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Exploration of the Amadori Rearrangement for Bioconjugation of Carbohydrates

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Meinen Eltern und Rene

„Wissen ist das Kind der Erfahrung."

Leonardo da Vinci
(1452-1519)

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ABSTRACT

Carbohydrates, one of the most abundant class of biomolecules in nature, serve as energy source as well as structural material in plants. They play a significant role in cell-cell communication and recognition, cellular differentiation and in immune response, both in healthy and disease states of living organisms.

Glycoconjugates, in which carbohydrates are covalently linked to other biomolecules such as proteins, lipids or peptides, have manifold functions in cell-cell interaction. The investigations of their biological functions are of high interest and thus synthetic methods to form glycocconjugates are required. The conjugation through a *C*-glycosidic linkage promises to be an attractive approach, as it was recognized that this *C*-glycosides could serve as functional analogues of the corresponding more common *O*-as well as *N*-glycosides, whereas the *C*-glycosidic linkage show an increased stability towards enzymatic or chemical hydrolysis compared to the *O*- and *N*-glycosidic bonds.

In this thesis, the Amadori rearrangement - a reaction between aldoses and amines which leads to *C*-glycosyl type 1-amino-1-deoxy ketoses without the requirement of protecting group manipulations - was investigated as a new conjugation method for the synthesis of *C*-glycosyl type neoglycoconjugates. The scope and limitation of this rearrangement reaction in terms of the amino components as well as the sugar moiety have been a part of the investigation. In order to optimize the reaction conditions, the commercially available D-*glycero*-D-*gulo* aldoheptose was employed as model substrate, which gave access to D-*gluco* configured Amadori products.

Furthermore, the Amadori rearrangement was applied to synthesize D-*manno* configured *C*-glycosyl types glycoconjugates, which have been investigated in terms of their biological interaction with various D-mannose specific lectins.

KURZFASSUNG

Eine der häufigsten Klassen von Biomolekülen in der Natur sind Kohlenhydrate, die sowohl als Energiequelle als auch als Strukturmaterial für Pflanzen fungieren. Sie spielen eine wichtige Rolle in Prozessen, wie der Kommunikation und Erkennung von Zellen, der Zelldifferenzierung und der Immunreaktion, im gesunden als auch im kranken Stadium von lebenden Organismen

In Glycokonjugaten sind Kohlenhydrate kovalent an andere Biomoleküle wie Proteinen, Lipiden oder Peptiden gebunden und weisen eine vielfältige Funktion in der Zell-Zell Interaktion auf. Aus diesem Grund ist die Untersuchung und die Aufklärung der biologischen Funktion von Glycokonjugaten von großem Interesse und dies wiederum erfordert die Entwicklung von entsprechenden Methoden für deren Synthese. Eine vielversprechende Möglichkeit liefert die Konjugation über eine *C*-glykosidische Verknüpfung. Diese *C*-Glykoside können als synthetische Analoga zu den natürlichen *N*- als auch *O*-Glykosiden fungieren, jedoch besteht deren Vorteil darin, dass diese *C*-glykosidische Bindung eine erhöhte Stabilität gegenüber enzymatischer und chemischer Hydrolyse aufweist.

Im Rahme dieser Arbeit wurde die Amadori Umlagerung – eine Reaktion zwischen Aldosen und Aminen die zu 1-Amino-1-deoxyketose führt, wobei keine Schutzgruppenstrategie notwendig ist – als neue Methode für die Synthese von C-glycosyl Neoglycokonjugaten untersucht. Durch die Verwendung von unterschiedlichen Aminokomponenten und Zuckersubstraten wurde die Amadori Umlagerung im Hinblick auf ihren Anwendungsbereich eruiert und die Limitierungen dieser Umlagerungsreaktion untersucht. Für die Synthese von D-*gluco* konfigurierten Umlagerungsprodukten als auch für die Optimierung der Reaktionsbedingungen wurde als Modelsubstrat die kommerziell erhältliche D-*glycero*-D-*gulo* aldoheptose herangezogen.

Des Weiteren, wurde die Amadori Umlagerung für die Synthese von D-*manno* konfigurierten Glycokonjugaten herangezogen, die im Hinblick auf deren biologischen Wechselwirkung mit unterschiedlichen D-mannosespezifischen Lectinen untersucht wurden.

ABBREVIATIONS

$[\alpha]_D^{20}$	specific rotation
Å	angstrom
AD	Alzheimer´s disease
AEGs	advanced glycation end products
AcCl	acetyl chloride
Ala	alanine
Asn	asparagine
Asp	aspartic acid
BC2L-A	*Burkholderia cenocepacia lectin A*
Boc	*tert*-butyloxycarbonyl
BSA	bovine serum albumin
Bu_2SnO	dibutyltin oxide
Cbz	benzyloxycarbonyl
$CHCl_3$	chloroform
CH_2Cl_2	dichloromethane
Con A	*Canavalia ensiformis*
CRD	carbohydrate recognition domain
CS_2	carbon disulfide
CsF	cesium fluoride
CuI	copper iodide
C	cyclohexane
DCC	*N*,*N*'-dicyclohexylcarbodiimide
DC-SIGN	Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin
DEAD	diethyl azodicarboxylate
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformaide
DMSO-d_6	dimethyl sulfoxide-d_6
DNA	deoxyribonucleic acid
D_2O	deuterium oxide
Dol-P.	dolichol phosphate
EDCl	1-ethyl-3-(3-dimethylaminopropyl)carbodiimid
Et_3N	triethylamine

EtOAc	ethyl acetate
Et_2O	diethyl ether
EtOH	ethanol
Equiv.	equivalents
Gal	galactose
GalNAc	*N*-acetyl-D-galactosamine
GFP	green fluorescent protein
Gln	glutamine
Gluc	glucose
GlcNAc	*N*-acetyl-D-glucosamine
Gly	glycine
GNL/GNA	*Galanthus nivalis*
GPI	glycosyl phosphatidylinsitol
HBUT	*O*-(1H-benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium hexafluorophosphate
HCl	hydrochloric acid
HCN	hydrogen cyanide
HHL/HHA	*Hippeastrum hybrid*
HIV	*Human immunodeficient virus*
HOAc	acetic acid
HOBt	hydroxybenzotriazole
HPLC-MS	high performance liquid chromatography mass spectrometry
HRMS	high-resolution mass spectrometry
H_2SO_4	sulfuric acid
IC_{50}	half maximal inhibitory concentration
Ile	isoleucine
LCs	Langerhans cells
Leu	leucine
LPS	lipopolysaccharides
M	mol/L
Man	mannose
MBP	mannose-binding protein
MeCN	acetonitrile
MeMan	methyl α-D-mannopyranoside
MeOH	methanol

MeOH-d_4	methanol-d_4
Met	methionine
MTBE	methyl *tert*-butyl ether
mwco	molecular weight cutoff
Mw	molecular weight
$NaCO_3$	sodium carbonate
$NaIO_4$	sodium periodate
Na_2SO_4	sodium sulfate
NH_4OH	ammonium hydroxide
NMR	nuclear magnetic resonance
$NaOCH_3$	sodium methoxide
OAc	acetoxy
OD_{600}	optical density
OMe	methoxy
OsO_4	osmium tetraoxide
PBS	phosphate buffered saline
PBST	phosphate buffered saline with tween-20
Pd/C	palladium on charcoal
$Pd(OH)_2/C$	palladium hydroxide on charcoal
Phe	phenylalanine
PPh_3	triphenylphosphine
PVA	polyvinyl alcohol
R_f – value	retardation factor
RIP	relative inhibitory potency
RNA	ribonucleic acid
rt	room temperature
Satd.	saturated
Ser	serine
SGC	silica gel chromatography
TBTU	*O*-(benzotriazol-1-yl)-*N*,*N*,*N*',*N*'-tetramethyluronium tetrafluoroborat
THF	tetrahydrofuran
Thr	threonine
TLC	thin layer chromatography
Thy	tyrosine
UDP-GalNAc	uridine 5'-diphospho-*N*-acetylgalactosamine

UDP-GlcNAc	uridine 5'-diphospho-*N*-acetylglucosamine
UPEC	uropathogenic *Escherichia coli*
VNTR	variable number of tandem repeat
VEA	*Vicia ervilia*

1. INTRODUCTION

1.1. Carbohydrates

Carbohydrates represent one of the most abundant class of biomolecules besides proteins, peptides and nucleosides on earth. Nearly all organisms synthesize and metabolize carbohydrates.[1] They serve as energy source and as structural material in plants. Even more important is their role in intra- and intermolecular communication processes.[2] Carbohydrates are present at both the surface and inside the cell, mediating versatile biological processes which are essential for the cell functioning but also for diseased stages of living organisms.[3]

The term carbohydrate was first implemented in 1844 by Carl Schmidt, who included all substances with the empirical formula $C_n(H_2O)_m$ as "hydrates of carbon". Historically, carbohydrates consist of carbon, hydrogen and oxygen in a ratio of 1:2:1, however, this definition is not strictly applicable since many naturally occurring carbohydrates differ in this atom ratio. In fact, there are many compounds showing the same chemical formula, such as lactic acids ($C_3H_6O_3$) and acrylic acid ($C_3H_4O_2$) that vary significantly in their properties compared to those of carbohydrates. Furthermore, many sugars are known to contain heteroatoms for example nitrogen or sulphur.[4]

Actually, carbohydrates are defined as polyhydroxyaldehydes, polyhydroxyketones or compounds which provide these products after hydrolysis. Carbohydrates exist in a large elemental as well as sterochemical diversity and are built up from monosaccharides, compounds which cannot be converted through hydrolysis into smaller units. These monosaccharides are linked together *via* a glycosidic linkage forming a large variety of linear or branched oligosaccharides as well as polysaccharides.[5]

Monosaccharides can be classified according to the number of carbon atoms, including triose (3 C), tetrose (4 C), pentose (5 C), hexose (6 C), heptose (7 C). Depending on the nature of the carbonyl group, monosaccharides are called aldose when containing an aldehyde function and those monosaccharides that contain a keto function are called ketose.

Polysaccharides are constituted of typically more than 10 monosaccharide units. Among the most important polysaccharides are starch (**1**) and glycogen (**2**), with D-glucose as monosaccharidic units conjugated *via* a α-1,4-glycosidic linkage, which possess the function of energy storage for plants and mammals. In plants, the major constituent of supporting tissues is cellulose (**3**), a β-1,4-glycosidically linked polysaccharide built up from D-glucose as

monosaccharidic unit (Figure 1). Furthermore, carbohydrates are also essential as structural components. Chitin (**4**), for instance, is found as a major component of the exoskeleton of arthropods (Figure 1).

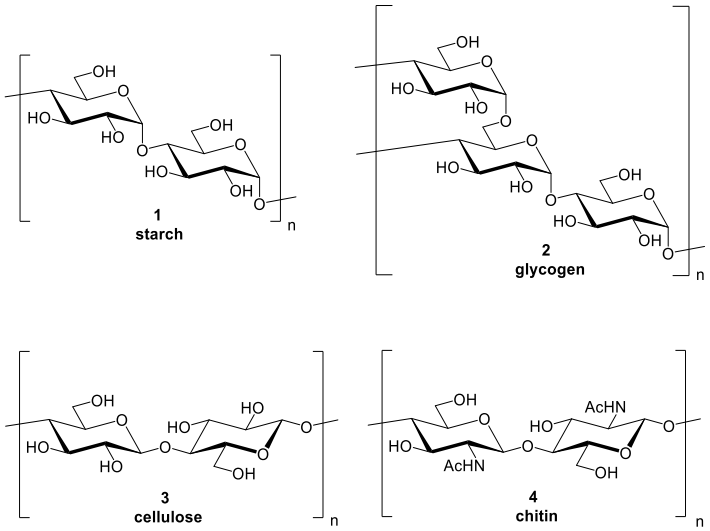


Figure 1: Structure of starch (**1**), glycogen (**2**), cellulose (**3**) and chitin (**4**).

Since carbohydrates possess at least one carbonyl function, several hydroxy groups and often other kinds of functional groups, such as amines, phosphates or sulfates, the structural appearance of this compound class is highly diverse (Figure 2). Carbohydrates are not only found as most common 5- and 6-membered heterocyclic rings but also as open chain alditols, carboxylic acids and deoxy derivatives. By the replacement of the endocyclic oxygen by a carbon or nitrogen, so-called carba- or iminosugars are obtained.[5] Additionally, carbohydrates are found to be linked covalently to other biomolecules, such as lipids, peptides or proteins, generating various glycoconjugates which are subdivided into glycolipids, glycopeptides or glycoproteins.

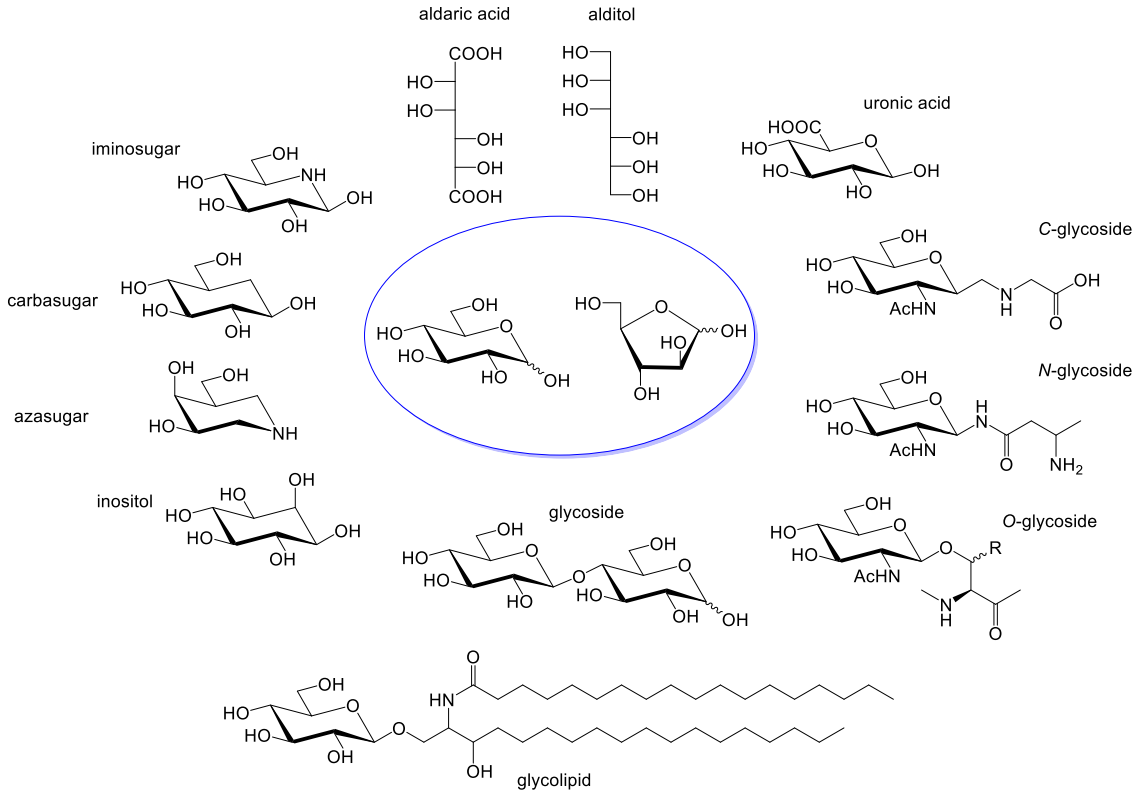


Figure 2: Examples of different structural appearance of carbohydrates.[5]

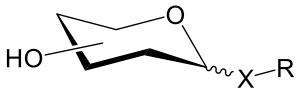
Historically, carbohydrates were thought to be important mainly as energy source for metabolism and were only seen as structural components of natural products. The biological roles of carbohydrates especially that of oligosaccharides have been underestimated for a long time compared with the roles of proteins and nucleic acids. The structural complexities of carbohydtrates, such as the appearance of isomers, and the synthetic challenges facing glycans have complicated efforts to investigate their multifaceted role and the utilization in drug development. Due to the increased knowledge of biological processes and the emerging field of glycobiology, which integrates traditional carbohydrate chemistry and biochemistry with a modern understanding of the cell and molecular biology of glycans and their conjugates with proteins and lipids, the role of carbohydrates in biological processes became accepted and elucidated and consequently their synthesis became of enormous interest.[6]

Due to the structural complexity of carbohydrates, synthesis of carbohydrate and their analytical characterization is a highly demanding field for organic chemists. The biggest challenge in carbohydrate chemistry is the region- and stereoselective formation of the glycosidic linkage. Moreover, the conceptual design of straightforward strategies for protecting group manipulations on saccharidic building blocks followed by time consuming purification and characterization steps is often a big challenge.[7]

Nevertheless, after the effect of carbohydrates in biological processes became known, such as in cancer biology, cell-cell adhesion in metastasis, diabetes as well as inflammation, investigations in the field of carbohydrate chemistry in terms of their synthesis, analytics and their biochemical functions became relevant.

1.2. Glycoconjugates

In nature, oligosaccharides (glycans) are typically found as glycoconjugates, where the glycan core is covalently linked to other biomolecules, such as lipids, proteins or peptides (Figure 3).[8]



X = O or N
R = lipid, peptide, protein, saccharide

Figure 3: Structure of glycoconjugates.

Glycoconjugates are very important biomolecules in cell biology (Figure 4) and are responsible in particular for cell-cell interaction, recognition and communication events[9] and processes like cellular differentiation,[10] cell signalling regulation[11] as well as immune response.[12] Complex oligosaccharides found in glycoconjugates can occur in soluble form as well as part of a "sticky", nano-dimesioned sugar layer on cell surfaces (*glycocalyx*).[5]

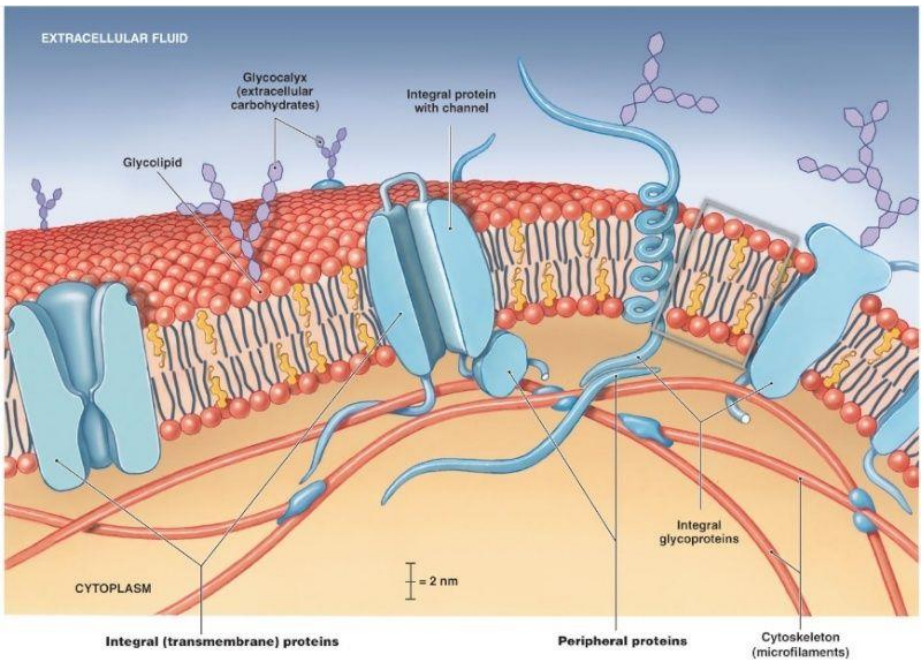


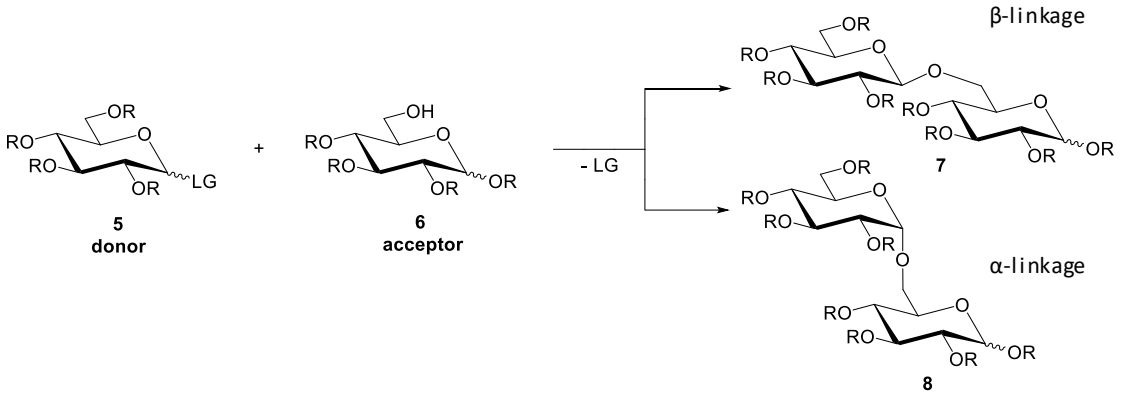
Figure 4: Glycoconjugates in cell membrane.[13]

The oligosaccharide moieties of glycoconjugates consist of up to 20 monomers, and although there is a very high variety of possible monosaccharide stereoisomers, only a relatively small set of monosaccharide building blocks is utilized by nature in the biosynthesis of glycoconjugate oligosaccharides. Due to that, common oligosaccharides patterns, as well as linking stereochemistries, are found in glycoconjugates (Table 1).

Table 1: The main monosaccharides used in biosynthesis of glycoconjugate oligosaccharides and their stereochemistries.[5]

Monosaccharide	Glycosidic linkage	Occurrence in glycoconjugates
D-Glucose	α or β	Mainly in collagen
D-Galactose	α or β	Ubiquitous
D-Mannose	α or β	Ubiquitous
N-Acetyl-D-glucosamine	α or β	Ubiquitous
N-Acetyl-D-galactosamine	α or β	Hardly in plants, blood group determination
N-Acetyl-neuraminic acid	α	Only in higher invertebrates and vertebrates
L-Frucose	α	Ubiquitous
D-Xylose	β	In plants and proteoglycans
3-Deoxy-D-manno-octulosonic acid	α	In lipopolysaccharides

The synthesis of oligosaccharides is based on their inherent chemical properties very complex, as carbohydrates exhibit several different hydroxyl groups which can be glycosylated. Thus, protecting group manipulation of the hydroxyl groups is essential for the synthesis of glycans. The selective exposure of one hydroxyl group allows the regioselective addition of another monosaccharide unit, which leads to the formation of common *O*-and *N*-linked glycans. Another synthetic challenge in glycan synthesis is to generate stereospecific glycosidic linkages, which depends on the steric and electronic nature of the glycosylating agent (glycosyl donor (**5**) and the nucleophilic hydroxyl group on the glycosyl acceptor (**6**) (Scheme 1).[14]



Scheme 1: Stereospecific formation of glycosidic bonds as either an α or β linkage. (LG: Leaving group)[14-15]

In nature, enzymes control the region- and stereospecific coupling of monosaccharide units to form glycans. In contrast to the chemical synthesis, which allows the preparation of various natural and non-natural structures with the need of sophisticated protecting group manipulation, the enzymatic synthesis does not require protecting groups and generate the desired glycosidic linkage with perfect stereo- and regiospecific control.[14]

Typically, three linkages are found in glycoconjugates (Figure 5):

- *O*-glycosidic linkage (**9**): The resulting conjugates are called O-glycans, where the glycans are attached to a hydroxyl oxygen of e.g. serine, threonine or an oxygen on lipids
- *N*-glycosidic linkage (**10**): Glycans are attached *via* the nitrogen of asparagine or arginine side chains
- *C*-glycosidic linkage (**11**): Glycans are attached to the carbon on a tryptophan side chain. This kind of glycosidic linkage is rather rare in nature.[16]

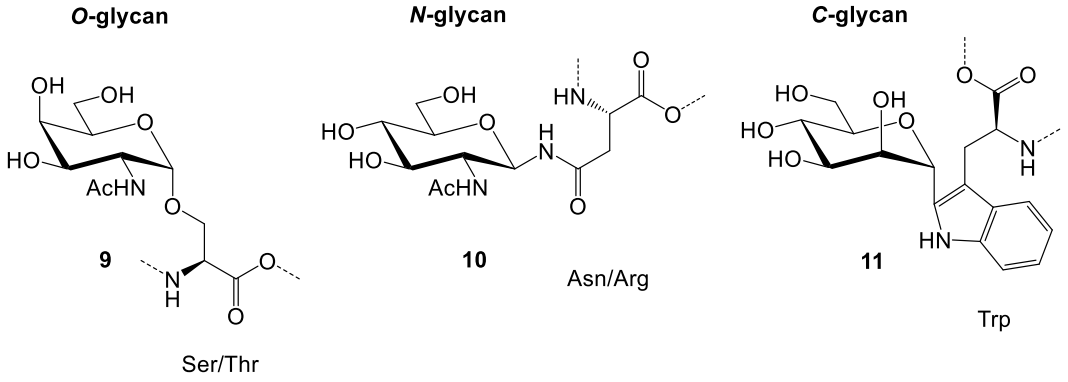


Figure 5: Three glycosidic linkages in glycoconjugates.[5, 17]

1.2.1. Glycolipids

Lipids which are attached covalently to a carbohydrate moiety *via* a glycosidic linkage, are called glycolipids. These glycolipids are membrane components and are omnipresent in all organisms, such as bacteria, plants and mammals. They represent amphiphilic molecules comprising a hydrophilic and a lipophilic part (Figure 6). The apolar hydrophobic hydrocarbon (acyl) chain consists of acylglycerol or ceramide, whereas the hydrophilic headgroup of the lipid is provided either by a phosphate group forming glycerophospholipids as well as shingophospholipids, or by a monosaccharide obtaining glycoglycerolipids and glycoshingolipids.[18] The hydrophobic moiety, like ceramide, anchors the molecule in the outer half of the plasma membrane, so that the carbohydrate part is exposed to the cell.[5] Due to the polar hydroxyl groups of carbohydrates, glycolipids are able to be soluble in the aqueous environment surrounding the cell.[19] Besides the function of membrane stabilization and cell surface rigidization, glycolipids play a significant role in diverse biological processes, including cell-cell communication, photosynthetic electron transport in plants, receptor modulation or single transduction.[18]

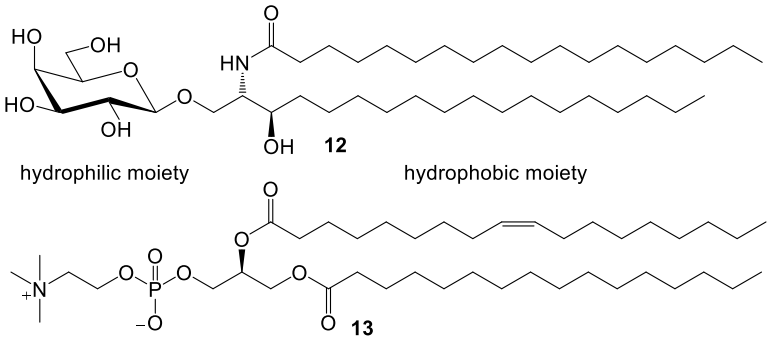
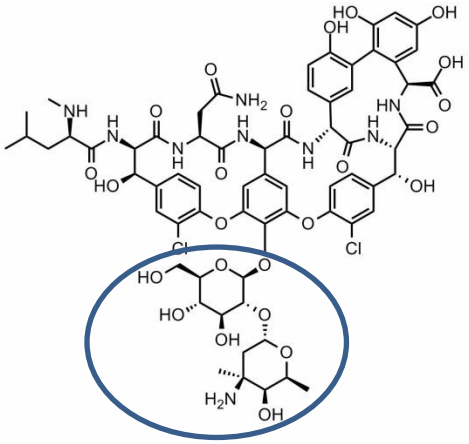


Figure 6: Examples of a glycolipid: galactosyl cerebroside (**12**) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (**13**).

