19th European Carbohydrate Symposium, Barcelona, Spain, 02.07.2017-06.07.2017 *accepted.*

2015

7. *"Glycolipid Mimetics: Lipophilic Carbasugars as Inhibitors and Chemical Chaperones for G_{M1} Gangliosidosis."*

   Schalli, M.; Fischer, R.; Gonzalez Santana, A.; Hojnik, C.; Paschke, E.; Stütz, A.E.; Thonhofer, M.; Withers, S.G.; Wrodnigg, T.M; Zoidl, M.:

   23rd International Symposium on Glycoconjugates. Split, Croatia, 15.09.2015.

8. *"Glycolipid Mimetics: Fluorine Containing Isoiminosugars as Chemical Chaperones for GM1 Gangliosidosis and Fabry's Disease."*

   Stütz, A.E.; Fischer, R.; Gonzalez Santana, A.; Hojnik, C.; Paschke, E.; Schalli, M.; Thonhofer, M.; Torvisco Gomez, A.; Withers, S.G.; Wrodnigg, T.M.; Zoidl, M.:

   23rd International Symposium on Glycoconjugates. Split, Croatia, 15.09.2015.

9. *"A Powerful Approach towards C-Glycosyl Type Iminoalditol Building Blocks for Biological Applications."*

   Zoidl, M.; Gonzales-Santana, A.; Hojnik, C.; Schalli, M.; Siriwardena, A.; Stütz, A.E.; Thonhofer, M.; Torvisco Gomez, A.; Wrodnigg, T.M; Withers, S.G.

   23rd International Symposium on Glycoconjugates. Split, Croatia, 15.09.2015.

10. *"Glycolipid Mimetics: Lipophilic 4-epi-Isofagomine Derivatives as Chemical Chaperones for G_{M1}-Gangliosidosis and Morquio B."*

    Thonhofer, M.; Fischer, R.; Gonzalez-Santana, A.; Hojnik, C.; Paschke, E.; Schalli, M.; Stütz, A.E.; Weber, P.; Withers , S.G.; Wrodnigg, T.M.; Zoidl, M.

    23rd International Symposium on Glycoconjugates. Split, Croatia, 15.09.2015.

11. *"The Amadori Rearrangement: A Versatile Reaction for the Synthesis of Neoglycoconjugates."*

    Hojnik, C.; Gloe, T.-E.; Lindhorst, T.K.; Schalli, M.; Stütz, A.E.; Thonhofer, M.; Wrodnigg, T.M.; Zoidl, M.

    23rd International Symposium on Glycoconjugates. Split, Croatia, 15.09.2015.

12. *"The Amadori Rearrangement as Key Step for the Synthesis of Biologically Relevant C-Glycosyl Type Neoglycoconjugates"*

    Wrodnigg, T.M.; Hojnik, C.; Gloe, T.; Lindhorst, T.; Schalli, M.; Stütz, A.E.; Thonhofer, M.; Zoidl, M.

    23rd International Symposium on Glycoconjugates. Split, Croatia, 15.09.2015.

2014

13. *"A Convenient One-Pot Synthesis for functionalised Iminosugar Scaffolds as Building Blocks for Glycoprobes"*

    Zoidl, M.; Schalli, M.; Siriwardena, A.; Stütz, A.E.; Withers, S.G.; Wrodnigg, T.M.

    13th Bratislava Symposium on Saccharides, Bratislava, 22.-26th of June 2014.

14. *"Synthesis of Carbasugar Derivatives as Active Site Ligands for Carbohydrate Processing Enzymes"*

    Schalli, M.; Thonhofer, M.; Zoidl, M.; Withers, S.G.; Wrodnigg, T.M.; Stütz, A.E.

    13th Bratislava Symposium on Saccharides, Bratislava, 22.-26th of June 2014.

15. "*The Amadori Rearrangement as key step for the Synthesis of D-Manno Glycosyl type Glycoconjugates"*

    Zoidl M.; Hojnik C.; Gloe, T.E.; Lindhorst, T.; Schalli M.; Stütz, A.E.; Thonhofer, M.; Wrodnigg, T.M.

    13th Bratislava Symposium on Saccharides, Bratislava, 22.-26th of June 2014.