Because of the structural variety of both the lipophilic chain as well as the carbohydrate moiety, there exist a vast number of different glycolipids.

1.2.2. Glycopeptides

Carbohydrate moieties which are covalently bound to the side chain of a specific amino acid in the peptide amino acid sequence are called glycopeptides. Although, glycopeptides are rather small in size and complexity, they exhibit relevant properties, such as antigenicity.[20] For example, vancomycin (**14**) (Figure 7), teicoplanin and telavancin belong to the family of glycopeptide antibiotics, which are essential for the control of infectious diseases caused by Gram-positive pathogenic bacteria.



14

Figure 7: Glycopeptide antibiotic vancomycin (**14**).[21]

The structures of such glycopeptide antibiotics are remarkable, the core heptapeptides are highly crosslinked and variously modified. These heptapeptides are synthesized by large "assembly line"-like multienzyme complexes, the nonribosomal peptide synthetases (NRPS), which assemble amino acids through diverse modules. The NRPS modules are the repetitive building blocks of these megasynthetases. One module corresponds to one amino acid and consequently the primary structure of the peptide product is dictated by the order of the modules in the NRPS. These modules can encode various catalytic domains including condensation (C), adenylation (A), thiolation (T, also known as peptidyl carrier protein (PCP)), epimerization (E) and thioesterase (Te) domains.[22] Three domains are necessary for each module, the A, C and PCP domain (Figure 8), which catalyzed the elongation of peptidic intermediates. Domain A is responsible for substrate recognition and activation. The PCP domain covalently binds all substrates and intermediates of the NRPS assembly line and the C domain is responsible for the peptide bond formation between two PCP-bound units.[23]

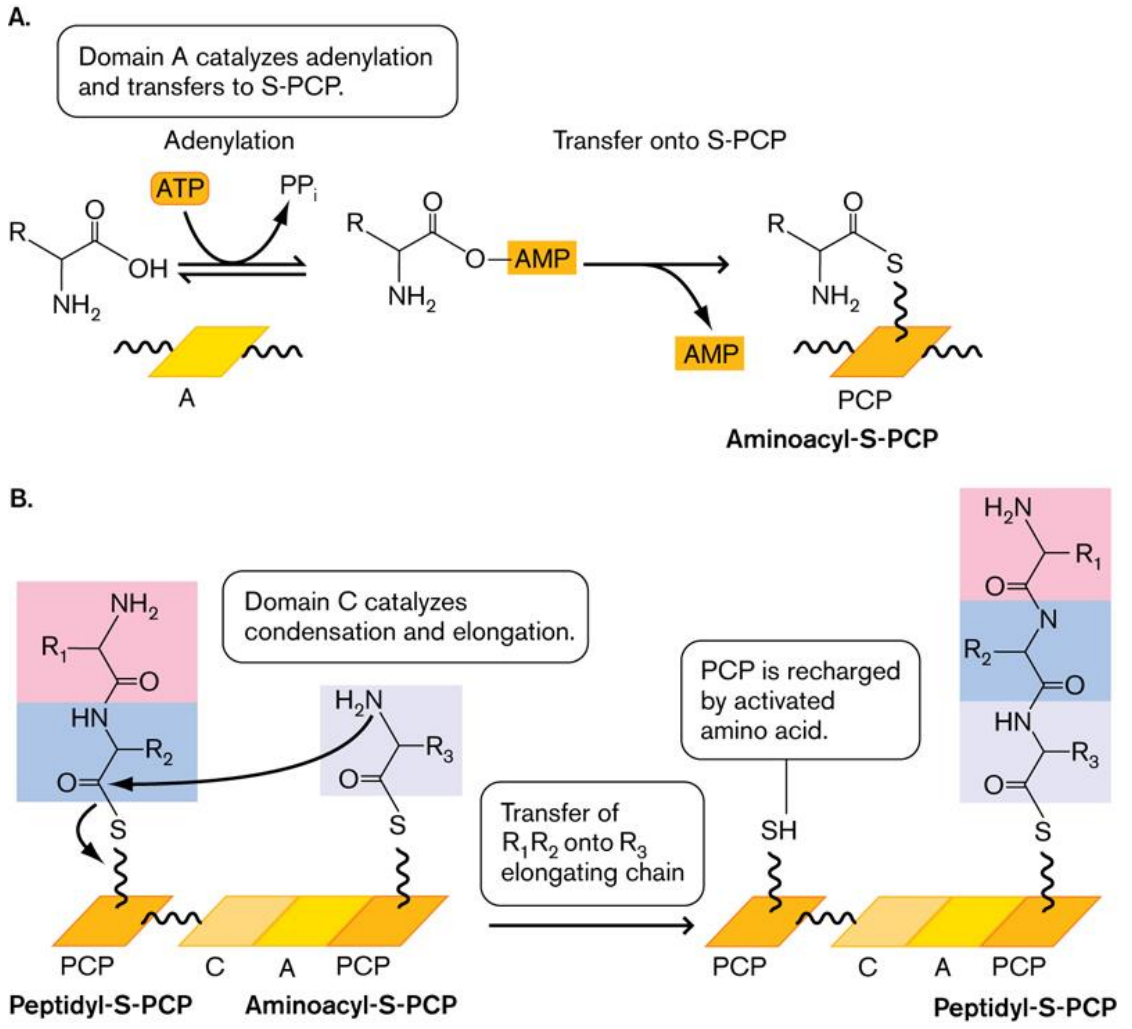


Figure 8: Elongation of nonribosomal peptide.[24]

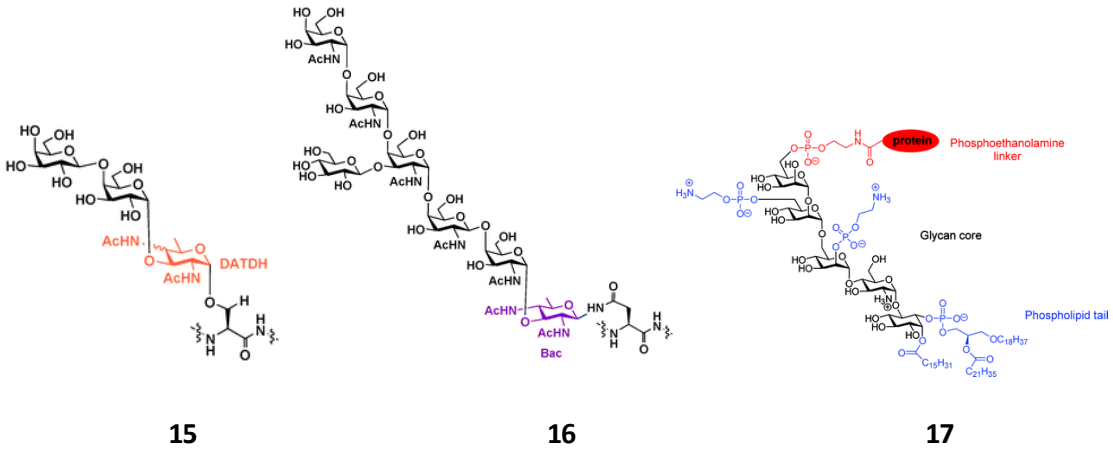
1.2.3. Glycoproteins

Glycoproteins are enzymes which contain covalently conjugated carbohydrate moieties and are found in all living organism in both soluble and insoluble forms. They are fundamentally involved in many important biological processes including immune defense, viral replication, fertilization, parasitic infection, cell growth, cell-cell adhesion, degradation of blood clots and inflammation.[25]

Proteins are macromolecules which are built up with four different levels of structures- primary, secondary, tertiary and quaternary. The primary structure of a protein is made up by a linear sequence of amino acids in a polypeptide chain. The secondary structure describes the highly regular local sub-domain on the actual polypeptide backbone chain. The two main types of secondary structures are the α-helix and the β-sheet. The tertiary structure describes the overall three-dimensional shape of an entire protein molecule, where the secondary structures are

folded into a compact globular structure to achieve maximum stability or lowest energy state. And the quaternary structure refers to the three-dimensional structure of a multi-subunit protein and describes how these subunits interact with each other and arrange themselves to form a larger aggregate protein complex.[26]

In glycoproteins, where carbohydrates are attached to proteins, different carbohydrate side chains are present and they are classified according to the linkage between the carbohydrate moiety and amino acid residue (Figure 9), depending on whether the binding occurs *via* an oxygen atom of the amino acid (*O*-glycosidic linkage **15**), *via* the nitrogen atom (*N*-glycosidic linkage **16**) or *via* an ethanolamine phosphate (GPI-anchor **17**). These glycans differ not only in their structure but also accomplish different functions.



15 **16** **17**

Figure 9: Bacterial glycans: *O*-linked glycan (**15**, *N. meningitides*),[27] *N*-linked glycan (**16**, *C. jejuni*)[27], GPI anchor[28] with ethanolamine phosphate linkage (**17**).

- ***N*-glycans:** In *N*-glycans, the carbohydrate moieties are covalently attached to an asparagine residue in the protein chain by an *N*-glycosidic linkage. The linkage between asparagine and *N*-acetylglucosamine (GlcNAcβ1-Asn) is the most common linkage. *N*-Glycosylation is a modification performed during the translation of mRNA to proteins and is available to all proteins with the Asn-X-Ser/Thr "sequons", whereby X can be any amino acid except proline.[29] The asparagine residue of this sequence binds covalently to the so called "core region", a peptide-linked pentasaccharide fragment which all *N*-glycoproteins consist of. This pentasaccharidic region includes a branched structure of Man-(α1,6)[Man-(α1,3)]Man-(β1,4)-

GlcNAc-(β1,4)GlcNAc, whereby the terminal *N*-acetylglucosamine (GlcNAc) is attached in the β-form *via* a *N*-glycosidic linkage to the asparagine residue of the peptide chain (Figure 10).[11]

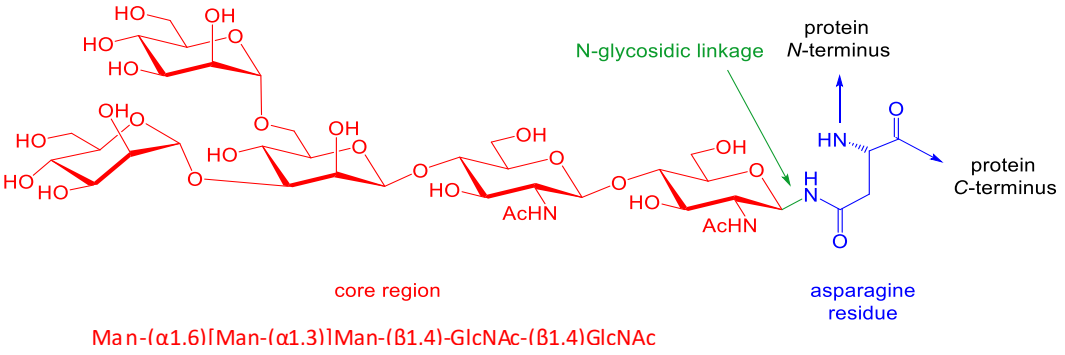


Figure 10: *N*-glycan with the core molecule Man-(α1,6)[Man-(α1,3)]Man-(β1,4)-GlcNAc-(β1,4)GlcNAc.

Although *N*-glycans possess the same core region, they show an extraordinary diversity in the structure due to the variety of monosaccharides leading to branched or unbranched molecules, so that *N*-glycans of the same protein exist of many different isoforms. Therefore, *N*-glycans are classified into three groups with respect to the "higher structure" of the carbohydrate moiety, namely the "high-mannose" type, the "complex" type and the "hybrid" type. Furthermore, *N*-glycan chains can differ in the number of side chains, forming branched structures, which are called "*antennae*". In high-mannose type oligosaccharides, the simplest *N*-glycans, only D-mannose (Man) residues are attached to the core, while in complex oligosaccharides two or more antennae are attached to the core. They contain diverse numbers of both sialylated *N*-acetyllactosamine units and L-fucose residues linked to GlcNAc and a bisecting GlcNAc residue attached to the β-linked Man of the core. Also there are hybrid-type oligosaccharides, which have the characteristic feature of both other classes. They have one or two complex-type antennae bound on the Manα-1,3-residue of the core and furthermore, a high mannose antennae linked to the Manα-1,6 of the core (Figure 11).[30]

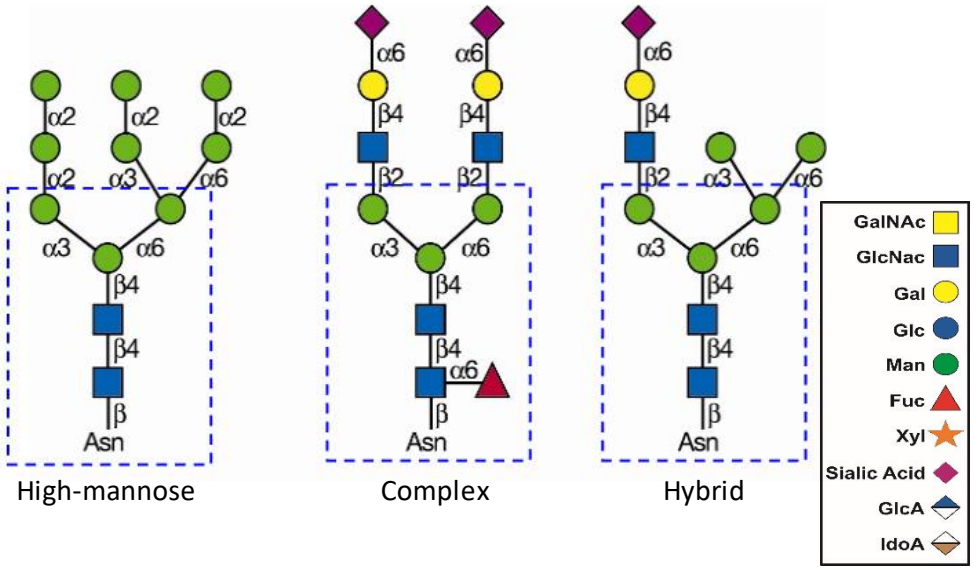
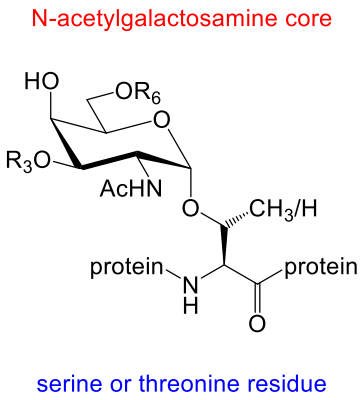


Figure 11: The three types of *N*-glycans: *N*-glycans added to protein at Asn-X-Ser/Thr sequons are of three general types in a mature glycoprotein: high-mannose, complex, and hybrid. Each *N*-glycan contains the common core Man-(α1,6)[Man-(α1,3)]Man-(β1,4)-GlcNAc-(β1,4)GlcNAc.[29b, 30]

Biologically, the synthesis of all eukaryotic *N*-glycans starts on the cytoplasmic face of the endoplasmatic reticulum membrane. GlcNAc-P is first transferred from UDP-GlcNAc to the lipid-like precursor dolichol phosphate (Dol-P), where dolichol pyrophosphate *N*-acetylglucosamine is formed. Gradually, fourteen sugar residues are added to Dol-P. The entire glycan structure *en bloc* is then transferred to the nitrogen of asparagine in a nascent peptide chain by oligosaccharyltransferase (OST), which is a glycosyltransferase. This protein-bound *N*-glycan is trimmed and processed by several membrane-bound glycosidases and glycosyltransferases.[29, 31]

- ***O*-glycans:** These glycoconjugates consist of sugar moieties which are covalently linked *via* the oxygen of a serine, threonine or tyrosine residue on a peptide backbone which occurs in lower and higher organisms. In contrast to *N*-glycosylation, the biosynthesis of an *O*-glycan occurs post-translationally in the Golgi apparatus with the addition of a single monosaccharide. This precursor is then transferred from UDP-GalNAc onto the peptide chain to a hydroxyl group of a serine or threonine residue.[32] The enzymes, which are responsible for the initiation of mucin-type *O*-linked glycosylation, are Golgi resident polypeptide α-GalNAc transferases.[31a, 33] Due to their high structural diversity, *O*-glycosidic glycoproteins exhibit no common core structure. They can be classified into eight "core" groups (Figure 12), which can be further elongated or modified. The most common form of *O*-linked glycosylation in eukaryotes is the "mucine" type (core 1), where *N*-acetyl-α-D-galactosamine (GalNAc) is attached covalently in α-position to the hydroxyl group of serine or threonine.[8]



Core 1: R^3 = β(1,3)-Gal
Core 2: R^3 = β(1,3)-Gal; R^6 = β(1,6)GlcNAc
Core 3: R^3 = β(1,3)-GlcNAc
Core 4: R^3 = β(1,3)-GlcNAc; R^6 = β(1,6)GlcNAc
Core 5: R^3 = α(1,3)-GlcNAc
Core 6: R^6 = β(1,6)-GlcNAc
Core 7: R^6 = α(1,6)-GalNAc
Core 8: R^3 = α(1,3)-Gal

Figure 12: Core structures of *O*-linked glycans.[8]

Mucines are glycoconjugates which are heavily glycosylated and of high molecular mass. They are found as a protective biofilm on the surface of various types of epithelial cells. Typically, they occur in clusters and are found in mucin domains on membrane-bound and secreted proteins. The "variable number of tandem repeat" (VNTR) - repeated peptide stretches - is the hallmark of mucins which are rich in serine or threonine *O*-glycan acceptor sites. They show an abundance of clustered mucin *O*-glycans comprising 80% of the molecular weight. Because of the rich proline residues in this region, which its role is to expose serine and threonine residues in a β-turn conformation leading to a more efficient *O*-glycosylation, the *O*-GalNAc glycosylation can be facilitated, up to hundreds of *O*-GAlNac glycans. These glycans are attached to serine or threonine residues in the VNTR regions adopting an extended "bottle brush" conformation (Figure 13).[29a, 34]

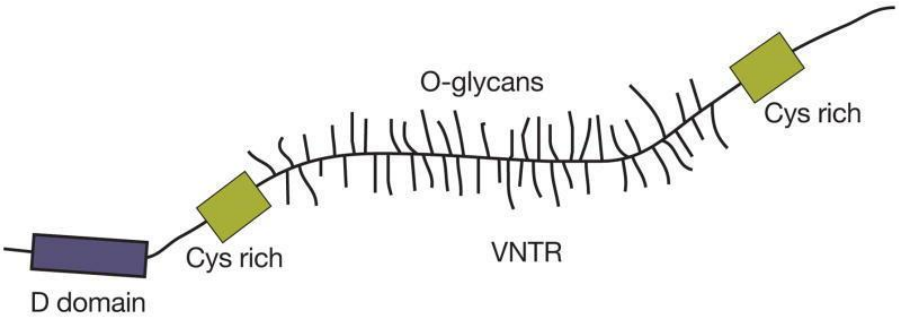


Figure 13: A simple model of a large secreted mucin: The "bottle brush" conformation in the VNTR regions, which is rich in serine, threonine and proline residues, and therefore highly *O*-glycosylated.[29a, 34]

Besides α-*O*-glycosidically linked GalNAc residues, other carbohydrates can also be attached to amino acids, such as GlcNAc in a GlcNAc-β-Ser/Thr linkage, which is found in nuclear and cytoskeletal proteins. In contrast to most other peptide linked monosaccharides, the GlcNAc-β-

Ser/Thr unit does not become further substituted by other sugars. Likewise, glycosylation can also occur with galactose, glucose, xylose, arabinose or mannose.[35]

Besides the *O*-glycosidic linkage to serine or threonine residues, also the linkage to tyrosine residues is known. For example, in glycogenin, the priming enzyme of glycogen synthesis, glucose is α-linked to the hydroxyl group of a tyrosine residue.[36] Moreover, β-arabinofuranose is attached to hydroxyproline[37] and found in plant glycoproteins.

- **GPI-Anchors**: Proteins attached to glycosylphosphatidylinsitol molecules (GPI) *via* their carboxyl termini are found in the outer leaflet of the cell membrane and face the extracellular environment. They are functionally diverse and are involved in numerous biological and physiological processes, such as in signal transduction, prion disease pathogenesis, immune response, and in the pathobiology of trypanosomal parasites.[38] All reported GPI-anchors show the same basic core structure, including a phosphoethanolamine linker, a glycan core and a phospholipid tail (Figure 14). The glycan part - a linear tetrasaccharide attached to the position O-6 of inositol - with phosphoinositol, glucoseamine and mannose residues can be modified with species-specific carbohydrates, like phosphoethanolamine groups and by variation in the lipid moiety. Proteins or glycoproteins are linked to the non-reducing mannose residue by their *C*-termini or *via* an ethanolamine phosphate bridge between the C-6 hydroxyl group of mannose and the α-carboxyl group of the carboxy-terminal amino acid.[8, 29a, 39]

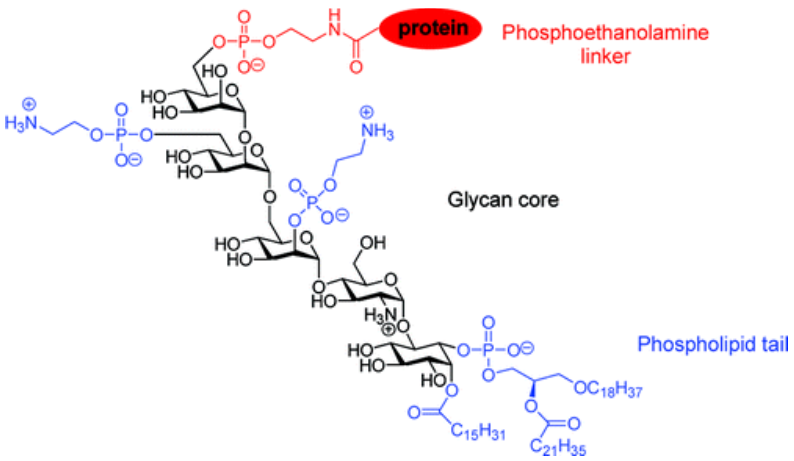


Figure 14: Structure of the GPI anchor from human erythrocyte acetylcholinesterase.[28]

- ***C*-glycans**: compounds in which the exo-cylic oxygen (anomeric oxygen) of the sugar moiety is replaced by a carbon atom and are attached to another biomolecule *via* a *C*-glycosidic linkage, are of high interest, as it was recognized that these *C*-glycosides could serve as functional analogues of the corresponding *O*-glycosides.[40] The difference between *C*- and *O*-glycosides is found within the chemical reactivities. Besides the absence of anomeric effects, *C*-glycosides are not sensitive towards hydrolytic or enzymatic cleavage in cellular environment and therefore, they exhibit a chemical stability comparable to that of cyclic acetals.[40-41] The sugar moiety of *C*-glycoside can be furanose and pyranose and the aglycon can be an aliphatic or an aromatic residue.[42] Due to the chemically stable nature of *C*-glycosides, this compound class became important for pharmaceutical and biotechnology industries as stable pharmacophores.

For example, in phlorizin (**18**), which is an inhibitor for the sodium-glucose cotransporter 2 (SGLT2) in kidneys, the *O*-glycosidic linkages renders phlorizin susceptible to rapid clearance *in vivo*. In contrast, the *C*-glycosidic analogue dapagliflozin (**19**) was designed as new inhibitor class exhibiting the advantages of the enhanced chemical stability of the glycosidic bond and its drug-like properties.[43]

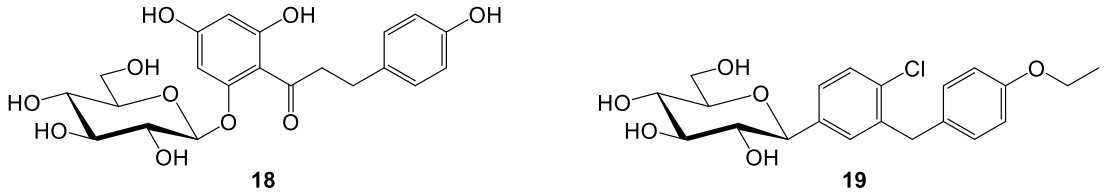


Figure 15: *O*-glycoside phlorizin (**18**) and the *C*-glycosidic analogue dapagliflozin (**19**).[40]

C-Mannosylation is a unique glycosylation in proteins. Herein, an α-mannosyl residue is directly attached at its C-1 position *via* a *C*-glycosidic linkage to the C-2 atom of a tryptophan residue (Figure 16). This *C*-glycosidic linkage was first recognized in ribonuclease 2 (RNas 2) in human urine,[44] whereas this modification can also take place in the cell, as RNas from erythrocytes also contain this *C*-mannoylated tryptophan.[45]

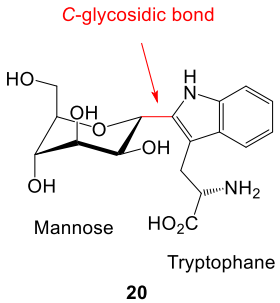


Figure 16: Structure of *C*-Man-Trp (**20**).[17]

C-Mannosylation usually takes place at the first tryptophan in the amino acid sequence Trp-X-X-Trp motif, where X could be any amino acid and the second Trp can be replaced with another aromatic amino acid such as Phe.[46] The transfer of the mannoside moiety to the target protein is catalyzed by the enzyme *C*-mannosyltransferase.[44] The acceptor sequence Trp-X-X-Trp is encountered on multiple proteins, such as in the thrombospondin type 1 repeat (TSR) and type I cytokine receptor superfamilies.[17]

To date, the function of *C*-mannosylation is not fully elucidated. However, it has been revealed that *C*-mannosylation is required for protein folding as well as for molecular recognition in the cell and plays an important role in the functional regulation of target proteins.[17]

Additionally, there are different natural *C*-glycosides known to be present in diverse plant genes, namely Scoparin (**21**), Aloin (**22**) (Figure 17), Saponarin and Cucumerins.[42]

Moreover, many efforts have been devoted to investigate appropriate synthesic routes for the preparation of complex *C*-glycosides which show potent antibiotic activity, such as the synthesis of Showdomycin,[47] Vineomycin B2,[48] Aurodox,[49] Herbicidin[50] and the hyperfunctionalized molecules Spofongistatin[51] and Palytoxin.[52]

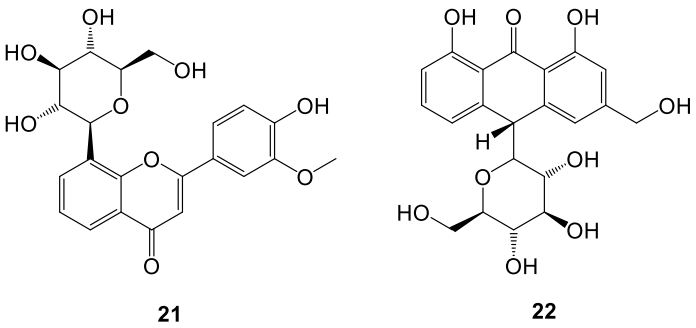


Figure 17: Natural *C*-glycosides: Scoparin (**21**) and Aloin (**22**).

1.3. Bioconjugation methods

Bioconjugation involves the coupling of two or more biomolecules *via* a covalent linkage to form bioconjugates, which exhibit the properties of each individual component. Bioconjugation is an important and growing field of interest which covers a wide range of science between chemistry and molecular biology. The goal of bioconjugation chemistry is to develop an efficient and straightforward chemical strategy for the linkage of molecules to biomolecules. In addition to the fusion of two biomolecules forming different types of bioconjugates such as protein-protein or protein-carbohydrate conjugates, bioconjugation also involves the linkage of synthetic labels

(such as isotope labels, fluorescent dyes as well as affinity tags) to biological moieties (such as carbohydates, peptides, proteins, glycans etc.).[53]

Due to the enormous number of reactions and components used to form bioconjugates, the strategies to develop bioconjugates for particular applications can be very manifold. Therefore, the choice of the appropriate compounds, methods, as well as the proper crosslinking reagents should be carefully considered.[54] Furthermore, the condition under which the bioconjugates will be used should be taken under account. Typically, biological systems furnish best in aqueous solution. Thus, reagents which are required for bioconjugation should be stable in water as well as the ligation method/reaction need to proceed in water. In addition, due to the low concentration in which biomolecules and their reactive groups are present, the reaction rate should be fast enough to obtain significant modifications within an adequate time span.[55]

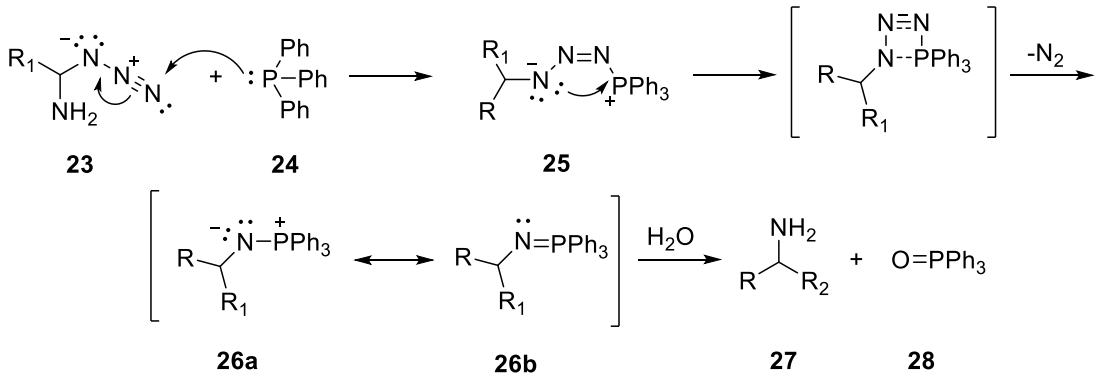
Generally, two types of covalent bioconjugation strategies exist: the random (modification at multiple sites) and the site-specific (modification at a single site) bioconjugation. Traditional bioconjugation methods preclude control over the regiochemistry of reaction which leads to heterogeneous reaction products and this often results in the loss of the biological function of the target biomolecule. A promising approach to overcome these limitations was the development of site-specific, bioorthogonal conjugation strategies, which need to be selective for their target and should not give cross-reactivity with any of the naturally occurring functional groups. With bioorthogonal reactions controlled introduction of labels is possible and not only in single proteins, but also on cell-surfaces, in living cells as well as in live animals which enable new opportunities for studying biological processes.[55]

The most well-known reactions that have been employed in bioconjugation methodology include Staudinger ligation, “click”-chemistry, Diels-Alder ligation, Native chemical ligation and Tetrazine ligation, which will be described in the following sections.

1.3.1. Staudinger ligation

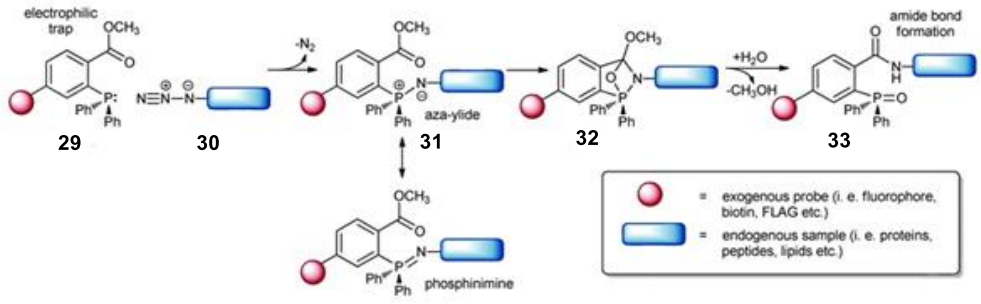
The bioorthogonal Staudinger ligation was the first example of bioorthogonal ligation reactions and derived from the Staudinger reduction[56] (Scheme 2) in which an azide (**23**) reacts with a phosphine (**24**) generating a phosphazide (**25**) which decompose with the loss of N_2 to form an iminophosphorane (**26**). This iminophosphorane carries a very nucleophilic nitrogen atom which can react with a range of electrophilic reagents. In terms of aqueous work-up, the

iminophosphorane is hydrolyzed quickly to form the primary amine (**27**) and phosphane oxide (**28**).[57]



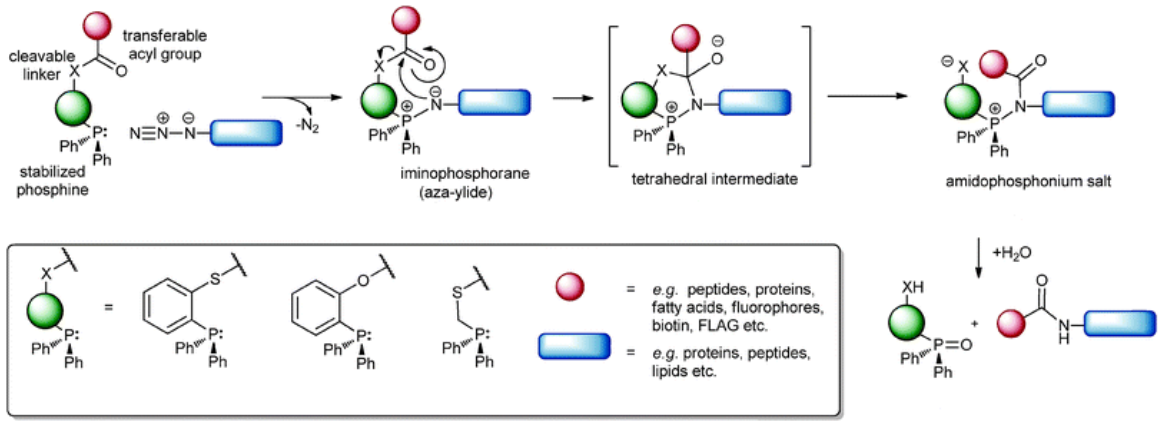
Scheme 2: Staudinger reaction.[57]

In 2006 the biorthogonal Staudinger ligation was first introduced by Bertozzi and co-workers. The aza-ylide (**31**) undergoes spontaneous hydrolysis which leads to the formation of a primary amine and the corresponding phosphine oxide as side product. Bertozzi and co-workers modified the phosphine reagent by introduction of an intramolecular electrophilic trap, such as an ester moiety, which captures the nucleophilic aza-ylide intermediate by an intramolecular cyclization generating a stable amide bond (**33**) after hydrolysis in water (Scheme 3).[58]



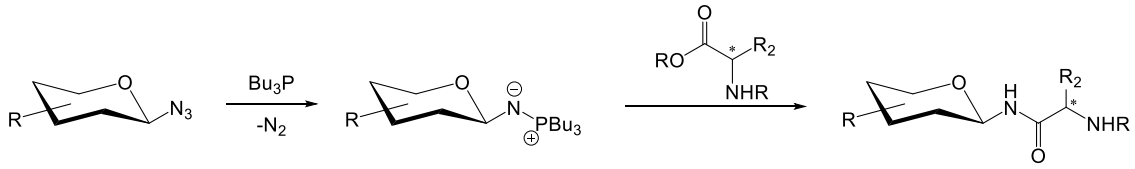
Scheme 3: Non-traceless Staudinger ligation of an ortho-phosphine terephthalic acid derivative.[59]

This nontraceless Staudinger ligation works well in biological environment, however, the so-called traceless Staudinger ligation[60], in which an amide bond is formed between the two coupling partners, and subsequent removal of the phosphine oxide from the final amide-linked product in the hydrolysis step was getting even more attractive, as this reaction leaves no residual atoms in the product (Scheme 4).[57]



Scheme 4: Traceless Staudinger ligation.[61]

This ligation method is used for the conjugation of two peptide fragments[60b] and enables the selective labeling of biomolecules in living cells.[62] Furthermore, the traceless Staudinger ligation is an attractive conjugation method for the synthesis of glycoamino acids. David and co-workers[63] developed the three-component Staudinger ligation which allows the formation of biologically relevant *N*-linked glycopeptides (Scheme 5).



Scheme 5: Three-component Staudinger ligation.[63]

Lindhorst and co-workers[64] investigated this ligation method for the synthesis of amide-linked glycomimetics, such as *N*-glycosyloctanamide (**34**), *N*-mannosyloxyethyl amino acids (**35**) as well as to generate trivalent glycocluster amides (**36**) (Figure 18).

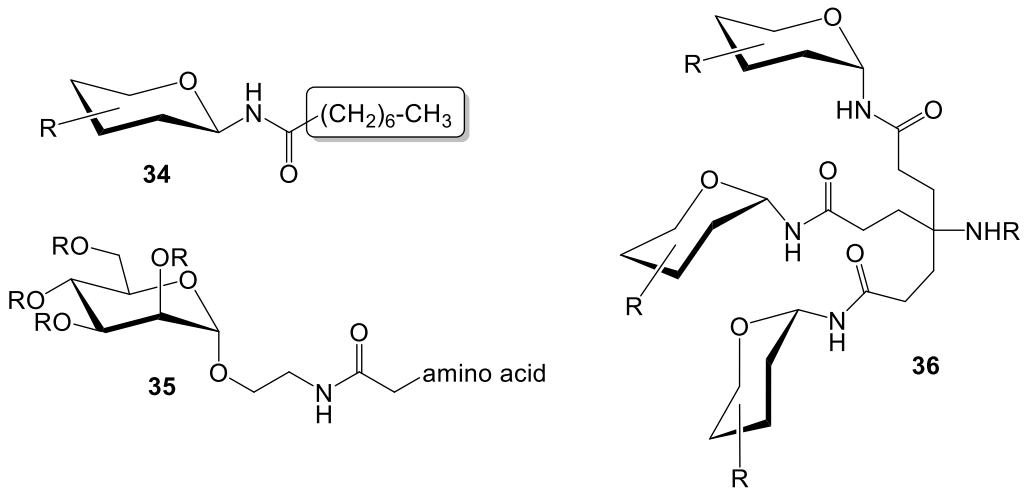
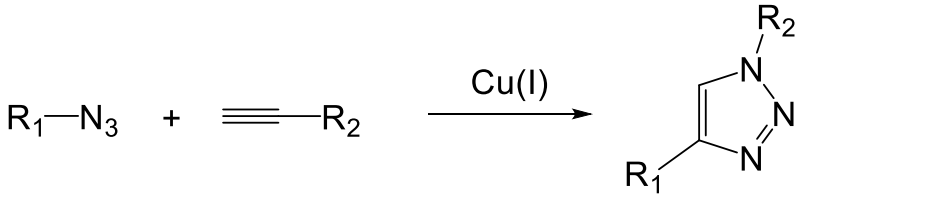


Figure 18: *N*-glycosyloctanamide (**34**), *N*-mannosyloxyethyl amino acids (**35**) and trivalent glycocluster amide (**36**).[64]

1.3.2. "Click" chemistry

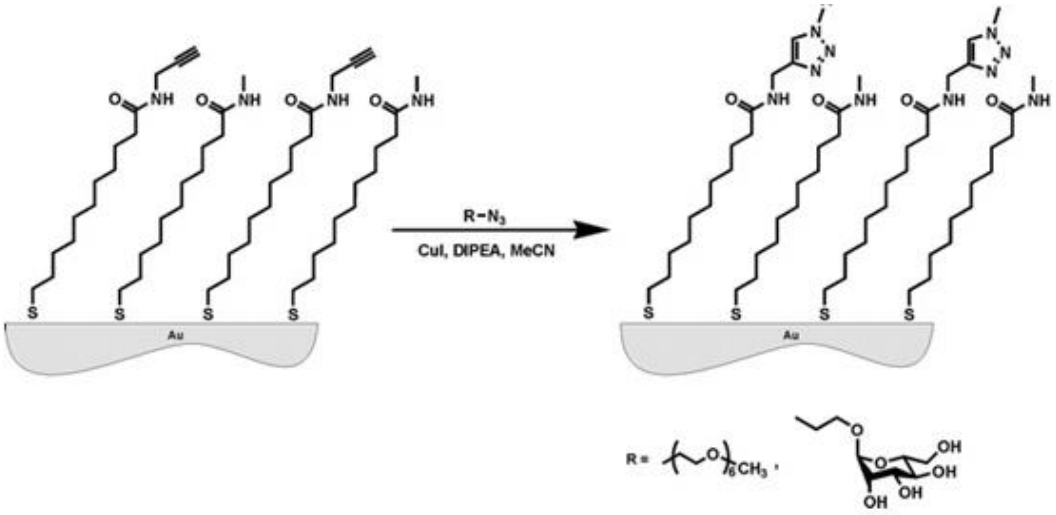
One of the most widely used bioorthogonal reactions is the "click" reaction – a cycloaddition reaction between azides and alkynes – which was first reported in 1890s by Michael.[65] However, the Huisgen 1,3-dipolar cycloaddition is undoubtedly the first example of a "click" reaction.[66] Due to the slow kinetics and the extreme reaction conditions, the conventional cycloaddition is not suitable as a bioconjugation method.[58b] Meldal[67] and Sharpless[68] demonstrated that the reaction could be dramatically accelerated in aqueous solution when Cu(I) is applied as catalyst (Scheme 6) and that under these reaction conditions several functional groups are completely stable. With this biorthogonal reaction, various biomolecules including DNA, proteins, peptides, oligosaccharides and glycoconjugates can be labeled which are applied as tools for studying biological systems.[69]



Scheme 6: Copper-catalyzed alkyne-azide cycloaddition.

The "click" reaction is an attractive approach for immobilization of carbohydrates and proteins, as this ligation method do not generate unwanted side products.[69] Using clickable self-assembled monolayers (SAM), the coupling with azido sugars including mono-, di-, or

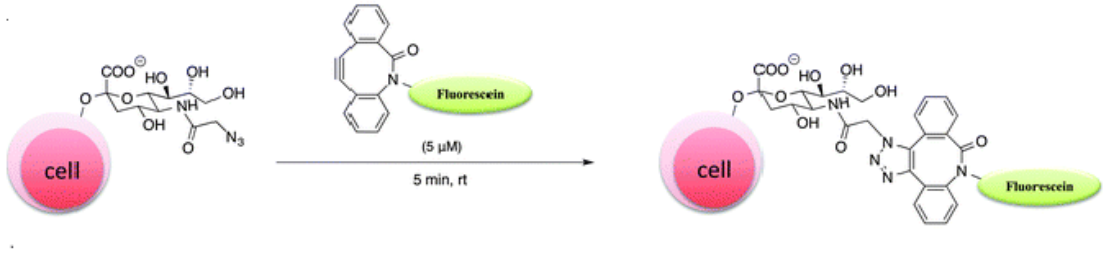
trisaccharides can be realized using standard Cu(I)-catalyzed conditions (Scheme 7). These glyco-SAMs are powerful tools for example of high-throughput characterization of carbohydrate-lectin interactions.[70]



Scheme 7: Surface modification of a mixed SAM on gold by coupling of azides.[70]

However, due to the toxicity of the Cu(I) catalyst this ligation method is incompatible with living cells. Since both alkyne as well as azide groups can be introduced in biomolecules without changing their function or metabolic processing,[71] several ligands have been investigated which can increase the reactivity of Cu(I) and as a consequence reduce the amount of the catalyst decreasing its toxicity.[58b, 72]

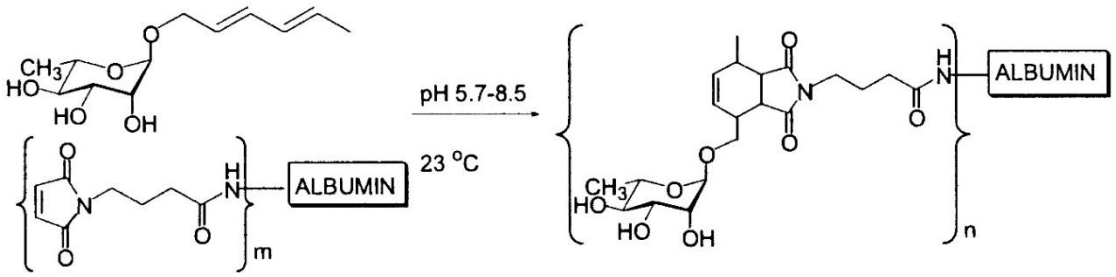
To overcome the use of a Cu(I) catalyst, alkyne substrate activation was investigated to accelerate the rate of azide-alkyne cycloaddition, which is known as copper-free "click" chemistry. Bertozzi and co-workers[73] introduced strained cyclooctynes instead of linear alkynes. This activated reaction partner is highly reactive and accelerates the reaction with azide under physiological environments. To improve the kinetics of the activated reaction partner, several chemical modifications on cyclooctyne have been investigated, such as introduction of fluoride substituents or fusion of two benzene rings to cylcooctyne.[72] This copper-free "click" ligation is widely used for bioconjugation in living cells.[74]



Scheme 8: Bioorthogonal labeling *via* strain-promoted azide–alkyne cycloaddition, employing a fluorescently labelled biarylazacyclooctynone as cyctooctyne component.[72]

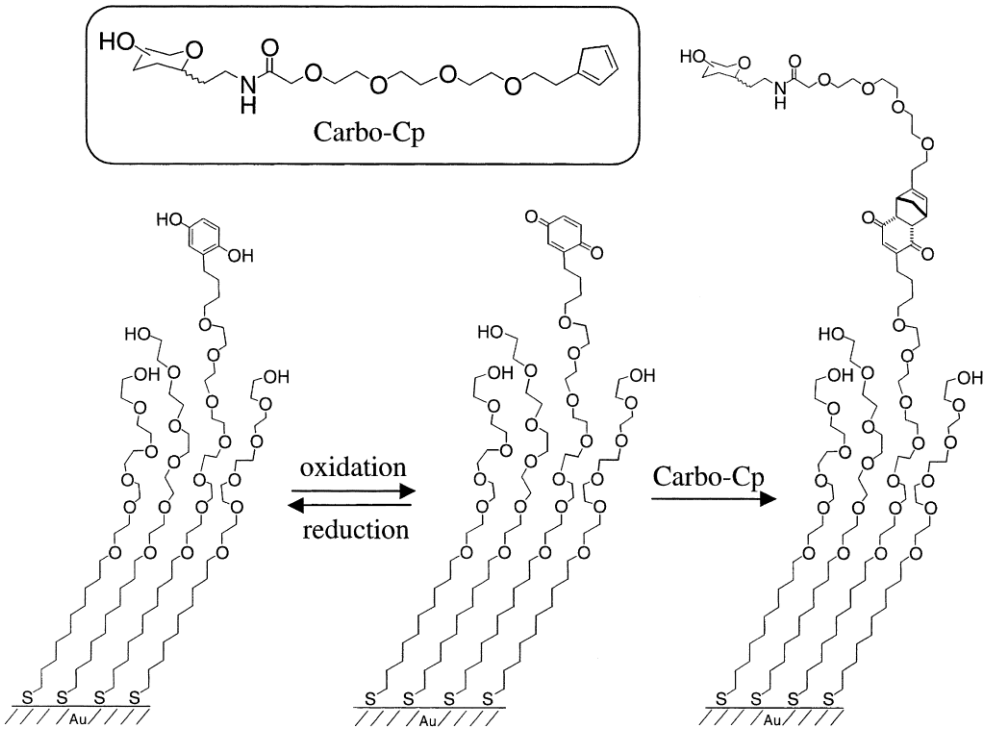
1.3.3. Diels-Alder ligation

The reaction between an electronically matched pair of a double bond (dienophile) and a conjugated diene generating a six-membered unsaturated ring is called after their discoverer, Diels-Alder reaction.[75] Due to its high velocity and selectivity, this reaction can be performed in water and is compatible with many biomolecules which makes this reaction very valuable as a bioconjugation or immobilization method of oligonucleotides and other biomolecules.[76] Pozsgay *et al.*[77] demonstrated that the Diels-Alder cycloaddition reaction is a suitable method to conjugate saccharides and proteins under biocompatible reaction conditions (Scheme 9).



Scheme 9: Diels–Alder-type cycloaddition of saccharide-linked conjugated dienes and a dienophile-equipped protein yields in the formation of neoglycoproteins.[77]

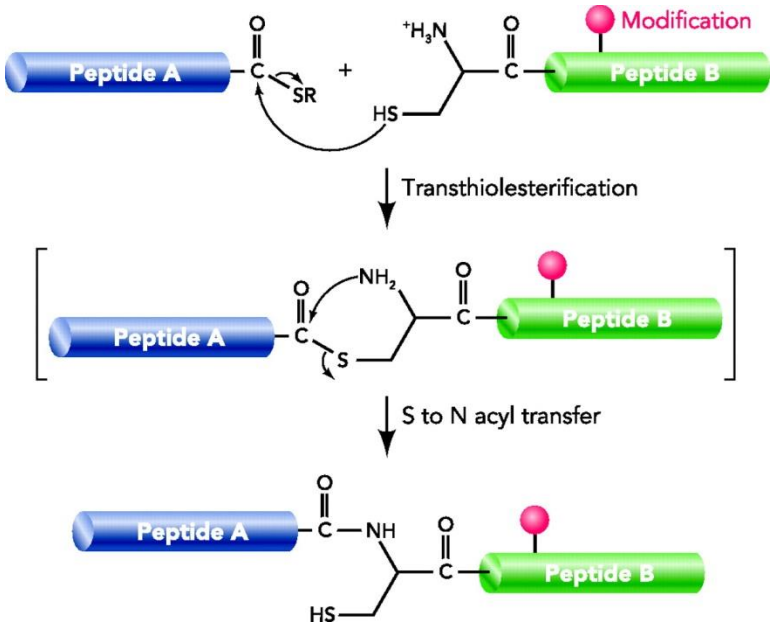
To characterize carbohydrate-protein interactions, Houseman and Mrksich[78] employed the Diels-Alder-ligation method for the immobilization of carbohydrate-cyclopentadienes conjugates to self-assembled monolayers on a gold surface containing benzoquinone groups (Scheme 10). Such carbohydrate arrays are widely used today for the investigation of carbohydrate interactions.