16. *"Synthesis of substituted Inososes and their Enantiomers."*

    Schalli M.; Eibel, A.; Fischer, R.; Torvisco Gomez, A.; Zoidl, M.; Wrodnigg, T.M.; Stütz, A.E.

    13th Bratislava Symposium on Saccharides, Bratislava, 22.-26th of June 2014.

17. *"A Multicomponent Approach towards functionalised Iminoalditols as Building Blocks for Glycoprobes"*

    Zoidl, M.; Müller, B.; Hojnik, C.; Schalli, M.; Siriwardena, A.; Stütz, A.E.; Thonhofer, M.; Withers, S.G.; Wrodnigg, T.M.

    97th Canadian Chemistry Conference and Exhibition, Canada, 01.-05th of June 2014.

18. *"Carbacyclic Amines as Active Site Ligands for Carbohydrate Processing Enzymes."*

    Schalli, M.; Hojnik, C.; Stütz, A.E.; Thonhofer, M.; Withers, S.G.; Wrodnigg, T.M.; Zoidl, M.

    97th Canadian Chemistry Conference and Exhibition, Canada, 01.-05th of June 2014.

19. *"New 4-epi-Isofagomine Derivatives for Glycosidase Research"*

    Thonhofer, M.; Hojnik, C.; Schalli, M.; Stütz, A.E.; Withers, S.G.; Wrodnigg, T.M.; Zoidl, M.

    97th Canadian Chemistry Conference and Exhibition, Canada, 01.-05th of June 2014.

20. *"The Amadori Rearrangement as Key Step for the Synthesis of Inhibitors of Type 1-Fimbriated E. coli Bacteria."*

Hojnik, C.; Gloe, T.; Lindhorst, T.; Schalli, M.; Stütz, A.E.; Thonhofer, M.; Wrodnigg, T.M.; Zoidl, M.

97th Canadian Chemistry Conference and Exhibition, Canada, 01.-05th of June 2014.

21. *"Synthesis and Biological Evaluation of Potential Therapeutic Compounds for G_{M1} Gangliosidosis."*

Stütz, A.E.; Hojnik, C.; Paschke, E.; Schalli, M.; Thonhofer, M.; Withers, S.G.; Wrodnigg, T.M.; Zoidl M.

97th Canadian Chemistry Conference and Exhibition, Canada, 01.-05th of June 2014.

2013

22. *"Saw MCR of Azido-aldoses for the Synthesis of Cyano-iminoalditol Derivatives as Building Blocks for Glycoprobes"*

Zoidl, M.; Müller, B.; Hojnik, C.; Schalli, M.; Thonhofer, M.; Stütz, A.E.; Siriwardena, A.; Wrodnigg, T.M.

15th Austrian Chemistry Days, 23.-26th of September 2013, Graz, Austria

23. *"Synthesis of Lithium Salt Imidazole Derivatives as Electrolytes for Lithium-ion Batteries"*

Zoidl, M.; Koller, S.; Schmuck, M.; Wrodnigg, T.M.; Saf, R.

15th Austrian Chemistry Days, 23.-26th of September 2013, Graz, Austria

24. *"New Synthetic Approaches to 4-epi-Isofagomine and its Derivatives."*

Thonhofer, M.; Hojnik, C.; Schalli, M.; Zoidl, M.; Wrodnigg, T.M.; Stütz, A.E.

15th Austrian Chemistry Days, 23.-26th of September 2013, Graz, Austria

25. *„Carbasugars as Active Site Ligands for Carbohydrate Processing Enzymes."*

Schalli, M.; Hojnik, C.; Thonhofer, M.; Zoidl, M.; Wrodnigg, T.M.; Stütz, A.E.

15th Austrian Chemistry Days, 23.-26th of September 2013, Graz, Austria

26. *"Exploration of the Amadori Rearrangement as Bioconjugation Method towards C-Glycosyl type Glycoconjugates of Carbohydrates."*

Hojnik, C.; Gloe, T.-E.; Schalli, M.; Thonhofer, M.; Zoidl, M.; Stütz, A.E.; Wrodnigg, T.M.; Lindhorst, T. K.

15th Austrian Chemistry Days, 23.-26th of September 2013, Graz, Austria