Scheme 10: Carbohydrate arrays by employing the Diels-Alder-mediated immobilization.[78]

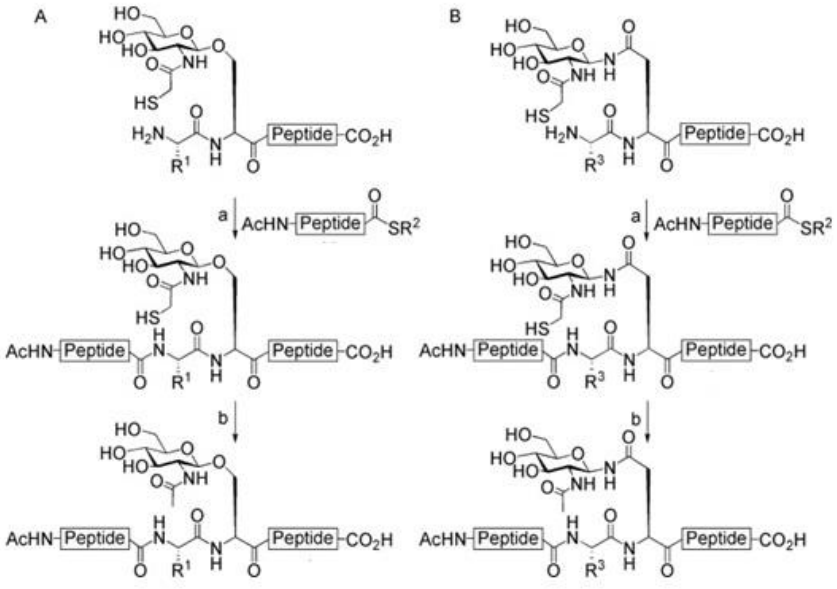
1.3.4. Native chemical ligation

The native chemical ligation (NCL) is a chemoselective ligation method which allows the coupling of two peptide units; one peptide carries a thioester and the second peptide presents a *N*-terminal cysteine unit, which leads *via* a transesterification reaction followed by an intramolecular amidation to the ligation of the two peptide moieties. (Scheme 11).[79]



Scheme 11: Native chemical ligation.[80]

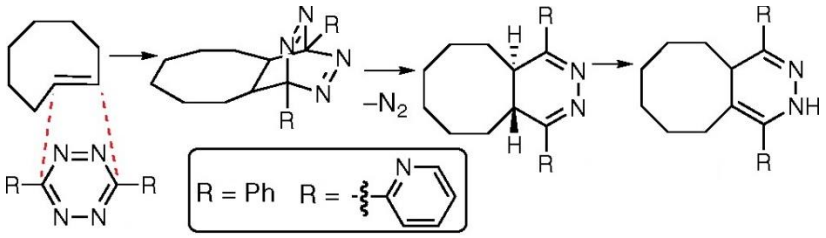
Furthermore, the NCL is an important non-recombinant approach for the synthesis of glycoproteins because it enables a stoichiometrically and site-specific glycosylation, while maintaining their native linkage.[81] Wong and co-workers[82] reported the sugar-assisted glycopeptide ligation using a peptide thioester and a glycopeptide, in which *N*-acetylglycosamine is β-anomerically linked to serine side chain and is modified at the C-2 acetamide with a thiol handle, which mimics the cysteine function at the ligation site (Scheme 12).[83] With this method, the access to *O*- as well as *N*-linked glycopeptides[84] is possible.



Scheme 12: Sugar-assisted glycopeptide ligation: (A) Ligation of *O*-linked glycopeptide (B) Ligation of *N*-linked glycopeptide. Reagents and conditions: (a) buffer (6.0 *M* guanidine hydrochloride, 0.2 *M* Na_2HPO_4, 50 m*M* TCEP, pH 7.5–8.5), 2% PhSH, 37°C, ~76%; (b) buffer (6.0 *M* guanidine hydrochloride, 0.1 *M* Na_2HPO_4, 10 m*M* TCEP, pH 5.8); Pd/Al_2O_3, H_2, ~90%. (R^1 = amino acid side chain: Gly; R^2 = $CH_2CH_2C(O)NH_2$; R^3 = amino acid side chain: Gly, Ala, Val, Asn, Asp, and His).[83]

1.3.5. Tetrazine ligation

The tetrazine ligation was first reported by Sauer in 1990s. He demonstrated that the rates of cycloaddition reaction between electron deficient tetrazines and diverse dienophiles were extremely fast.[85] The bioorthogonal tetrazine ligation was reported in 2008, in which the most common dienophiles for this type of reaction, norborene [86] and *trans*-cyclooctene,[87] wereused which react with tetrazine in aqueous solution (Scheme 13). This ligation method offers the opportunity for live-cell imaging of small molecules, which are biosynthetically incorporated into biopolymers such as proteins and glycans.[88]



Scheme 13: Diels–Alder reactions of tetrazines with *trans*-cyclooctene.[87]

Beckmann *et al.*[89] reported a series of carbohydrate-dienophile conjugates (Figure 19) that were immobilized onto tetrazine-modified glass slides (Scheme 14), an important tool for the high-throughput screening of carbohydrate-protein interaction.

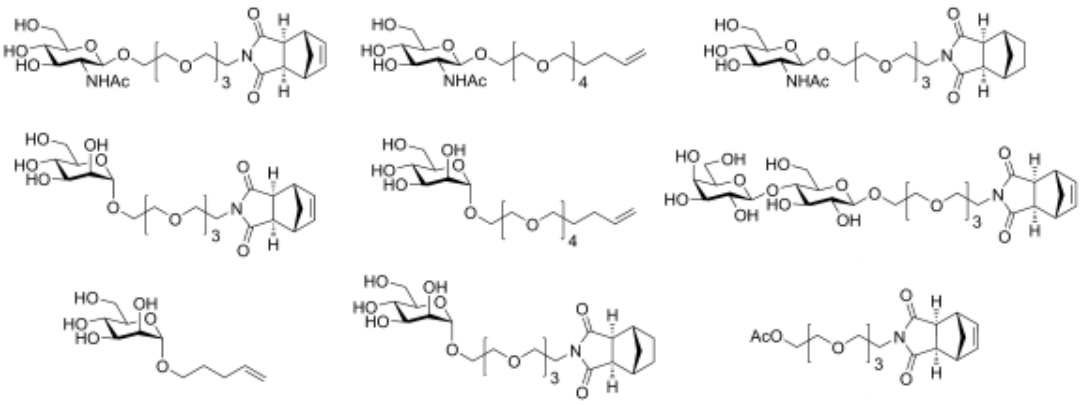
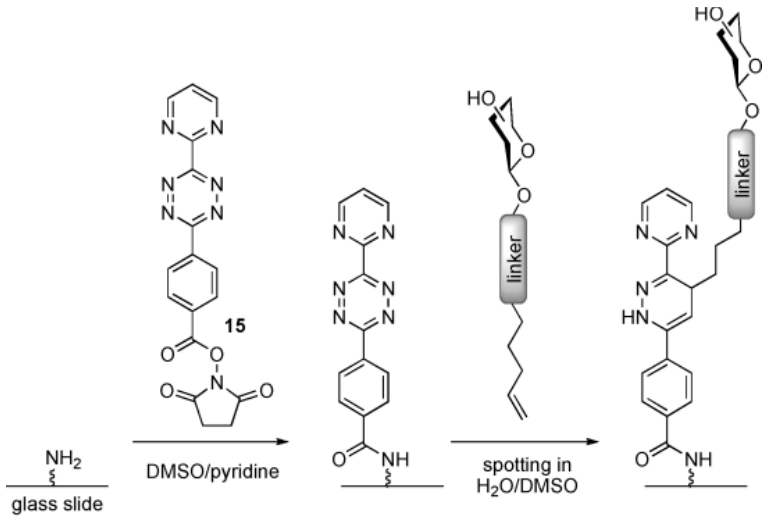


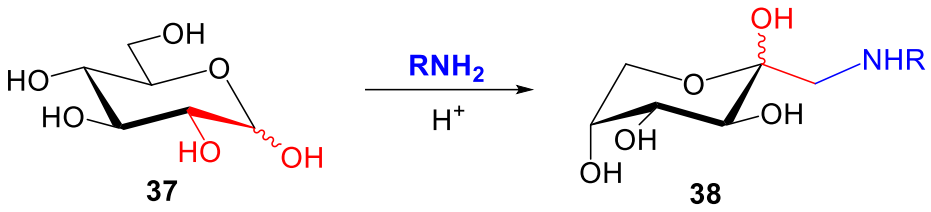
Figure 19: Series of carbohydrate-dienophile conjugates.[89]



Scheme 14: Carbohydrate array using tetrazine-modified glass slides.[89]

1.4. Amadori Rearrangement

The Amadori rearrangement is the reaction of α-hydroxy-aldehydes such as aldoses with suitable amines forming *N*-substituted 1-amino-1-deoxy-ketoses *via* an acid catalyzed rearrangement. For example, when glucose (**37**) reacts with suitable amines, the corresponding 1-deoxy-ketosamine (**38**) is formed (Scheme 15). This rearrangement reaction is known as the initial step of the Maillard cascade pathway,[90] the non-enzymatic browning of food.[91] The Maillard reaction takes place in cooking, baking and preservation processes and is responsible for aroma, taste, flavor and color of food.[92]



Scheme 15: Amadori rearrangement of D-glucose to 1-amino-1-deoxy-D-fructose.[93]

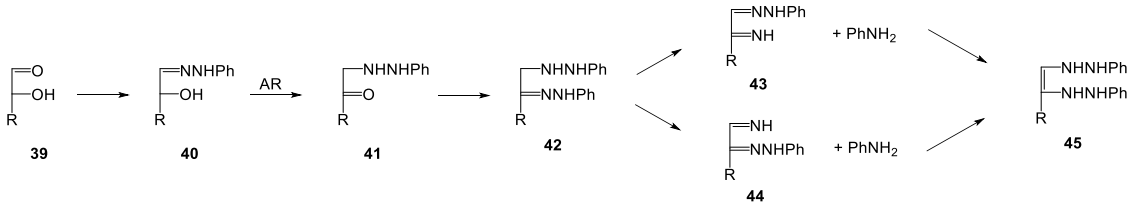
From 1925 to 1931, Mario Amadori demonstrated that during the reaction of D-glucose with aromatic amines, such as *p*-toluidine, *p*-anisidine and *p*-phenetidine two structurally different isomers were obtained (Scheme 17) using different reaction conditions. One of them was labile towards hydrolysis (**III**) and also more sensitive to decomposition, which he recognised as *N*-glycosylamine. And the other isomer was stable towards hydrolysis (**VIII**), which he thought mistakenly to be a Schiff´s base.[94]

In 1936, Kuhn and Dansi found out during the reaction of D-glucose with *p*-toluidine in substantia as well as in alcoholic solution that the stabile isomer was not a Schiff´s base, as is was previously assumed from Amadori, but its rather the product of a novel rearrangement reaction. [95]

Before Amadori made his important contribution, several other workers had been investigating the reaction between D-glucose and various amines. In 1866, Schiff heated D-glucose and aniline in substantia and obtained amorphous condensation products, which were proved at some later stage as the rearrangement products. [96] Using the same starting materials but different reaction conditions Sorokin reported in 1886 that he isolated a crystalline compound after the treatment of D-glucose and aniline at lower temperature in ethanol. He was able to isolate the comparably less stable glycosyl amines.[97]

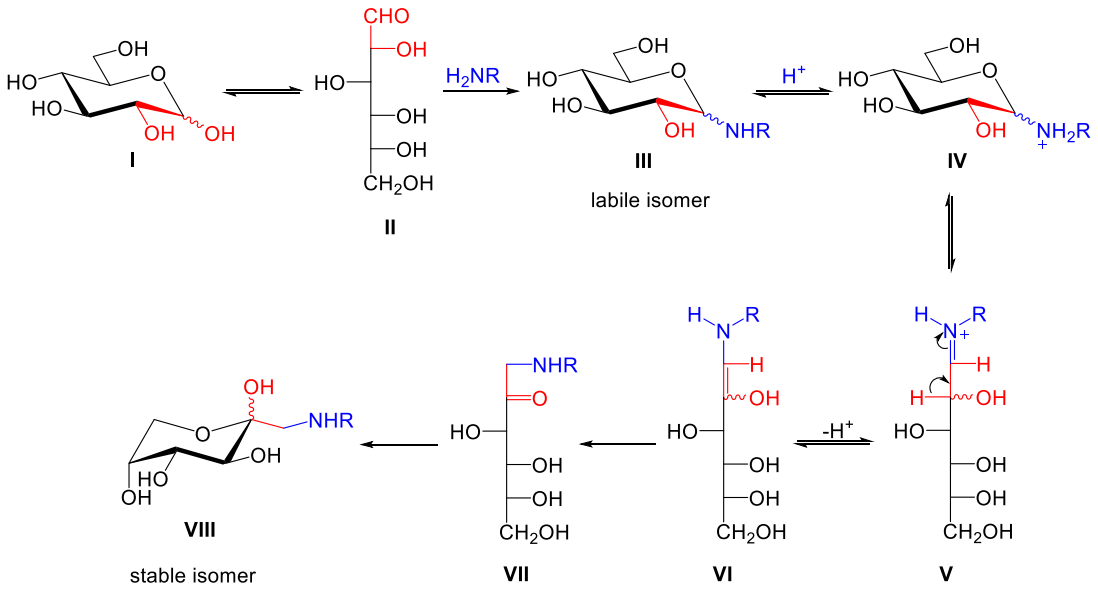
Furthermore, Fischer, for example, used phenylhydrazine as the basic component and demonstrated during his investigations of the constitution and configuration of carbohydrate

moieties using D-glucose and D-fructose as starting materials, that in both cases the reaction with phenylhydrazine led to the same product.[98] In the case of the reaction of cane sugar, sulfuric acid and phenylhydrazine, Fischer isolated "phenylglucosazone". After dissolving this product in ethanolic acetic acid, he obtained the rearrangement product "isoglucoseamine" (1-amino-1-deoxyfructose).[99] During these investigations, he found out that the properties of "isoglucosamine" are closely related to fructose and therefore, during the formation of "isoglucosamine", a change from the dextrose to the fructose series must be involved. In course of time and many studies later into the mechanism of the osazone (**45**) formation step, it turned out that an Amadori rearrangement was involved in this reaction cascade[100] (Scheme 16). Amadori products (**41**) were obtained after isomerization of phenylhydrazones (**40**) which was formed during the reaction of aldoses (**39**) with phenylhydrazine. The keto function of products (**41**) can react with a further phenylhydrazine to obtain compound **42**. After the loss of aniline at position C-1 or C-2 two possible iminophenylhydrazones, **43** and **44**, were formed. In the final step the imino moiety is exchanged through a third molecule of phenylhydrazine to give the desired osazone (**45**).



Scheme 16: Mechanism of osazone formation step.[93]

The mechanism for the Amadori rearrangement was proposed in 1937 by Kuhn and Weygand[101], which is still widely accepted (Scheme 17).



Scheme 17: Proposed mechanism of the Amadori rearrangment by Kuhn and Weygand.

In the first step, the amine reacts with the aldose, here D-glucose (**I**), at the anomeric centre forming an *N*-glycoside (**III**). The nitrogen of the *N*-substituted aldosylamine is protonated leading to the formation of the ammonium ion (**IV**) which is in equilibrium with the aldimine (**V**). Further tautomerization of the Schiff base (enamine-aldimine tautomerism) leads to the corresponding enaminol (**VI**) which is stabilized through the formation of 1-amino-1-deoxy-ketohexose (**VII**) (keto-enol tautomerism). Finally, ring closure leads to the desired Amadori rearrangement product (**VIII**). The loss of hydrogen at C-2 of Schiff base (**V**) is considered as the rate determing step.[102] If the hydroxyl group at position C-2 is protected, the rearrangement reaction cannot occur.[103]

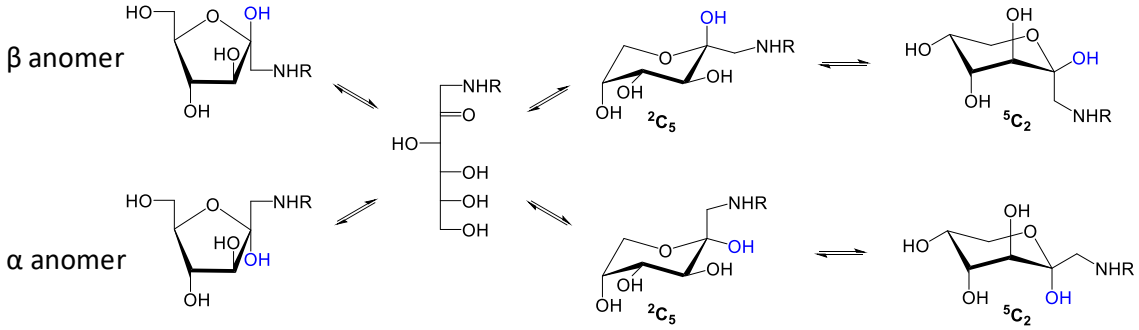
In 1940, Weygand[104] demonstrated that the addition of acid had a catalytical effect resulting in higher yields as well as improved purity. Rosen *et al.*[105] investigated in 1957 the influence of the basicity of the amino component in the Amadori rearrangement. Applying different reaction conditions by using aqueous pyridinium chloride as well as pyridinium acetate puffered glacial acetic acid, they found out that this rearrangement is an acid-base catalysed reaction.

In the early stages of the investigations of the Amadori rearrangement, it was believed that only primary aromatic amines were suitable for this reaction, which was refuted by Hodge and Rist[91], due to their results obtained from the reaction of D-glucose with *N*-alkylamines in the presence of malonic acid. Later on, Heyns demonstrated the reaction of α-hydroxycarbonyl compounds with aromatic[106] or aliphatic amines.[107]

In addition to the investigations with aldoses and simple aryl and alkyl amines, the Amadori rearrangement was performed employing various combinations of sugars and amines. Using amino acids as the amino component in this rearrangement, where the carboxyl group of the amino acid acts as the acidic catalysts,[108] Gottschalk was able to synthesize the first amino-acid-fructose adducts.[109]

During the studies of the Amadori rearrangement, it was concluded that four factors are crucial for this reaction, namely the reaction time, the temperature, the employed catalyst and the sugar as well as the amine which are utilized for this rearrangement.

Due to the fact that Amadori products are unprotected sugars, they are able to occupy different tautomeric forms. According to the thermodynamic equilibrium, they can occur as acyclic, furanoide and pyranoide $^{5}C_{2}$ and $^{2}C_{5}$ forms and α as well as β configurations (Scheme 18).[93]



Scheme 18: Possible tautomeric forms of Amadori rearrangement products.[93]

Although the Amadori rearrangement is a highly useful synthetic method generating *C*-glycosyl type glycoconjugates, this reaction also exhibits some limitations. If the reaction is conducted at excessive temperature and time, the easily dehydrated Amadori ketose can enter the so-called Maillard reaction cascade, during which a wide range of diverse products are formed.[110] Because of the reversibility of this reaction, the glycosyl amine precursor can isomerise to 1-amino-ketose or can undergo a hydrolysis back to the starting material which results in mixtures of many compounds that require quite sophisticated separation techniques. Consequently, this complex mixture of products, which are challenging to separate, leads to a low yield of the rearrangement products itself.[93]

Depending on the pH value of the system, Amadori rearrangement products can undergo degradation in the Maillard reaction as follows: a 1,2-enolisation can take place at pH 7 or below, which leads to furfural or hydroxymethylfurfural depending on the involved starting material (pentose or hexose). At a pH value higher than 7, a 2,3-enolisation takes place forming diverse

fission products, like pyruvaldehyde or diacetyl. All these formed products exhibit a high reactivity, so a range of further reactions can take place (cyclisation, rearrangement, dehydration, retro-aldolisation and much more) leading in the final stage to the formation of brown nitrogenous polymers and co-polymers, known as melanoidins (Figure 20).[110]

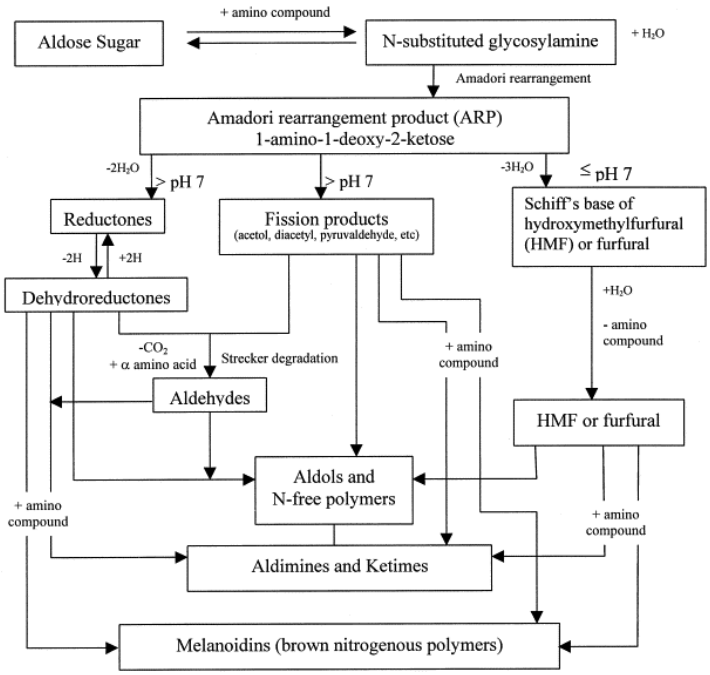
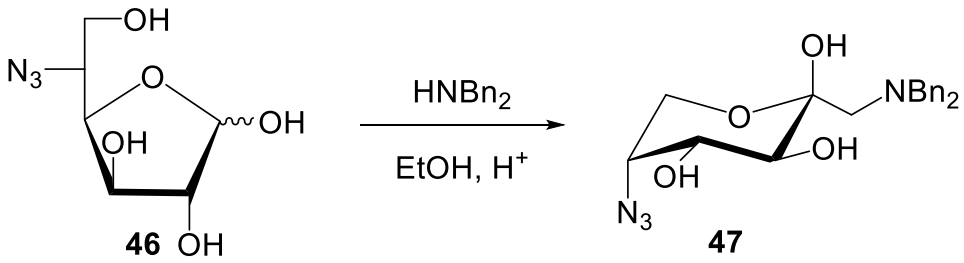


Figure 20: Maillard reaction pathway.[111]

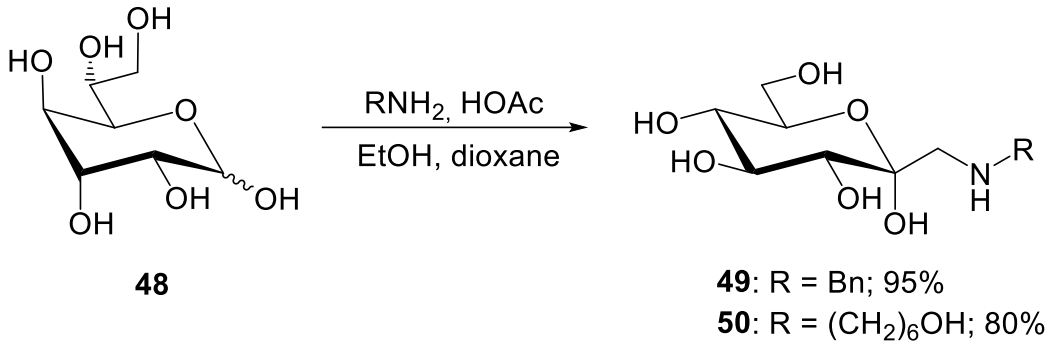
However, studies showed that the Amadori rearrangement is an attractive, high yielding and efficient synthetic method for the synthesis of *C*-glycosyl-type glycoconjugates when applied to selected carbohydrate moieties.

In case of 5-azido-5-deoxy-α,β-D-glucofuranose (**46**) as starting material in the Amadori rearrangement, only the pyranoid form of 1-amino-5-azido-1,5-dideoxy-D-fructose (**47**) was formed (Scheme 19). The additional driving force is the ring enlargement of the aldofuranose to the ketopyranose. The relatively bulky amino substituent, dibenzyl amine, in this particular case is oriented in the equatorial position in the β-form.[112]



Scheme 19: Amadori rearrangement using 5-azido-5-deoxy-α,β-D-glucofuranose (**46**) as staring material.[112]

By choosing aldoheptose as the starting material, the respective 1-amino-1-deoxyketo-heptose can be obtained exclusively in one anomeric form. For instance, reaction of the commercially available D-*glycero*-D-*gulo* aldoheptose (**48**) with benzylamine or 6-aminohexanol as amino component gave exclusively the α-anomers **49** and **50** in excellent yields (Scheme 20). The driving force in this particular case is the all-equatorial arrangement of the hydroxyl groups in the D-*gluco* configured products. Furthermore, the $^5C_2$ pyranoid conformation in the α-anomeric form enables the amino substituent to be oriented in the more comfortable equatorial position.[113]



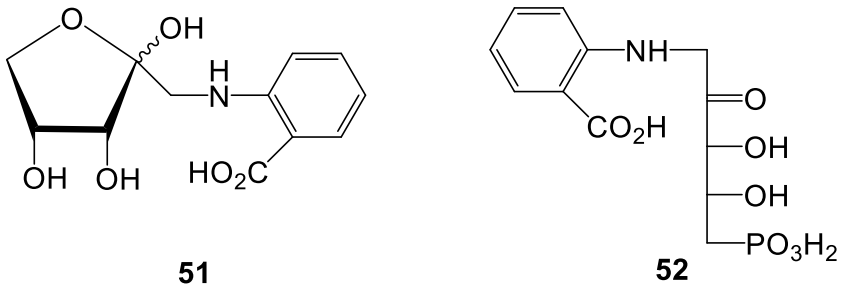
Scheme 20: Amadori rearrangement using D-*glycero*-D-*gulo* aldoheptose (**48**) as starting material.

In addition, the nature of amino component employed in the Amadori rearrangement is crucial for the outcome of the reaction. Kort[114] reported that the Amadori rearrangement failed when ammonia, which is a nucleophile as well as a strong base, was applied as amino component. In literature, there are many examples where several different reaction conditions were examined. For example, applying short reaction times, low temperatures and no catalyst, a Lobry de Bruyn - Alberda van Ekenstein rearrangement takes place which leads epimerization reactions. By increasing the reaction time, mainly the formation of degradation products were observed. In case of strongly basic amines, the probability of epimerization at C-2 increases, leading to subsequent side-reactions and unwanted degradation of the intermediates.[93]

1.4.1. Biological aspects

The formation, as well as the biological significance of Amadori rearrangement products is of particular interest for biological investigations, as these products exhibit a *C*-glycosyl type linkage which is not sensitive towards enzymatic hydrolysis, contrary to the more common *O*- and *N*-glycosidic bonds.

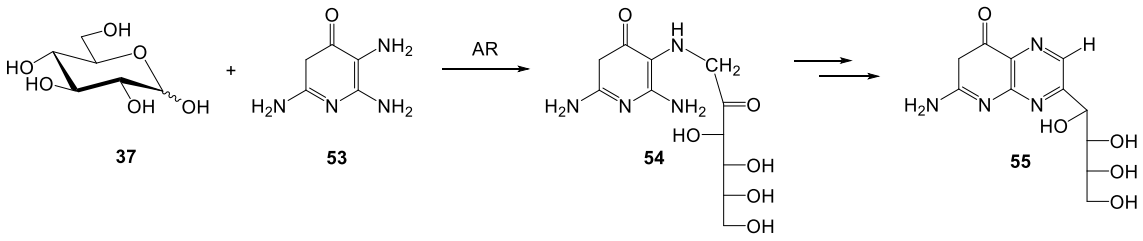
For example, during the biosynthesis of tryptophan, some intermediates (**51**) are formed *via* the Amadori rearrangement reaction of aldoses and anthranilic acid, which are present in cells as the corresponding phosphorylated form (**52**) (Figure 21).[115]



51 **52**

Figure 21: Amadori rearrangement product (**51**) as intermediate in the biosynthesis of tryptophan and the corresponding phosphorylated form (**52**).

During the synthesis of substituted pterine, an Amadori rearrangement was observed which took place as the first step in the reaction of D-glucose (**37**) with 2,4,5-triamino-6-hydroxypyrimidine (**53**) (Scheme 21) leading to compound (**54**) which is a significant intermediate for the synthesis of folic acids and hydroxyalkylpterine.[116] Furthermore, these compounds are intermediates of the biogenesis of leucopterines (**55**).[93, 117]



37 **53** **54** **55**

Scheme 21: Amadori rearrangement of D-glucose (**37**) with 2,4,5-triamino-6-hydroxypyrimidine (**53**) leading to substituted pterine (**54**).

Various Amadori products containing an aromatic amino acid moiety were demonstrated to occur as direct-acting mutagens in *Salmonella tryphimurium* his$^-$ strains.[118]

Further Amadori products, such as nitrosated fructose-tryptophane and fructose-serotonine compounds are notable for inducing DNA repair synthesis in cells of human HeLa S3 cell line.[119]

Additionally, it has been reported that Amadori compunds or their degradation poducts are able to effect the adhesion and aggregation properties of cancer cells.[120]

During kinetic studies on *N*-(5´-*O*-phosphono-β-D-ribosyl)anthranilate ketol isomerase (EC 5.3.1.24), which is involved in the biosynthesis of tryptophan, it turned out that this enzyme catalyzes the reversible Amadori reaction of antranilic acid with ribose 5-phosphate. This property might limit protein glycation and prohibit further tissue damage.[121]

Enkephalins, such as Tyr-Gly-Gly-Phe-Met or Tyr-Gly-Gly-Phe-Leu, are neuropeptides and endogenous ligands of opiate receptors and are of central importance for physiological suppression of pain,[122] albeit because of rapid hydrolysis of these enkephalins, their effect is of limited duration.[122-123] Therefore, the investigation of increasing the efficiency of natural enkephalins by inhibiting their breakdown is of interest. Vértesy *et al.*[124] reported Amadori rearrangement products which have a high biological activity and a selective endopeptidase-24.11-inhibiting action. Reaction of D-glucose with dipeptides, such as L-isoleucyl-L-aspartic acid and L-valoyl-L-aspatic acid leads, *via* the Amadori rearrangement, to *N*-(1-deoxyfructos-1-yl) dipetides **56** and **57** (Figure 22), also known as enkephalinase, which were found to be specific inhibitors of endopeptidase (EC 3.4.24.11).[124] These two compounds were isolated after the Amadori rearrangement with the unprotected peptides in more than 70% yield.

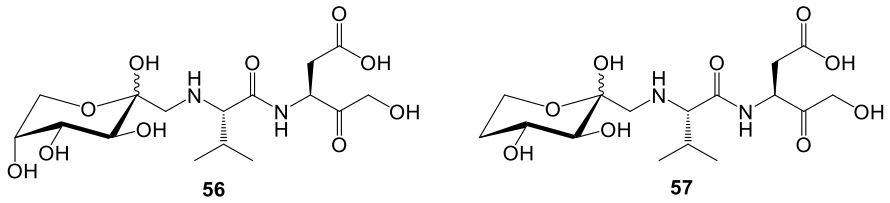


Figure 22: *N*-(1-Deoxyfructos-1-yl) dipetides.[124]

During the studies towards the synthesis of leucine enkephaline (Tyr-Gly-Gly-Phe-Leu),[125] it was determined that depending on the employed solvent, this related 6-*O*-glycoconjugates can undergo different rearrangement reactions. In a dry methanol solution, cyclic sugar-related imidazolidinones[126] were isolated, whereas in a solvent mixture of pyridine-acetic acid 1:1, bicyclic Amadori rearrangement products (**58** and **59**)[127] were obtained, which can be transformed by hydrolysis into the corresponding 1-amino-1-deoxy-D-fructose (**60**) and -D-tagatose (**61**) Amadori products of leucine-enkephalin (Figure 23).[128]

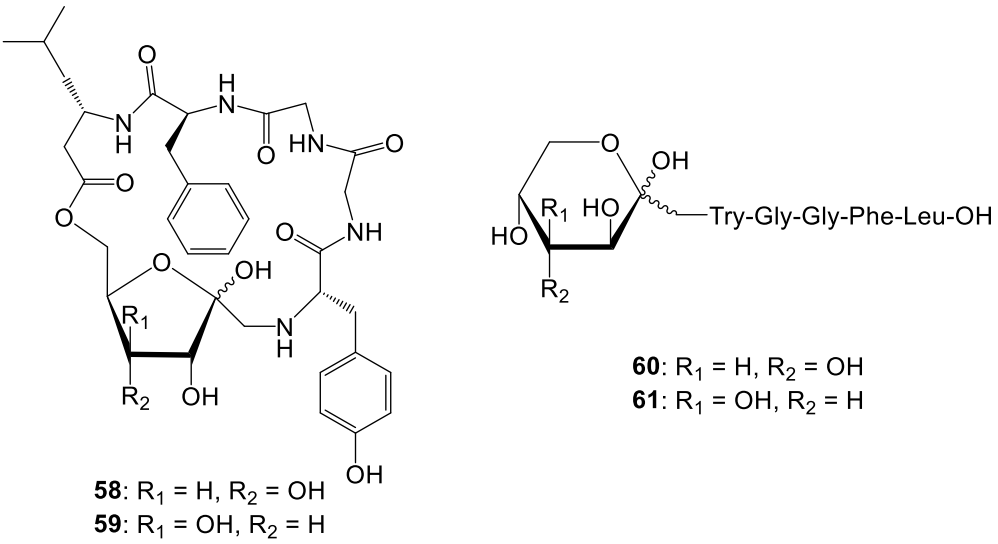


Figure 23: Bicyclic Amadori rearrangement products (**58** and **59**) and the corresponding 1-amino-1-deoxy-D-fructose (**60**) and –D-tagatose (**61**) Amadori products of leucine-enkephalin.[127]

Recently, it has been found that products generated in the Maillard casacade pathway are involved in Alzheimer´s disease,[129] in diabetic cataract formation[130] as well as in age-related postsynthetic glycosylation of hemoglobin[131] and collagen.[132]

1.4.2. Advanced glycation products

Glycation is the non-enzymatical reaction of reducing sugars such as D-glucose or D-fructose with amine residues of proteins, lipids or nucleic acids leading to advanced glycation end products *via* a Amadori rearrangement. This reaction has to be distinguished from glycosylation, which is an enzymatic reaction occurring on defined sites of the target molecule (Figure 24).

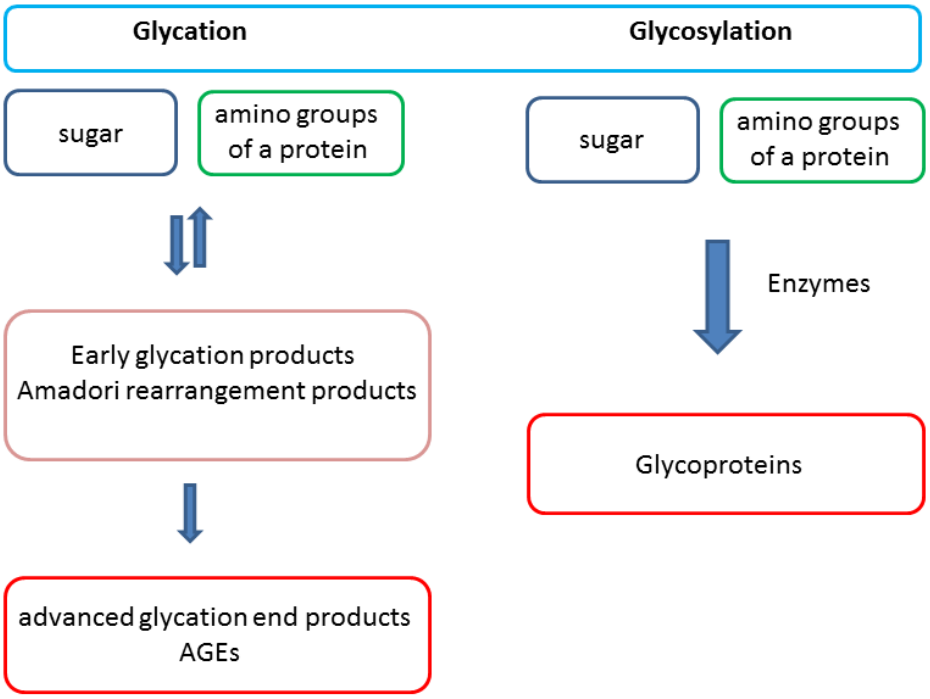


Figure 24: Non-enzymatical glycation vs enzymatical glycosylation.

Glycation takes place in food browning during thermal processes as well as in living systems and is involved in diverse pathologies in the human body, such as aging and diabetes. The formation of advanced glycation end products (AEGs) is an intricate molecular process with straightforward as well as more complex reactions. As mentioned above, this cascade starts with the reaction of the electrophilic carbonyl group of reactive sugars with free amino groups of amino acids forming an instable Schiff base which leads, *via* an Amadori rearrangement, to the stable ketosamine (Amadori rearrangement product). Both compounds can undergo several reactions forming a number of AGEs. The first discovered glycated protein was glycated hemoglobin in diabetes and to date many other AGEs have been detected.[133]

Carboxymethyl-lysine (CML) is the most prevalent AGE in vivo,[134] a non-fluorescent protein adduct which is formed *via* oxidative degradation of the Amadori product or directly *via* the addition of glyoxal to lysine (Figure 25) and is one of the major epitope of the commonly used polyclonal anit-AGE antibodies.[135] The oxidative degradation or auto-oxidation of Amadori products leads to the formation of dicarbonyl compounds, such as 3-deoxyglucosome, methylglyoxal and glyoxal, which are very reactive compounds leading to protein crosslinks.[136]

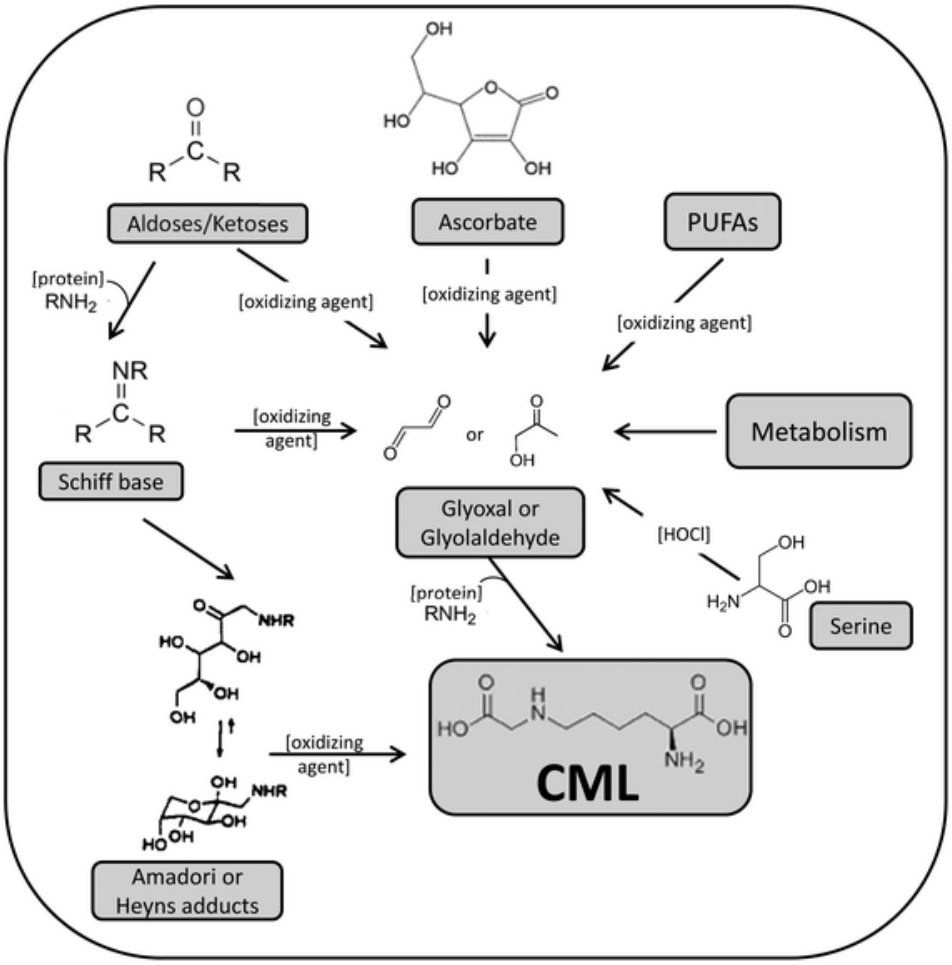


Figure 25: Proposed routes for CML formation as described by Thorpe & Baynes.[137]

Glycation may occur both inside the cell (endogenous glycation) and outside the cell (exogenous glycation). AGEs which are ingested exogenously are produced during cooking or grilling when carbohydrates are heated with proteins or fat forming compounds, such as acryl amide which are evidentially carcinogenic.[138] The formation of endogenous AGEs is increased in diabetes, however AGEs can also be generated in normal metabolic processes of organisms at lower rates.[133]

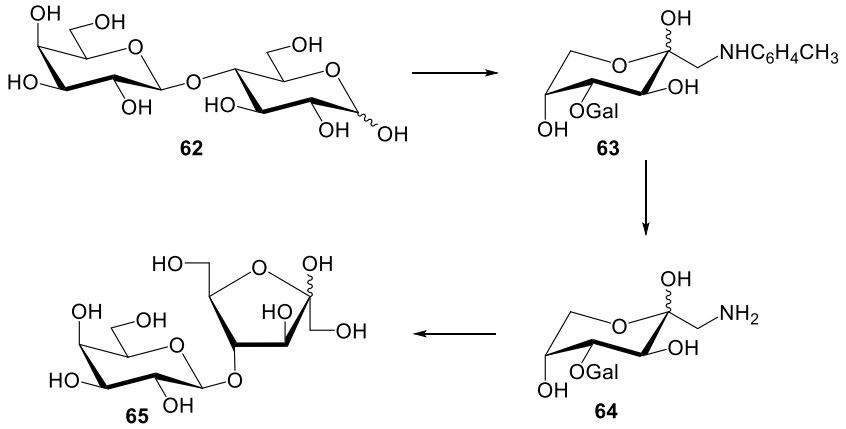
Furthermore, AGEs also play an important role in the pathogenesis of Alzheimer´s disease (AD) which is characterized pathologically by the presence of neurofibrially tangles (NFTs) and senile plaques (SPs). NFTs and SPs are the major constituents of amyloid β protein and tau protein, respectively. AGEs can be identified immunohistochemically in both SPs and NFTs in AD patients[139] and can cause oxidative stress, extensive protein crosslinking (β-amyloid and MAP-τ) as well as neuronal cell death, which are neuropathological and biochemical features of AD.[140] It was shown that AGEs regulate the amyloid β peptide aggregation and amyloid

accumulation,[141] but the role of AGEs in the development of Alzheimer´s disease is still unclear.[142]

1.4.3. Applications of the Amadori rearrangement

In the following selected examples are excerpted from the literature.

One of the first applications of the Amadori rearrangement has been the synthesis of lactulose (**65**) in 1959 (Scheme 22).[143] In this route, lactose (**62**) was treated with *p*-toluidine in the presence of pyridine and acetic acid generating rearrangement product **63**. After catalytic hydrogenolysis**,** the corresponding 1-amino-1-deoxyketose (**64**) was obtained which gave after deamination the desired lactulose (**65**). This synthetic route was the first alternative to the initial approach, in which lactulose was synthesized *via* a Lobry de Bruyn – Alberda van Ekenstein rearrangement.[144] Lactulose is a non-absorbable sugar and acts as a laxative or is used in the treatment of hepatic encephalopathy, a decline in brain function that occurs as a result of liver disease.

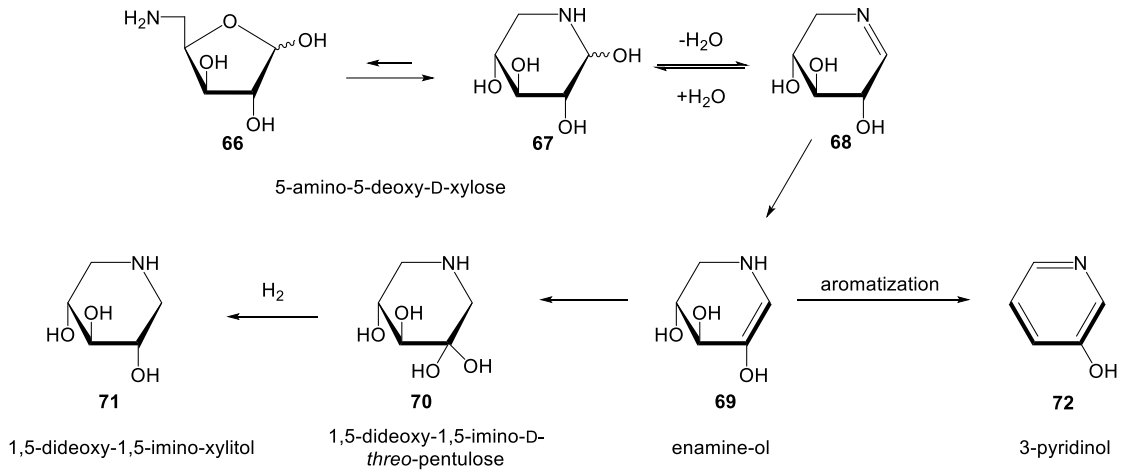


Scheme 22: Synthesis of lactulose via the Amadori rearrangement.[93]

A few years later in 1969, Grünnagel and Haas[145] applied the same strategy for the synthesis of D-tagatose from D-galactose *via* the Amadori rearrangement product 1-deoxy-1-dibenzylamino-D-tagatose.

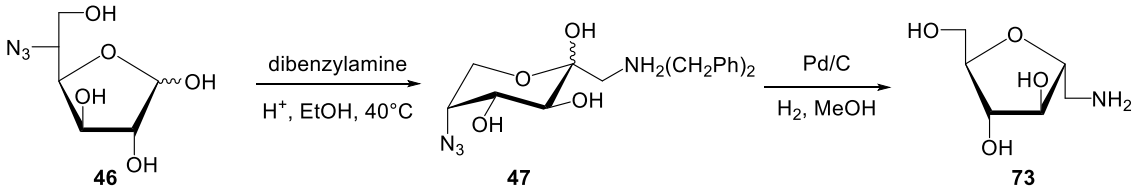
Paulsen showed in the late 60s that suitable amino sugars can undergo an intramolecular Amadori rearrangement leading to iminosugars. 5-Amino-5-deoxy-D-xylose (**66**) gave under appropriate reaction conditions (0 °C and moderate acidic conditions), 1,5-dideoxy-1,5-imino-D-*threo*-pentulose (**70**), which is a direct precursor of 1,5-dideoxy-1,5-imino-xylitol (**71**) (Scheme 23). Depending on the reaction conditions in terms of temperature and the nature of catalyst,

enamine-ol (**69**) undergoes an aromatization leading to 3-pyrinidol (**72**). The driving force for the formation of the Amadori rearrangement product is the release of strain by ring enlargement and the all-equatorial orientation of the substituents in the product after this rearrangement. This reaction leads to the access of an important class of sugar analogues exhibiting nitrogen instead of oxygen in the ring, so called iminosugars.[146]



Scheme 23: Synthesis of 1,5-dideoxy-1,5-imino-xylitol.[146]

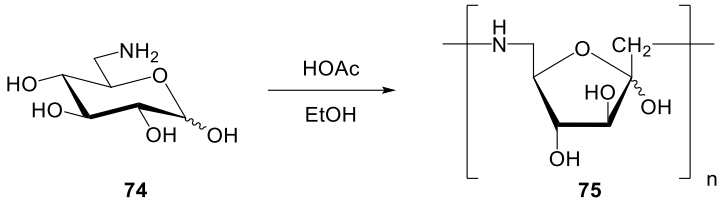
Furthermore, the Amadori rearrangement was investigated as a simple, efficient and versatile method for the synthesis of 1-amino-1-deoxy derivatives of 2,5-didesoxy-2,5-imino-D-mannitol, which is a powerful β-glucosidase and invertase inhibitor.[147] Starting from 5-amino-5-deoxy-D-glucofuranose (**46**), the corresponding 1-amino-1-deoxy-fructose derivative (**47**) was obtained in extraordinarily high yields of more than 95%. To furnish the corresponding C-1 amino modified inhibitors, such as 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol (**73**), compound **47** was cyclized by an intramolecular reductive amination (Scheme 24). These compounds have been found to possess high and powerful inhibitory activity against β-glucosidases.[112, 148]



Scheme 24: Synthesis of 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol.[112]

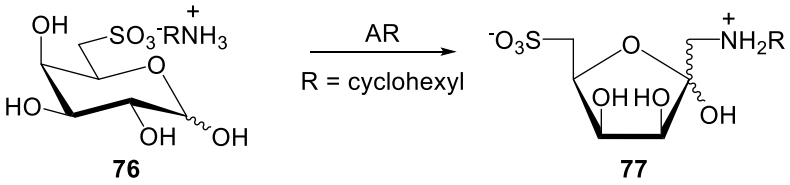
Aminopolysaccharides are of great interest because of their unique structures and their properties which differ from normal polysaccharides, such as cellulose. Therefore, the synthesis of such polymers is an important prerequisite to clarify the biological functions of naturally

occurring aminopolysaccharides. In Scheme 25, the synthesis of aminopolysaccharide (**75**) is depicted which takes place by terminal polymerization of 6-amino-6-deoxy-D-glucose (**74**) *via* the Amadori rearrangement having aminoketose-structure in the main chain.[149]



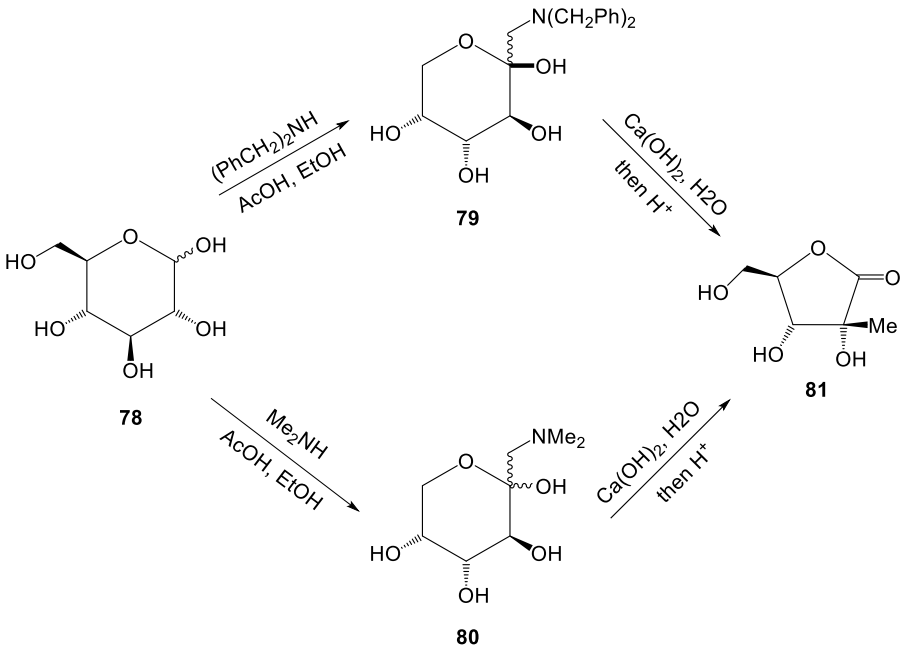
Scheme 25: Synthesis of aminopolysaccharides via the Amadori rearrangement.[149]

Fernandes-Bolanos *et. al.* observed during their studies in the synthesis of *C*-sulfosugars and *C*-sulfoalditols a spontaneous Amadori rearrangement of the cyclohexylammonium salt of sulfofucose (**76**) which leads to 6-deoxy-6-sulfo-D-tagatosamine (**77**) (Scheme 26).[150]



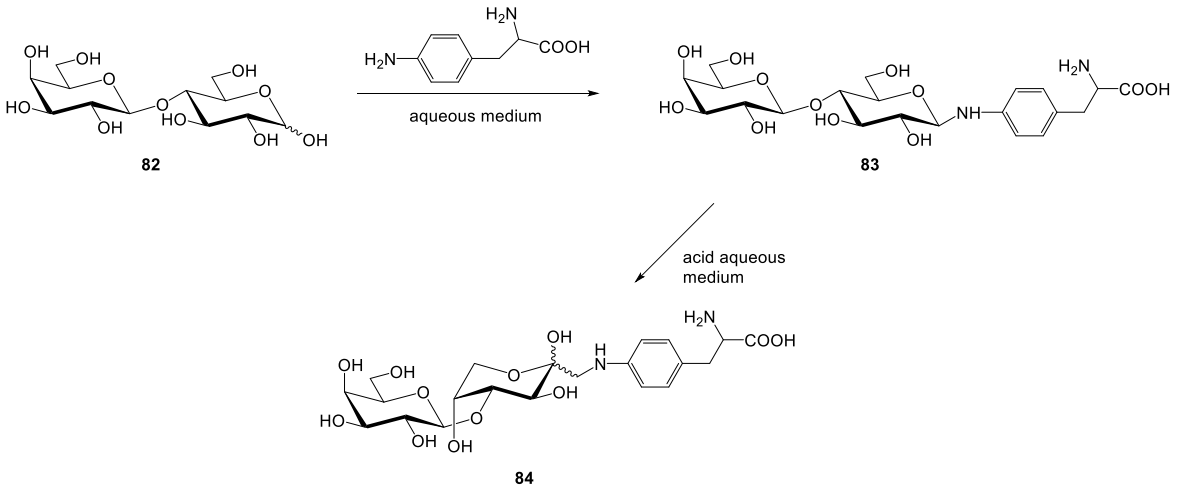
Scheme 26: Synthesis of 6-deoxy-6-sulfo-D-tagatosamine via the Amadoir rearrangement.[150]

The reaction of D-glucose with aqueous base leading to saccharinic acid is one of the oldest reactions, but one of the sophisticated reactions known due to the predominant formation of various side-products. Therefore, Fleet *et al.* [151] reported two alternative approaches for the synthesis of saccharinic acids. On of this strategy was the reaction of D-glucose with dibenzylamine or dimethylamine leading to the corresponding Amadori ketoses (**79** and **80**) which were treated with calcium hydroxide to generate saccharinic acid (Scheme 27).



Scheme 27: Synthesis of saccharinic acid by the treatment of Amadori ketoses with calcium hydroxide.[151]

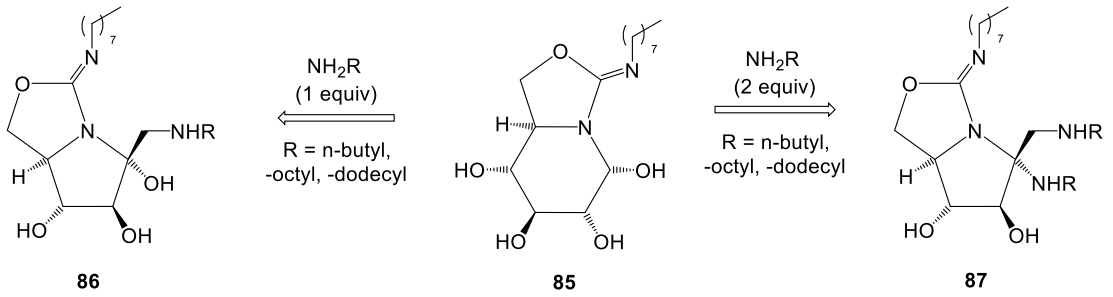
In order to investigate the multi-facetted biological function of *O*- as well as *N*-linked glycoconjugates and to understand the mechanism of cellular processes in which these glycoconjugates are involved, the synthesis of *C*-glycosides as chemically and metabolically stable carbohydrate analogues became of high interest. In particular, *C*-glycosyl amino acids were investigated as a novel tool to study such carbohydrate-based interactions. As their synthesis is usually very complicated due to the sophisticated protection and deprotection steps of the amino acids as well as the sugar moiety, Bridiau *et al.*[152] reported a facile synthesis of pseudo *C*-glycosyl amino acids employing the Amadori rearrangement. Therefore, unprotected D-lactose (**82**) was treated with an unprotected tyrosine analogue *p*-amino-DL-phenylalanine in aqueous solution (Scheme 28) to form in the first step the *N*-glycosylamine (**83**) which was stabilized in the second step by the formation of the Amadori product *N*-[β-D-galactosyl-1-4-(1-deoxyfructos-1-yl)]-*p*-amino-DL-phenylalanine (**84**).



Scheme 28: Synthesis of pseudo *C*-glycosyl amino acids employing the Amadori rearrangement.

In case of selected aldoheptoses, which are for example readily available by means of the Kiliani-Fischer C-elongation reaction of the corresponding aldohexoses, glycoconjugates with D-*gluco*, D-*manno*, D-*galacto* as well as GlcNAc motifs can be synthesized.[113, 153]

Mellet *et al.*[154] reported in 2014 the synthesis of iminosugar-type glycomimetics *via* the unprecedented Amadori rearrangement of *gem*-diamines as a protecting group-free method to obtain polyantennated pyrrolizidine glycomimetics (Scheme 29). With this strategy, two new types of sp^2-iminosugar structures, reducing pseudo-*C*-nucleosides (**86**) and dual-pseudo-*C*-nucleosides (**87**), can be synthesized by changing the reagents ratio of the applied amine. The resulting compounds act as selective competitive inhibitors of β-glucosidase.



Scheme 29: Synthesis of polyantennated pyrrolizidine glycomimetics via the Amadori rearragment of *gem*-diamines.[154]

1.5. Lectins

One class of carbohydrate-specific proteins are lectins, which are proteins that preferably recognize and bind the carbohydrate complex protruding from glycolipids and glycoproteins.[155] The name lectin is derived from the Latin *legere*, to select or to choose. Lectins specifically and reversibly bind monosaccharides, oligosaccharides as well as partial structures of saccharides but do not cause any antigenic stimulation within the immune system and are devoid of catalytic activity. These carbohydrate-specific proteins are omnipresent and are found in animals, plants and microorganism. The ability of lectins to bind carbohydrates, based on their individual amino sequence, is mediated by the carbohydrate recognition domain (CRD).[156]

Generally, lectins are classified into five groups[157] based on their affinity to bind with

- Glucose/mannose
- Galactose/*N*-acetyl-D-galactosamine
- *N*-Acetylglucosamine
- L-Fucose
- Sialic acids

The binding of lectins with simple or complex carbohydrate conjugates is non-covalent and reversible. Furthermore, binding can occur freely in solution or on the cell surface, which contains glycoconjugates acting as lectin receptors. Hemagglutionation, the major property of lectins and therefore often used for their characterization and detection, is the agglutination of the highly glycosylated red blood cells, erythrocytes, with lectins. Agglutinates, cell precipitates, are formed based on the ability of lectins to cross-link cells by combining with carbohydrate moieties on two or more cell surfaces. This phenomenon can take place because many lectins are di- or oligovalent concerning the number of CRDs. The carbohydrate-specificity of lectins based on their capacity to inhibit hemagglutination is determined by the so called “Hapten inhibition test”. Furthermore, lectins are often integral membrane proteins where the CRD is oriented to the cell exterior or to the luminal spaces of organelles. They differ in their size, structure, molecular organization and particularly in the carbohydrate recognition domain.[5, 158]

1.5.1. Plant lectins

Legume lectins, such as concanavalin A from jack bean (*Canavalia ensiformis*), are very useful model systems for the study of recognition processes between proteins and carbohydrates, as they exhibit a wide range of carbohydrate specifities and are easy to purify in large quantities.[159]

Concanavalin (Con A) is one of the best known and most investigated plant lectins, because of its various biological properties and it was the first legume lectin recognized as a Glc/Man-specific lectin. Con A exhibit a dome shaped monomer which is built up from two β-sheets of seven (front sheet) and six (back sheet) antiparallel strands of β-sheet. These β-sheets are interconnected by turns and loops. The so-called S-sheet, a small β-sheet of five short strands of β-sheet, enables the connection of the front sheet with the back sheet.[160] At the upper part of the dome four loops are located which form the monosaccharide-binding site of the lectin monomer. Each subunit has one ligand binding-site which enables the binding of mannose- or glucose-containing carbohydrate ligands. However investigations showed that mannose has a higher affinity.[161] Depending on the pH value, Con A associate into a dimer or a tetramer and is therefore a divalent or tetravalent lectin. Each monomer consists of 237 residues, one saccharide-binding site and a transition metal and calcium binding site (Figure 26).[160]

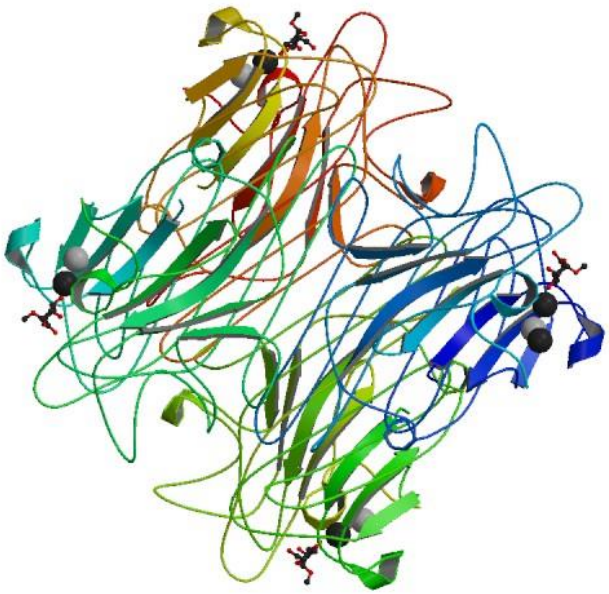


Figure 26: Structure of concanavalin A complexed with methyl α-D-mannopyranoside.[162]

Con A was the first lectin for which the primary sequence[163] and 3D structure[164] was estabilished, the latter by X-ray crystallography. The elaborate arrangement of extended beta

strands into two sheets, first observed in this structure, became known as the jelly roll or lectin fold.[165]

Many plant lectins display a significant activity against human immunodeficient virus (HIV) and other viruses with an envelope. This is the reason of the high interest of lectins as attractive targets for the development as novel antiviral drugs.[166]

The mannose-specific *Galanthus nivalis* agglutinin (GNA) or also called snowdrop bulb lectin was the first monocot mannose-binding lectin isolated. The original GNA is a homotetramer which is built up with four non-covalent associated monomers. Each monomer consists of a single polypeptide of 109 amino acid residues and is formed by three subdomains, which contains three or four strands of antiparallel β-sheet and the subdomains are connected by loops (Figure 27). GNA is specified towards Manα(1-3)Man containing oligosaccharides.[160]

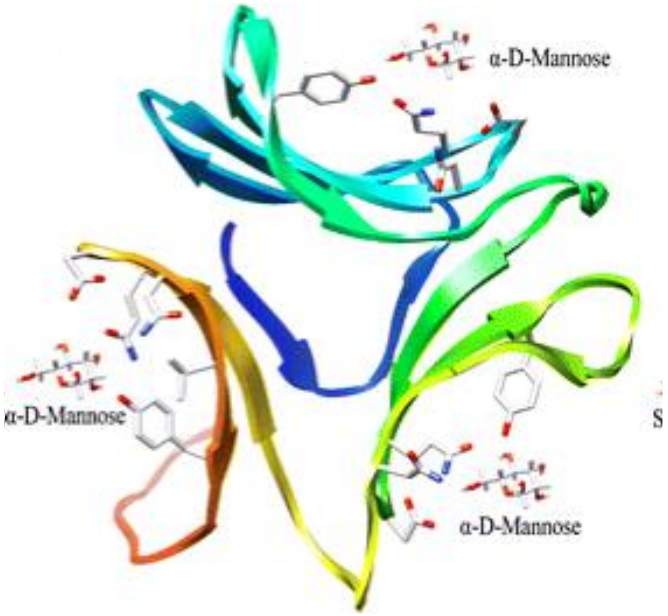


Figure 27: The three dimensional structure of GNA: Three binding sites are all active to bind α-D-mannose.[167]

GNA-related lectins show various biological activities, such as anti-tumor properties as well as anti-HIV and anti-HSV actions.[166]

Like GNA, the plant lectin from Hippeastrum hydrid (Amaryllis), the so-called HHA lectin, is a mannose-specified lectin and recognize especially linear (1-6)-linked α-mannosyl residues.[168] Caccia *et al*. demonstrated the interaction of lectin HHA with the brush border of midgut cells. The FITC-labeled lectin HHA binds to the cell membrane of columnar cells and is also internalized into columnar cells, which suggest that this lectin is able to cross the gut epithelial barrier and pass into the insect hemolymph.[168-169]

1.5.2. Bacterial lectins

Besides lectins found in plants, animal lectins also differ in their structure and in their function, albeit classified based on the structural similarities and sequence alignment of their CRDs. Animal lectins can be classified into two groups, lectins which typically contain an evolutionarily conserved carbohydrate recognition domain and sulphated glycosaminoglycan-binding proteins, which have evolved by convergen evolution.[5] Some examples of the subgroups of animal lectins are listed in Table 2.

Table 2: Animal lectin family.[5]

Lectin family	Typical sugar ligands	Examples of functions
Calnexin	Glc_1Man_9	Protein sorting in the endoplasmic reticulum
M-type	Man_8	ER-associates degradation of glycoproteins
L-type	Various	Protein sorting in the endoplasmic reticulum
P-type	Man 6-phosphate, others	Protein sorting, post-Golgi, glycoprotein trafficking, ER-associated degradation of glycoproteins, enzyme targeting
C-type	Various	Cell adhesion (selectins), glycoprotein clearance, innate immunity (collectins)
Galectins	β-galactosides	Glycan crosslinking in the extracellular matrix
I-type, siglecs	Sialic acid	Cell adhesion
R-type	various	Enzyme targeting, glycoprotein hormone turnover
F-box	$GlcNAc_2$	Degradation of misfolded glycoproteins
F-type	Fuc-terminating oligosaccharides	Innate immunity
Intelectins	Gal, galactofuranose, pentoses	Innate immunity, fertilization and embryogenesis

C-Type lectins are one of the most common animal lectin binding sugars in a Ca^{2+}-dependent manner which can be further classified into three families: selectins, collectins and endocytic lectins. Most of them are large and asymmetric in their structure, have one or more CRDs and are found in secreted or bound form.[1] Three different representatives of selectins are known, the L-, E- and P-types which mediate the selective adhesion of circulating leukocytes to endothelial cells of blood vessels, a process which removes leukocytes from the circulation and transporting them into the tissues. Endocytic lectins which exhibit different carbohydrate

specificities are membrane-bound proteins, like mannose-binding proteins expressed on macrophages. They play a significant role in innate immunity which describes the mantling and destruction of infectious organism after receptor binding with no dependance on antibodies. Mannose-binding proteins (MBPs) are another relevant group of endocytic lectins which bind to oligomannoside on the surface of infectious microorganism causing activation of complement and subsequent lysis of the pathogens. Only the terminal mannose residue of an oligosaccharide interacts with the CRD of MBP, which is a very weak binding event with a dissociation constant in millimolar range. Because of the structural occurrence of MBP as a trimer, they allow the binding of several carbohydrate ligands to the lectin concerning appropriate distance of the ligands.[5]

In some cases, lectins are very specific in terms of their interaction with carbohydrate moieties. They can bind only to one kind of monosaccharides, for example they can distinguish between glucose and galactose or between GalNAc and GlcNAc.[5]

Early in the history of lectins, it was established that besides plants, organisms also express this class of proteins. Many bacteria, especially from the *Enterobacteriaceae* family, possess the ability to agglutinate erythrocytes. This haemagglutination activity of bacteria is always associated with the presence of multiple filamentous protein appendages projecting from the surface of the bacteria.[170] These are called fimbriae (from the Latin word for "thread") or pili (from the Latin word for "hair") (Figure 28).

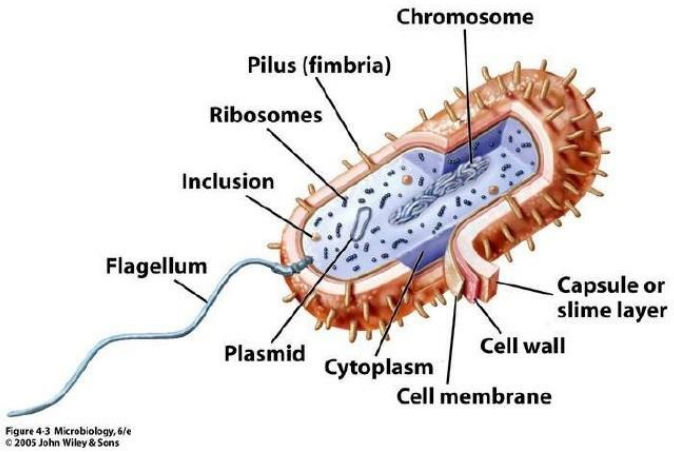


Figure 28: A majority of bacterial cells, such as *E. coli*, are equipped with three types of hair-like protein appendages, named pili, fimbriae and flagella.[171]

Fimbriae are adhesive organelles, containing lectin subunits, which mediate carbohydrate-spezific adhesion to the cell surface as well as cell agglutination. Thus, bacteria utilize the sugar decoration of cells – the glycocalyx – to colonize the cell surface, wherever cells are in contact with the outside environment, as for example in the case of epithelial cells.[172] Fimbriae can be identified and classified according to their carbohydrate specificity. In *Escherichia coli*, four types of fimbriae are found, type 1 fimbriae (specific for α-mannosyl residues), P fimbriae (specific for Galα1,4Gal), S fimbriae (specific for Neu5Acα2,3Gal) and type G fimbriae (specific for GalNAc). Nevertheless, type 1 fimbriae are the best characterized pili because it is the most common surface structure of both pathogenic and non-pathogenic Gram-negative bacteria. Type 1 fimbriae are mediated by a lectin called FimH, which forms from a minor component of the fimbrial protein complex.[173]

Hemagglutination mediated by type 1 fimbriae can be inhibited by mannose, mannan as well as exclusively by α-anomeric mannosides such as methyl α-D-mannoside (MeMan).[170] β-Mannosides cannot be complexed within the carbohydrate binding site, because the position, configuration and orientation of the D-mannose are unambiguously defined by the electron density.[174] The carbohydrate recognition domain of FimH is accommodated by the receptor-binding lectin domain of the protein, named $FimH_L$. This *N*-terminal FimH*L* domain selects out the α-configuration of D-mannose.[173] Further studies with more complex oligosaccharides confirmed the strict specificity of the type 1 fimbrial lectin for α-mannosides.[172] Type 1 fimbriae are very efficient adhesion tools of bacteria mediating the colonization of various biotic and abiotic surfaces.[175] They are presented in 90% of all known uropathogenic strains of *E. coli* (UPEC), known to be the main cause of urinary tract infections of humans and are important virulence and pathogenicity factors.[176]

In X-ray diffraction studies with lectins, it was observed that a carbohydrate ligand is complexed due to a well-defined array of hydrogen bonds. These hydrogen bonds can derive from hydrogen-bond donor and acceptor groups of the ligands and from diverse functional groups of the various amine residues, which are existent in the lectin CRD. Furthermore, water molecules can mediate these hydrogen bonds, occasionally also divalent metal ions such as Ca^{2+} or Mn^{2+} are involved in the carbohydrate binding.[172]

Type 1 fimbriae are distributed evenly on the bacteria between 100 and 400 per cell and their length varies between 0.1 to 2 micrometers, their width is approx. 7 nanometer. These organelles are composite structures made up of a right-handed helical rod consisting of repeating FimA subunits which are joined to distal tip fibrillum containing two adaptor proteins,

FimG and FimF, and the adhesin, FimH, which is responsible for receptor binding (Figure 29).[61] Assembly of *E. coli* type 1 pili need the conserved chaperone/usher pathway, like assembly of over 25 other adhesive organelles in Gram-negative bacteria. In this chaperone/usher pathway, a periplasmic chaperone controls the folding of pilus subunits and an outer membrane usher, Fim D, provides a platform for pilus assembly and secretion. In the outer side of the membrane, an oligomeric channel is formed by the usher mediating the subunit translocation across the outer membrane.[173] Moreover, they are built up by a "donor strand exchange" (DSE) process, in which the immunoglobin (Ig) fold of every subunit is completed by an amino-terminal extension from the following subunit.[172, 175]

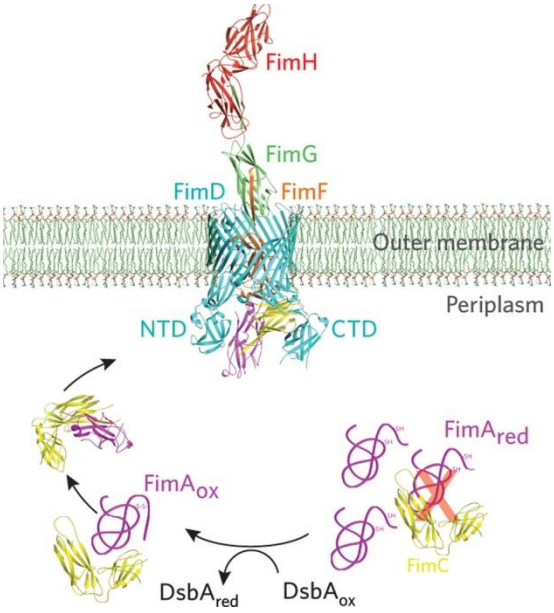


Figure 29: Model of type 1 pilus formation, including folding of FimA and its incorporation into the pilus fiber at the FimD usher.[177]

FimH mediates the bacterial adhesion to the surface of urothelial cells by binding to oligomannoside residues of the glycoprotein uroplakin Ia, which is prerequisite for bacterial invasion.[178]

FimH comprise two domains, a C-terminal pili domain, $FimH_P$, which anchors the protein at the fimbrial tip and a N-terminal lectin domain, $FimH_L$, which accommodates the α-D-mannoside-specific carbohydrate-binding site. In the crystal structure of FimH, only one CRD is seen located at the tip of the lectin domain which is able to bind one α-mannoside (Figure 30).[172-173, 179]

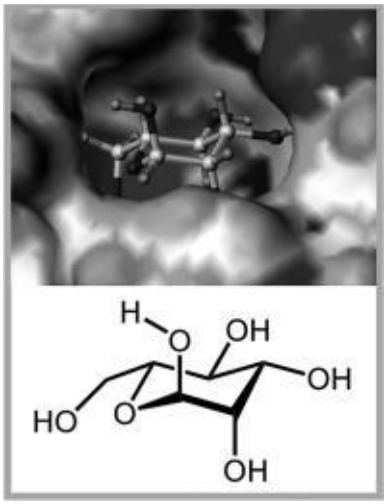


Figure 30: Complexation of mannosidis ligand within the CRD of FimH. The aglyon points toward the entrance of the binding pocket, which allows terminal mannose residues on complex oligosaccharides to be complexed by FimH.[172]

However, the exact structure has been unknown for a long time due to the inability to crystallizes the FimH exclusively, as it is missing a strand to complete the Ig fold of its pilus domain. In 2002, the crystal structure of the FimC/FimH chaperon-adhesion complex binding to its physiologically relevant ligand α-D-mannose was published and the amino acids that are of concern for mannose complexation were identified in detail.[174] It was concluded, that mannose is located in a deep and negatively charged pocket at the tip of $FimH_L$, the receptor-binding lectin domain. In the binding site, the mannose ring interacts with the side chains of the amino acids in ten direct hydrogen bonds and indirect water-mediated hydrogen bonds are formed. Excluding the hydroxyl group of the anomeric position, all of the hydroxyl groups of the sugar ring interact with the CDR of FimH, in particular with residues Phe1, Asn46, Asp47, Asp54, Gln133, Asn135, Asp140 and Phe142 (Figure 31).[173]

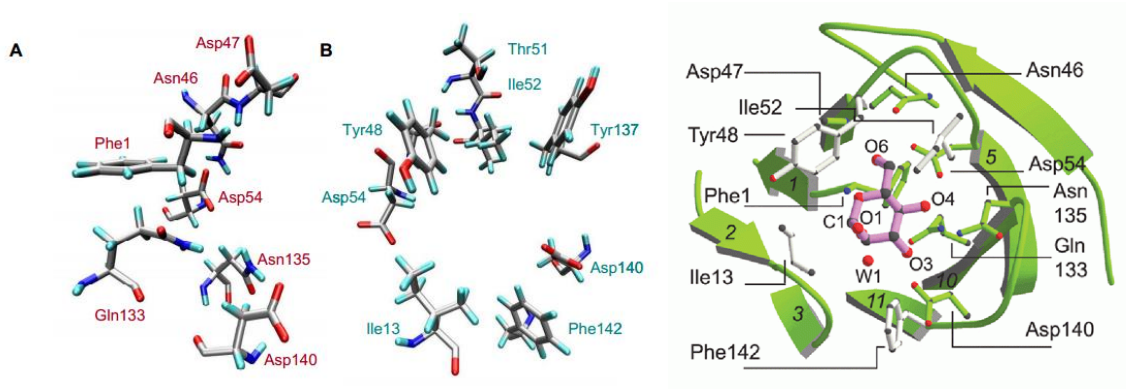


Figure 31: A) Amino acid residues lining the binding sites interior and responsible for complexation of the glycon part of a mannosidic ligand. B) Amino acid residues of the hydrophobic ridge at the entrance of the carbohydrate binding site, including the tyrosine gate formed by the side chains of residues Tyr48 and Tyr137. C) Amino acids residues interacting with mannose (pink) in the binding pocket of FimH (green).[173] [180]

The mannose-binding pocket is surrounded by a hydrophobic ridge comprised of Ile13, Tyr48, Ile52, Tyr137 and Phe142. The side chains of Tyr48 and Tyr137 are positioned such that they form a gate-like structure that has been named the "tyrosine gate".[172, 175] In this way, mannoside ligands with an aromatic aglycon, like *p*-nitrophenyl-α-D-mannopyranoside (*p*NPMan), can additionally interact with this tyrosine gate *via* π-π stacking to increase the binding.[172]

In 2005, by means of another crystallographic study, two FimH proteins named $FimH_{tr1}$ and $FimH_{tr2}$ from two different bacterial strains were identified, where butyl α-D-mannoside was bound in the CRD. In case of $FimH_{tr2}$, the Tyr48 and Tyr137 side chains were found in an almost parallel orientation as in the earlier FimC/FimH structures. On the other hand, in the $FimH_{tr1}$ structure, the Tyr48 is packed edge-to-face with Tyr137 in contrast to the parallel orientation in $FimH_{tr2}$. [174]

Moreover, in 2008 it was possible to crystallize $FimH_t$ in complexation with the natural ligand oligosaccharide-3, where the α-1,3-linked mannose residue is complexed within the CRD and the Manβ1,4GlcNAcβ1,4GlcNAc portion of "oligomannose-3" interacts with an extended region of the binding site.[172]

Since FimH-mediated adhesion to the glycosylated surface of host cells is a key step in infections caused by type 1-fimbriated bacteria, the investigation of FimH antagonists that inhibit bacterial adhesion is a focus of several studies because it can be valuable for the treatment of infectious diseases,[181] especially in the context of an anti-adhesion therapy against bacterial infections.[182]

Three classes of FimH ligands have been identified to exhibit a relatively high affinity, namely long-chain alkyl mannosides, mannosides with various substituted aromatic aglycon moieties and mannosides with extended aglycon moieties.

As mentioned above, butyl α-D-mannoside (**88**) was found to complex within the CRD and exhibited a 15 fold higher inhibitory potency than the standard inhibitor methyl α-D-mannoside (MeMan). The butyl residue interacts with the phenyl rings of Tyr48 and Tyr137 at the entrance of the binding pocket *via* van der Waals contacts. This hydrophobic interaction with the tyrosine gate increases with the length of the aglycon alkyl chain. For example, heptyl α-D-mannsoide (**89**) exhibits a 440 fold greater power as inhibitor of type 1-fimbriae-mediated bacterial adhesion than the MeMan (Figure 32). RI is the relative inhibitory potency, based on MeMan with $IP_{MeMan} \equiv 1$.

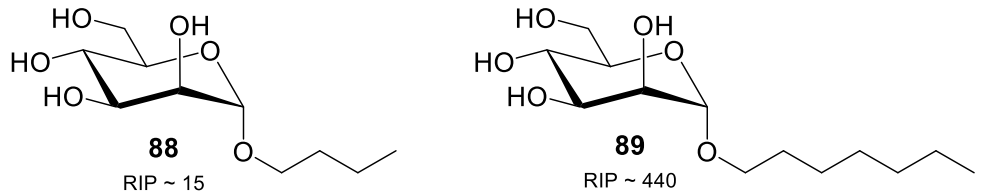


Figure 32: Alkyl mannosides: butyl α-D-mannoside (**88**) and heptyl α-D-mannoside (**89**).

It was shown that aromatic aglycon moieties enhanced the affinity of the mannoside ligand for FimH by a factor of 600 and more,[183] because of the π-π interaction of the aromatic aglycon with the amino acids side chains of Tyr48 and Tyr137 located in the tyrosine gate. Different variations of the aromatic aglycon of mannoside ligand have been synthesized. While variations of the *p*-substitution of phenyl mannoside had no influence on the affinity for FimH, the *o*-substitution showed an increased affinity. For example, a chlorine substituent in the *o*-position on the phenyl ring (*p*-nitro-*o*-chlorophenyl α-mannoside **90**, Figure 33) enhanced the inhibitory potency by a factor of 720.[183] Using fused ring systems as aglycon moiety, like in methylumbelliferyl mannoside (**91**), Figure 33, the π-π interaction between the phenyl rings and the tyrosine gate is very strong, thus the affinity was improved 116 times based on MeMan. [184]

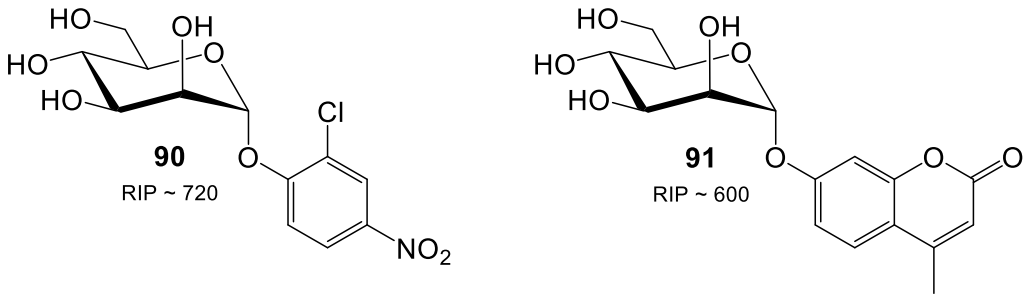


Figure 33: Mannoside with substituted aromatic aglycon moiety: *p*-nitro-*o*-chlorophenyl α-mannoside (**90**); mannoside with extended aglycon moiety: methylumbelliferyl mannoside (**91**).

In Figure 34, five potent representatives of small molecule FimH antagonists are depicted. Extending *p*-nitrophenyl α-mannoside by a squaric acid moiety (**92**), the resulting ligand showed a very high inhibitory potency. When a chloro substituent is introduced in the *o*-position (**93**), the resulted RIP value (relative inhibitory potency) of 223 was determined by ELISA.[185] The photosensitive ligand **94** is beneficial as a ligand for FimH because of its water-solubility.[186] Mannoside **95** is the first reported orally available FimH antagonist that is hydrolyzed to acid **96**.[187] Indolinylphenyl mannoside **97** exhibits at an IC_{50} of 2.4 nM.[181b] The IC_{50} value of an inhibitor is the concentration at which 50% of bacterial adhesion is prevented.

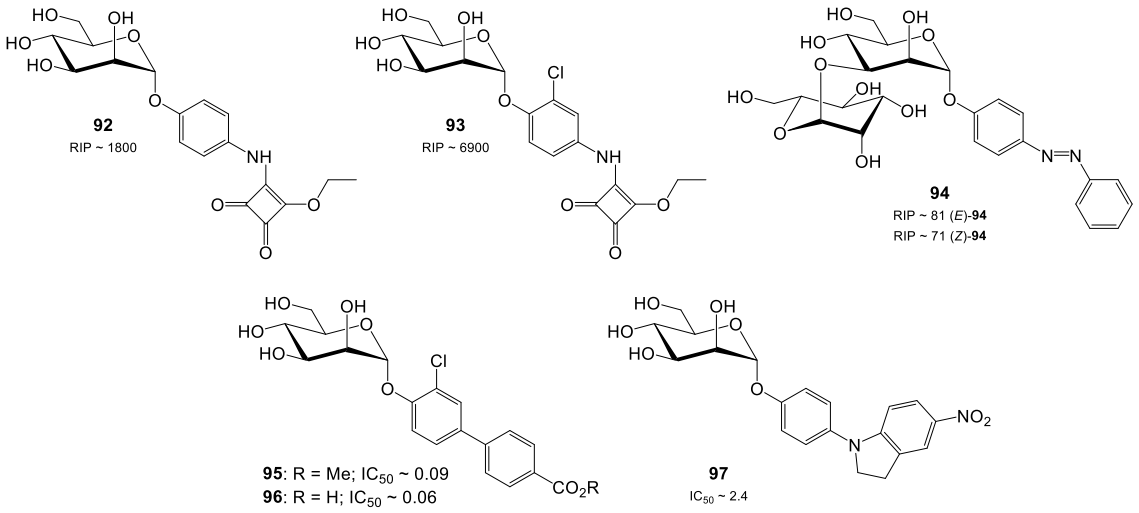


Figure 34: Structures of five potent representatives of small molecule FimH antagonists.

In addition, multivalent glycomimetics were investigated as inhibitors of type 1 fimbriae-mediated bacterial adhesion. Due to the multivalency effects which have been observed in other carbohydrate-lectin interactions, it was anticipated that multivalent or clustered mannosides would be a promising approach to high-affinity inhibitors of mannose-specific bacterial adhesion.[172] Tri-and tetravalent cluster mannosides are found to be potent inhibitors of type 1 fimbriae-mediated bacterial adhesion and additionally antagonists of FimH. Using an ethylenegylcol spacer , the obtained trivalent cluster **98** and **99** show a high RIP of 1063 (Figure 35).[172, 188]

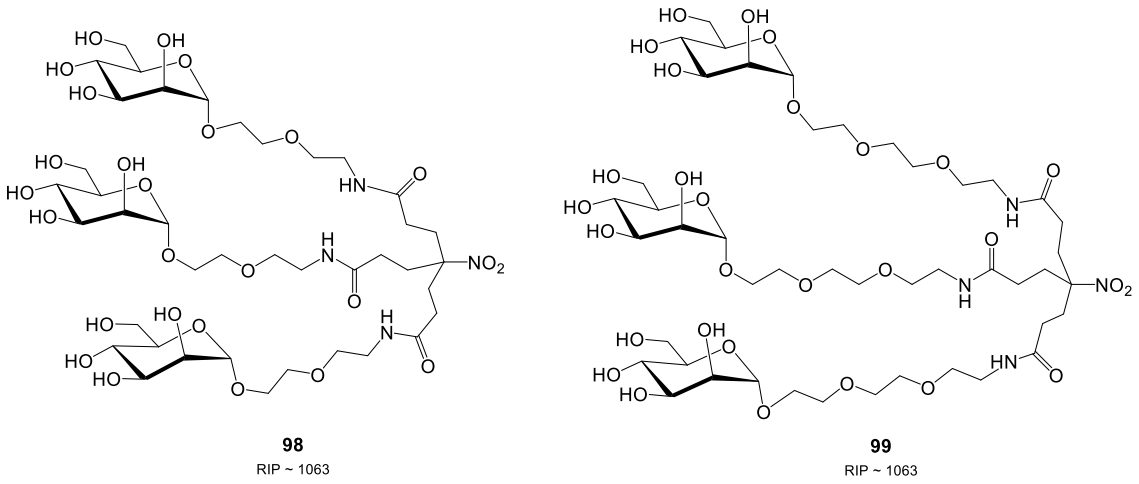


Figure 35: Trivalent cluster **98** and **99**.

Furthermore, by employing chemistry such as squaric acid conjugation,[189] "click"-chemistry[190] and Sonogashira coupling[191], squaric acid linked mannoside (**100**), triazol-linked multivalent mannoside (**101**) and alkyne-multivalent cluster (**102**) were synthesized, respectively. The

triazole-linked cluster **101**,[192] when tested in a hemagglutination inhibition assay (HAI), showed a relative inhibitory potency of 2670 based on D-mannose with $IP_{Man} \equiv 1$ (Figure 36).

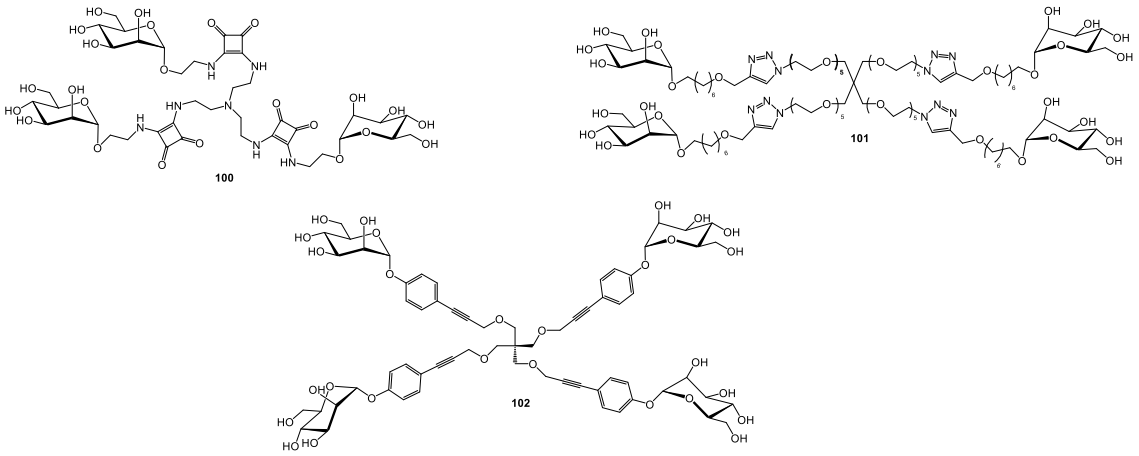


Figure 36: Cluster mannoside: squaric acid linked mannoside (**100**, RIP ~ 8, based on MeMan wit $IP_{MeMan} \equiv 1$), triazol-linked multivalent mannoside (**101**, RIP ~ 2670, based on D-mannose with $IP_{Man} \equiv 1$) and alkyne-multivalent cluster (**102**, RIP ~ 4489, based on MeMan with $IP_{MeMan} \equiv 1$).

Burkholderia cenocepacia, a Gram-negative bacterium, is known as an opportunistic human pathogen which provokes lung infections in immunocompromised individuals. This bacterium produces three soluble lectins, BC2L-A, BC2L-B and BC2L-C, which are important virulence factors of *B. cenocepacia*. As the bacterium is able to form a biofilm, the corresponding lectins play a significant role in biofilm formation as well as in host cell recognition and adhesion.[193] BC2L-A is the smallest *B. cenocepacia* lectin and is a mannose-specific lectin. This lectin exhibits a nine-stranded antiparallel β-sandwich which is built up of two sheets of four or five strands. It is a dimeric lectin and each monomer is associated with two Ca^{2+} ions and one mannose residue (Figure 37).

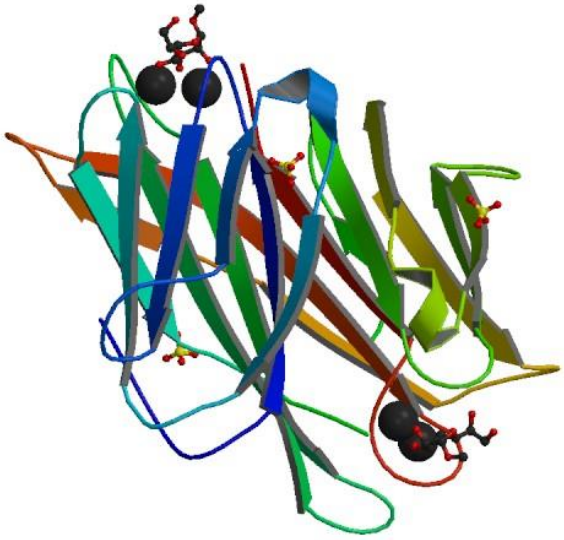


Figure 37: Crystal sturcute of BC2L-A lectin from Burkolderia cenocepacia in complex with methyl-heptoside.[194]

Methyl α-D-mannosides as well as α-(1-2), α-(1-3) and α-(1-6) linked mannosides are investigated as high-affinity ligands for BC2L-A, however, this lectin can also bind directly to bacterial lipopolysaccharides (LPSs) on the outer membrane. In this case, the lectin shows an affinity to L-*glycero*-D-*manno*-heptoses, a common compound of the core region of LPS form Gram-negative bacteria.[193] However, it has been proposed that interactions between plant lectins and LPS play an important role in the establishment of symbioses between legume plants and nitrogen-fixing bacteria. Furthermore, heptose mediates the interaction of human lectins of the innate immune system, such as surfactant protein D or mannan –binding protein with pathogenic bacteria. Machetti *et al.*[194b] investigated the particular binding of the bacterial lectin BC2L-A to LPS heptose, which can be crucial in bacterial social life, such as exist from dormancy, formation of microcolony or any other processes in which the detection of specific bacterial strain is required.

1.5.3. Human recombinant C-type lectins

Langerin, a C-type lectin which is expressed in humans only by Langerhans cells (LCs), is thought to play a role in pathogen recognition in terms of facilitating pathogen uptake and processing for antigen presentation.[195] Langerin is built up by an extracellular domain, a transmembrane region and a cytoplasmic trail which contains a proline-rich signaling domain.[196] This lectin belongs to the Ca^{2+}-dependent lectin family exhibiting a single carbohydrate recognition domain.[197] Langerin induces the formation on Birbeck granules which are subdomains of the

endoplasmal recycling compartment and Langerhans cells-specific intracellular organelles.[195] The extracellular domain of Langerin consists of a neck region, which is known to mediate trimer formation of Langerin (Figure 38A),[198] and contains a series of heptad repeats as well as a C-terminal C-type CDR. This CDR binds various monosaccharides, including mannose (Figure 38B) , fructose and *N*-acetyl-glycosamine, in a calcium-dependent manner, but the CRD is also able to bind oligosaccharides like mannan.[199]

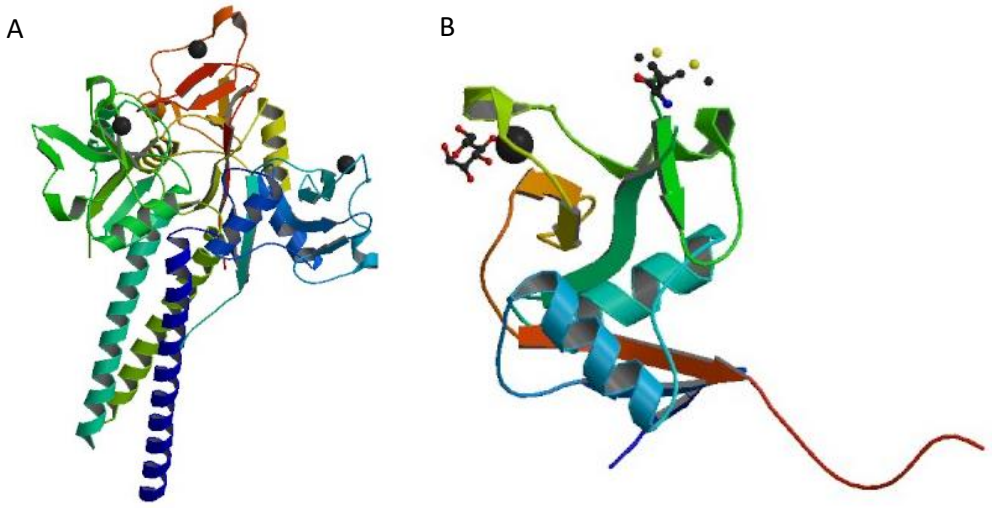


Figure 38: A) trimeric structure of Langerin;[200] B) structure of the human Langerin carbohydrate recognition domain in complex with mannose.[201]

Whereas langerin is characterized as a pathogen recognition receptor, only a couple of pathogens are reported to exhibit an interaction with Langerin such as HIV-1 and *Myobacteria leprae*. The lectin is important in protecting against HIV-1 transmission,[195] because capturing of HIV-1 by Langerin leads to viral internalization into Birbeck granules and degradation. Thereby, HIV-1 infection of Langerhans cells is prevented.[202] Further studies recently show that Langerin-mediated uptake can simplify the transfer of the virus from dendritic cells to T-cells, which occur *via* two routes. The transfer is possible *via* plasma membrane-linked (neutral pH) intracellular compartments or *via* receptors of enhanced de novo replication in dendritic cells, which facilitate virus transmission.[196] HIV-1 transmission by Langerhans cells can be enhanced strongly by Langerin inhibition with mannan or with a cognate antibody.[198]

DC-SIGN (Dendritic cell-specific ICAM-3 grabbing nonintegrin, CD209) is a type II trans-membrane C-type lectin with a single C-terminal CRD and was initially defined as an intracellular adhesion molecule-3 (ICAM) receptor which is essential in establishing the first contacts between dendritic cells and resting T cells.[203]

DC-SIGN is assembled as a tetramer in the cellular membrane. This is due to an extended coiled-coil region that enables simultaneous presentation of four CRDs. The lectin comprises three main domains, a cytoplasmic region, a transmembrane segment and an extracellular domain. This extracellular domain is built up with two structurally as well as functionally distinct regions, namely a neck region which is involved in the tetramerization of the receptor and a calcium-dependent CRD which is essential for molecular recognition processes mediated by DC-SIGN (Figure 39).[204]

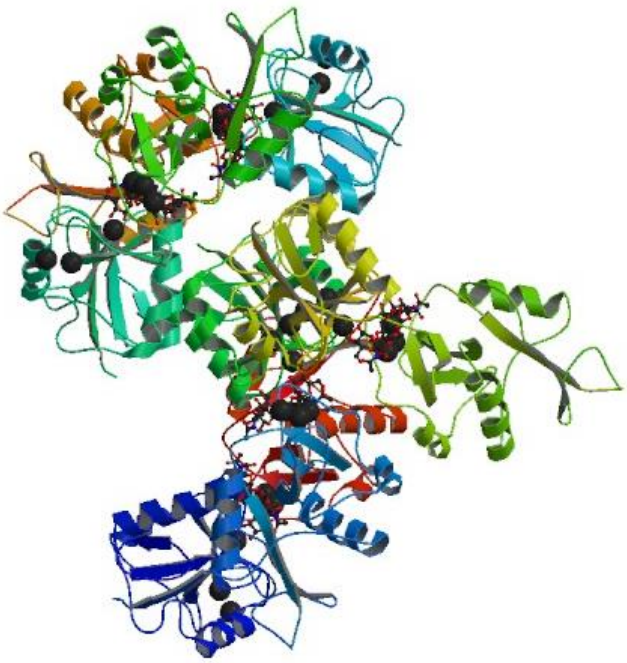


Figure 39: Complexation of DC-SIGN and $GlcNAc_2Man_3$.[205]

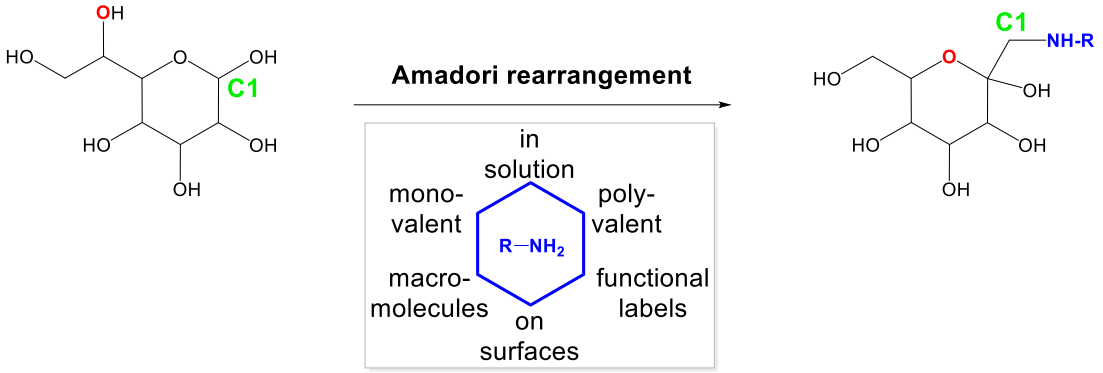
This lectin recognizes carbohydrate ligands, mainly the high mannose glycan $(Man)_9(GlcNAc)_2$, which is a branched oligosaccharide presenting in several different copies in diverse pathogen glycoproteins. This high mannose glycan is especially presenting by the gp120 envelope protein HIV. Moreover, DC-SIGN can also recognize branched fucosylated structures which contain a terminal fucose residue.[204d]

As DC-SIGN is one of the dendritic cells specific pathogen-uptake receptors, it is able to recognize glycoconjugates on the surface of several pathogens including viruses (HIV, Ebola, Dengue, SARS, Cytomegalovirus), bacteria (*S. pneumoniae*, *M. tuberculosis*), fungi (*C. albicans*, *A. fumigatus*) and parasites (*Leishmania*, *S. mansoni*). Studies showed that this lectin plays a significant role in the initial steps of infections caused by some pathogens. Besides the role of this lectin in infection processes, it was discovered that DC-SIGN is involved in immunoregulation processes

which makes this lectin very attractive for the investigations as a novel potential target in immunotheraphy.[204d]

2. AIM OF THE WORK

The aim of this thesis was to investigate a synthetic strategy for the synthesis of *C*-glycosyl type neoglycoconjugates employing the Amadori rearrangement which allows the conjugation of respective aldoses to suitable amino components in one single step without the need for protecting group manipulations (Scheme 30).



Scheme 30: Amadori rearrangement furnishes *C*-glycosyl type neoglycoconjugates (1-amino-1-deoxy ketose) from aldoses.

Following our main interest to explore if the Amadori rearrangement is a new valuable ligation method for bioconjugation, the scope and limitation of this rearrangement reaction in terms of the amino components, as well as the sugar configuration, has been investigated.

In order to obtain hexose analogues in the Amadori rearrangement, the appropriate heptoses have to be employed. Thus, a straight forward synthetic approach to a selected aldoheptose has been developed, which gave in consequence access to the desired D-*manno* configuration in the carbohydrate part of the Amadori products.

In addition, various amines, including simple amines, as well as diamines and amino-functionalized carbohydrates as well as scaffold-molecules, were employed in the Amadori rearrangement to explore the scope and limitations of this reaction.

This *C*-glycosyl type glycoconjugates offer two intrinsic advantages, (i) the stability towards enzymatic or chemical hydrolysis and (ii) the option to compare the activity of non-natural *C*-glycosides with that of their *O*-glycosidic counterparts.

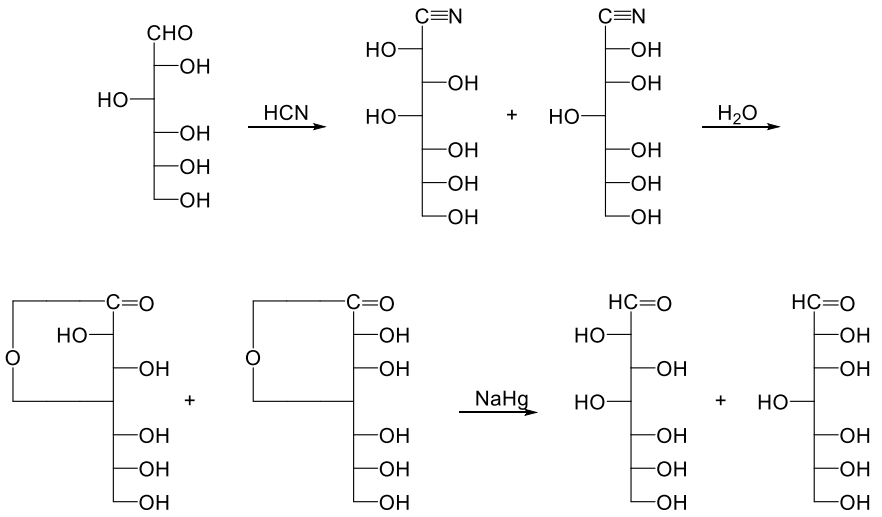
Biological evaluation of D-*manno* configured Amadori products as novel potential inhibitors for different D-mannose specific lectins were implemented.

3. RESULTS AND DISCUSSION

3.1. Synthesis of D-*glycero*-D-*galacto*/D-*talo* aldoheptose

In this section, the development of a straight forward approach for the synthesis of D-*glycero*-D-*galacto*/D-*talo* aldoheptose (**110a** and **110b**), which leads to D-*manno* configured products after the Amadori rearrangement, will be presented. Such D-*manno* configured *C*-glycosyl type glycoconjugates will be applied to investigate their function as novel potential inhibitors for different D-mannose specific lectins

The most common method for chain elongation of carbohydrates is the Kiliani-Fischer reaction, where aldoses react in aqueous solution with hydrogen cyanide, followed by saponification of the obtained nitriles and acidification to obtain aldonic acids.[206] The corresponding acids can be isolated as lactones, if the pH value is adjusted accordingly. Reduction of these lactones under weak acid conditions with sodium amalgam results in the formation of the respective C-elongated aldoses, which are lengthened by one carbon (Scheme 31).[207] With this synthetic method, two diastereomers are obtained distinguished by their configuration at position C-2, the former C-1.



Scheme 31: Reaction scheme of the Kiliani-Fischer cyanohydrin synthesis from D-glucose by Richtmyer.[208]

As mentioned above, in the original Kiliani-Fischer C-elongation strategy, hydrogen cyanide has to be applied to generate, in the first step, the corresponding cyanohydrins at position C-1. In order to avoid the inconvenient handling of hydrogen cyanide in terms of its high toxicity, a modified method was chosen, where hydrogen cyanide was generated *in situ* in an organic solvent.[209] To obtain a 2 M HCN solution in methyl *tert*-butyl ether (MTBE), sodium cyanide was dissolved in H_2O, followed by addition of MTBE and slow acidification with 30% aqueous HCl

solution. The evolved HCN is then concomitantly extracted into MTBE and separated finally from the aqueous phase. Next for the synthesis of the desired aldononitriles, four different reaction mixtures were prepared, listed in Table 3, which were treated each with 8 mL of HCN/MTBE solution.

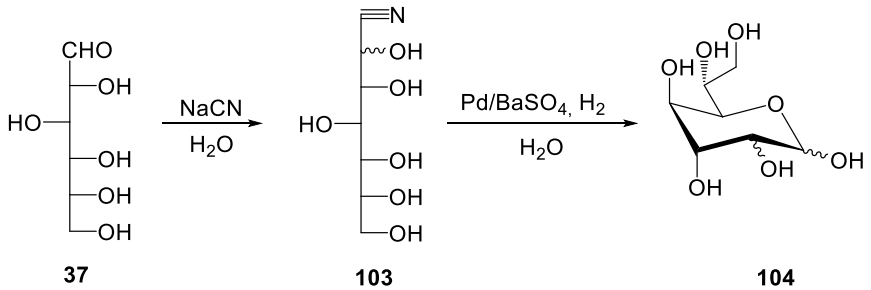
Table 3: Different reaction mixtures for the synthesis of aldononitriles with *in situ* generated HCN in organic solvent.

Entry	Aldehyde (3.0 mmol)	Solvent
1	D-glucose	Pyridine (10 mL)
2	D-glucose	Et_3N (10 mL)
3	D-glucose	Pyridine (10 mL) + Et_3N (40 µL)
4	D-glucose	H_2O dest. (10 mL)

The progress of the reaction was followed by TLC. However, no consumption of the starting material was observed in any case, as cyanohydrins can undergo hydrolysis back to the starting material induced by the slightly acidic conditions of the TLC surface. Thus, to facilitate the detection, a small amount of each reaction mixture was per-*O*-acetylated. However, the formations of the desired aldononitriles were not observed in any case.

Therefore, another modified and very often used method of the Kiliani-Fischer synthesis was chosen. Serianni *et al.*[210] concluded that cyanohydrins are formed quickly and are stable at low pH values. Furthermore, they observed that these aldononitriles can be reduced with palladium followed by rapid hydrolysis of the intermediate imine at pH 4.2 ± 0.1 to generate the aldoses.

Following the literature[210] (Scheme 32), an aqueous solution of sodium cyanide (1.5 M) was charged in a three-necked flask and the pH value (pH = 7.5) was adjusted by addition of a 3 M acetic acid solution. Next, an aqueous solution of D-glucose (**37**) (0.5 M) was added slowly to the reaction mixture, wherein the pH value was adjusted frequently during the reaction by addition of acetic acid. After a reaction time of 5 hours, the pH was lowered to 4.2 and the corresponding aldononitriles (**103**) were reduced over palladium-barium sulfate.



37 **103** **104**

Scheme 32: Synthesis of D-*glycero*-D-*gulo*/D-*ido* aldoheptose (**104**) using the protocol from Serianni.[210]

Therefore, palladium-barium sulfate (5%, 62 mg per mmol of nitrile) was charged in a reduction flask and 10 mL H_2O was added. The solution of C-2 epimeric aldononitriles (**103**) at pH 4.2 was transferred and the reaction mixture stirred under hydrogen atmosphere overnight. The reaction mixture was acidified to pH 2 with an acid ion-exchanger (IR-120 H^+). The NMR measurement as well as TLC showed that no desired heptose (**104**) was formed, unfortunately only the starting material D-glucose was isolated.

Investigations have revealed that already formation of the desired aldononitriles (**103**) in the first step failed when applied to the reaction condition mentioned above. In order to find the appropriate reaction conditions for the preparation of the aldononitriles, a range of different pH values were investigated and are listed in Table 4.

Table 4: Reaction conditions for the C-elongation from D-glucose.

Entry	[NaCN]	[Glucose]	pH (Preparation of aldononitriles)	pH (Reduction of aldononitriles)	Product
1	1.5	0.5	6.5	4.2	D-glucose
2	1.5	0.5	6.5	6.8	D-glucose
3	1.1	0.36	7.0	4.0	D-glucose
4	1.5	0.5	8	4.2	D-glucose as well as D-*glycero*-D-*gulo*/D-*ido* heptose

In preliminary studies, the pH value in the first step was reduced to 6.5, whereas the pH value during the reduction step was kept at 4.2. Unfortunately, this reaction condition was not successful. Also, the attempt to increase the pH value in the reduction step failed. However, by increasing the pH value to 8 in the first step forming the cyanohydrin and performing the reduction step at pH 4.2, the desired D-*glycero*-D-*gulo*/D-*ido* heptose (**104**) was obtained in a ratio 1:1 to starting material D-glucose (**37**), which was proven *via* HPLC-MS measurements (Figure 40).

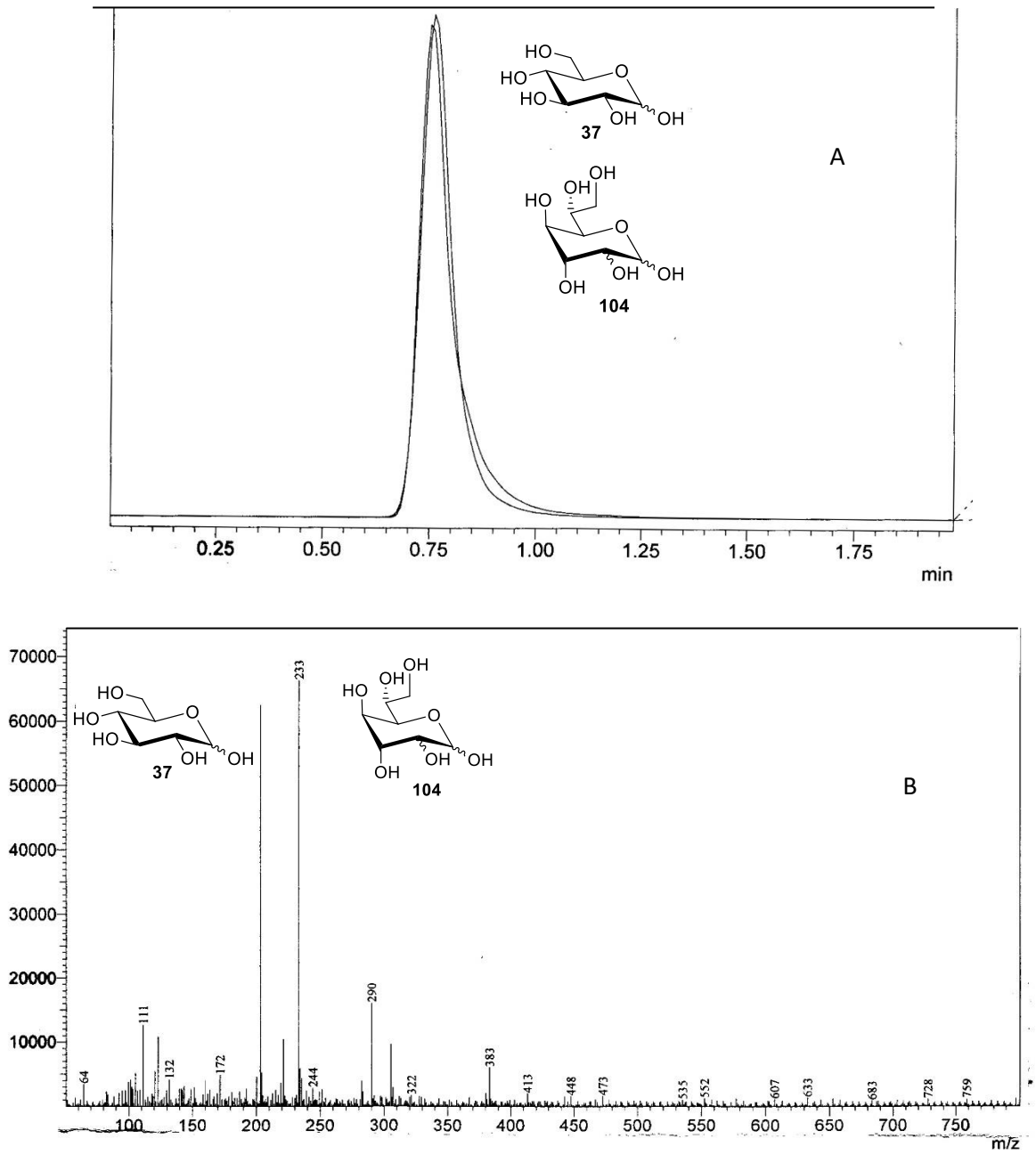
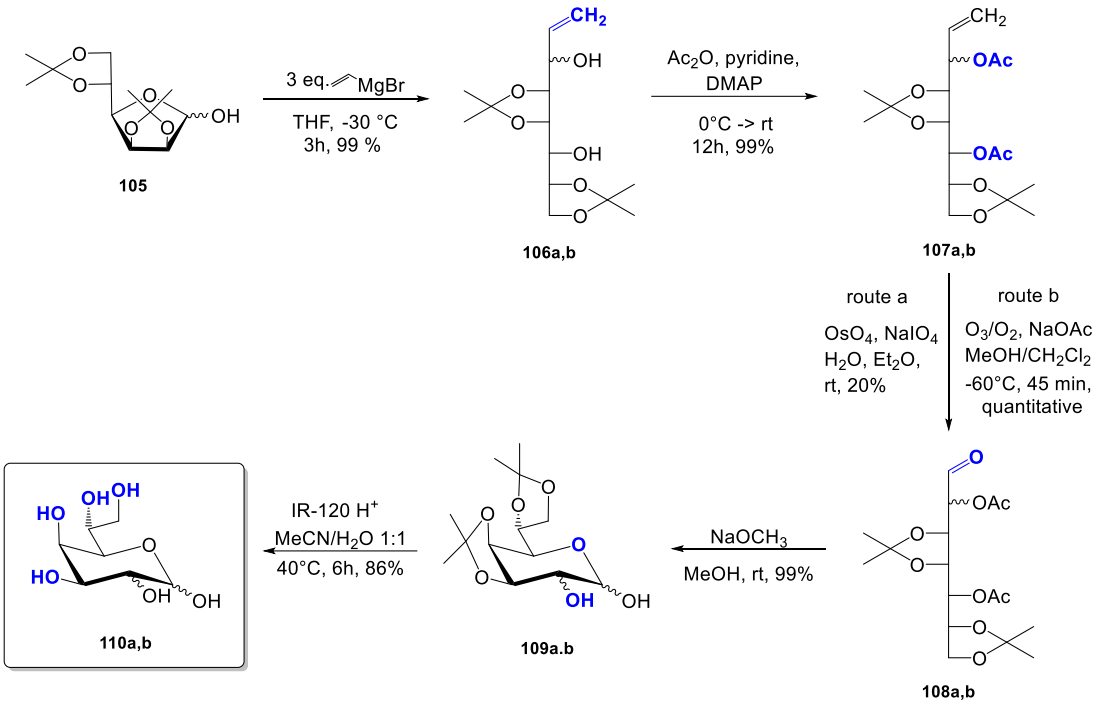


Figure 40: HPLC Measurement of compound 104: A: Chromatogram; B: Scan-mode (MNa^+ = 203 for compound (**37**); MNa^+ = 233 for compound **104**).

With this C-elongation method, formation of the two C-2 epimers, D-*glycero*-D-*gulo*/D-*ido* aldoheptose, each one possible as mixture of α and β anomers occurring in their pyranoid as well as furanoid form is possible. This leads in the end to eight different tautomers of the desired heptose **104**. Furthermore, due to the similar polarity of the starting material, D-glucose (**37**) and the synthesized heptose **104**, product isolation by conventional column chromatography was not successful.

Therefore, another synthetic pathway was examined to generate D-*glycero*-D-*galacto*/D-*talo* aldoheptose (**110**) as starting material (Scheme 33), which gave access to D-*manno* configured Amadori rearrangement products:

D-Mannose was treated in acetone with sulfuric acid to obtain 2,3:5,6-di-*O*-isopropylidene-D-mannose **105** after recrystallization from dichloromethane and cyclohexane as a white powder in a yield of 82%.[211] The next step has been a Grignard reaction[212] using commercially available vinylmagnesium bromide solution (1 M in THF) to synthesize compounds **106a** and **106b** in 76% yield. The consumption of the starting material as well as product formation by TLC was challenging. Employing 10% sulfuric acid in EtOH and heat treatment of the TLC plate, a differentiation in colour for the two compounds has been visually detectable. The isopropylidene-protected mannose became visible as a tan spot whereas the product spot was brown. This C-elongation approach resulted in a mixture of C-2 diastereomers. However, during the Amadori rearrangement this centre is converted to a keto group and thus separation of the C-2 epimeric mixture prior to the Amadori rearrangement is not necessary. After acetylation[213] of compounds **106a** and **106b** with acetic anhydride in pyridine using catalytic amounts of DMAP, compounds **107a** and **107b** were obtained. Introduction of an aldehyde function in the following step was achieved by two different approaches. First, a dihydroxylation using osmium tetraoxide (OsO_4) as oxidant to generate a vicinal diol, followed by oxidative cleavage with sodium periodate method was employed to obtain the desired aldehydes[214] **108a** and **108b** in 20% yield (route a, Scheme 33). This reaction sequence is also known as the Lemieux-Johnson oxidation[215], where an excess of sodium periodate is used to regenerate the osmium tetraoxide, thus it can be used in catalytic amounts. However, due to the high toxicity and the high price of OsO_4, we investigated a more economic method for the introduction of the aldehyde function. Therefore, a simple ozonolysis (route b, Scheme 33) of the terminal double bound in compound **107a,b** was carried out. The diasteriomeric mixture of compounds **107a** and **107b** was treated with ozone until TLC showed complete consumption of the starting material. After nitrogen was passed through the reaction mixture to supersede the excess of ozone, the reaction was treated with dimethylsulfide to obtain, after reductive cleavage, the desired compounds **108a** and **108b** in quantitative yields. Next, using Zemplén conditions, the acetyl groups were removed[216] and compounds **109a** and **109b** were obtained in quantitative yields after purification on silica gel chromatography employing Cy/EtOAc in a mixture of 4:1. Finally, acidic cleavage of the isopropylidene protecting groups led to the formation of a mixture of D-*glycero*-D-*galacto*/D-*talo* heptose **110a** and **110b** (Figure 41 and 42, Table 5 and 6) in an overall yield of 83% from **105**.



Scheme 33: Synthesis of D-*glycero*-D-*galacto*/D-*talo* aldoheptose (**110**).

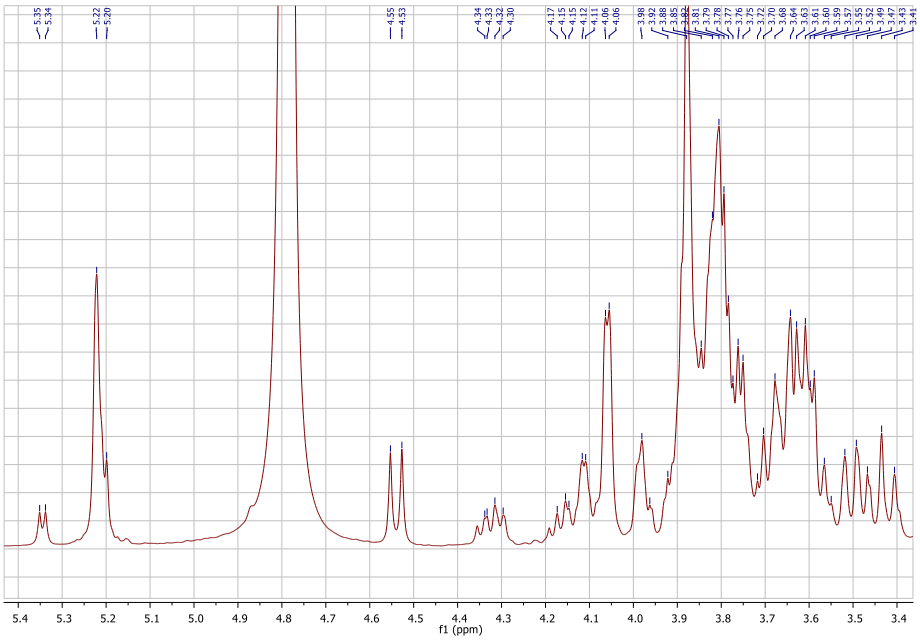
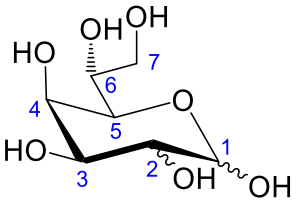


Figure 41: ^{1}H spektrum of D-*glycero*-D-*galacto*/D-*talo* aldoheptose **110a** and **110b**.

Table 5: ^{1}H NMR signals of aldoheptose **110a** and **110b**.

	H-1, H-1´(α- and β-pyranose)	5.35, 5.30-5.19, 4.57, 4.47 – 4.29 ppm
	H-7, H-7´, H-6, H-5, H-4, H-3, H-2 ´(α- and β-pyranose)	4.24 – 3.4 ppm

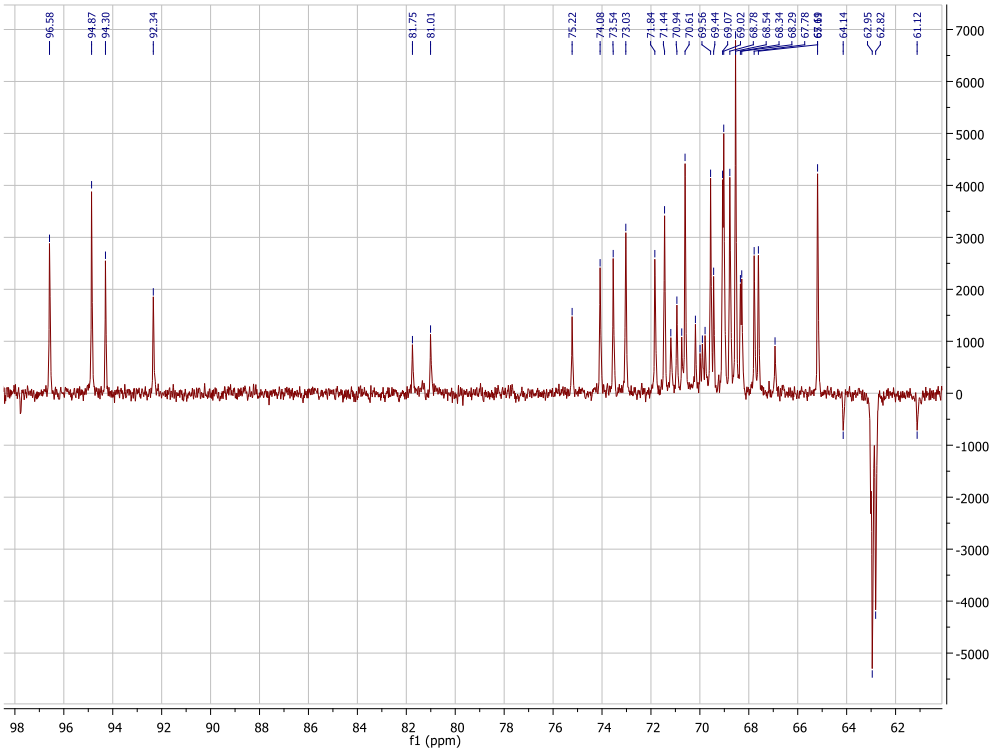
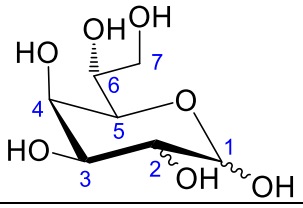


Figure 42: APT spektrum of D-*glycero*-D-*galacto*/D-*talo* aldoheptose **110a** and **110b**.

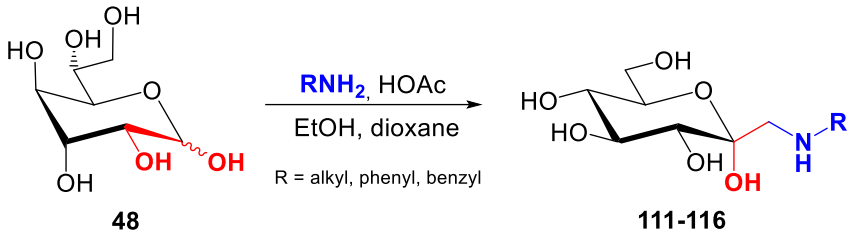
Table 6: ^{13}C NMR signals of aldoheptose **110a** and **110b**.

	anomeric; C-1	96.6 – 92.3 ppm
	alc. sec.; C-2, C-3, C-4, C-5, C-6	75.2 – 67.6 ppm
	alc. prim.; C-7	62.8 – 63.0 ppm

3.2. Scope and limitation of different amino components in the Amadori rearrangement

3.2.1. Simple amines

In order to optimise the reaction conditions for the Amadori rearrangement employing diverse amines, the commercially available D-*glycero*-D-*gulo* aldoheptose[a] (**48**) has been used as model substrate (Scheme 34).

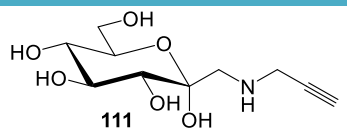
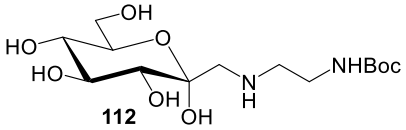


Scheme 34: Amadori rearrangment employing D-*glycero*-D-*gulo* aldoheptose (**48**) for the synthesis of D-*gluco* configured products (**111-116**).

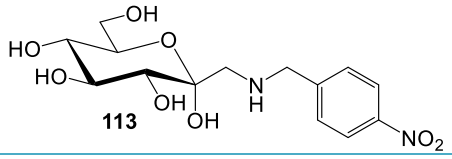
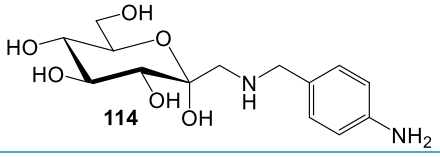
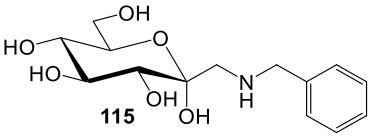
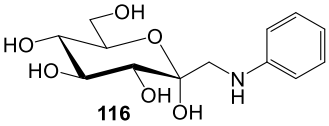
For the Amadori rearrangement, the respective aldoheptose was dissolved in EtOH, followed by addition of a small amount of 1,4-dioxane and H_2O dest. to improve the solubility of the aldoheptose. After addition of the desired amine and an acid catalyst (HOAc), the reaction mixture was kept to 70 °C. The conversion was detected by TLC using a solvent mixture of $CHCl_3$/MeOH/NH_4OH in individual ratios as indicated in the experimental section.

A broad range of simple amines were investigated leading to Amadori products depicted in Table 7.

Table 7: Synthesized Amadori rearrangement products in the D-*gluco* series employing simple amines.

Amadori rearrangement products in D-*gluco* series	Yields [%]	pK_a-value of the amine
 **111**	81	8.2
 **112**	98	7.8

[a] D-*glycero*-D-*gulo* aldoheptose (**48**) is no longer commercial available since 2016.

Amadori rearrangement products in D-*gluco* series	Yields [%]	pK_a-value of the amine
 **113**	64	8.1
 **114**	73	9.3
 **115**	67	9.3
 **116**	26	4.6

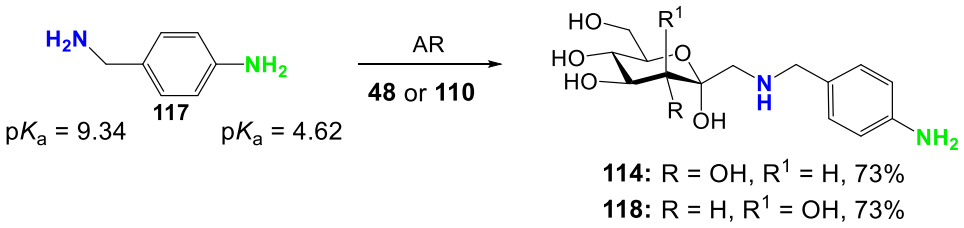
By employing aliphatic amines, the corresponding Amadori rearrangement products (**111** and **112**) were isolated after purification by silica gel chromatography in high yields. To increase the formation of the desired product, an excess of the respective amine was added. Nevertheless, to avoid the degradation of the Amadori products and the increased formation of compounds along the Maillard reaction cascade pathway by prolonged reaction time or elevated temperature, the reaction had to be stopped in some cases with a notable amount of starting material still present. Furthermore, the distinct polarity as well as basicity of the polar and unprotected compounds were the decisive factor in the rather challenging purification method using silica gel chromatography. To prevent the absorption of the compounds on silica gel, a high amount of ammonia of up to 25% had to be added to the eluent. In some cases, the R_f-value of the amine and the corresponding Amadori products were only slightly distinguishable, hence, a time-consuming and accurate silica gel chromatography was necessary.

NMR Analysis revealed that the Amarodri rearrangement leads to one anomeric form exclusively. The rearrangement products exist in their $^5C_2$ pyranoid conformation and the amino substituents at the anomeric position is found oriented in the sterically favored equatorial position located toward the β-face of the sugar ring. The corresponding hydroxyl group at anomeric position C-2 is positioned in the α-direction.

Furthermore, benzylic as well as aromatic amines were investigated in the rearrangement reaction which afforded Amadori products (**113**-**116**) in good to moderate yields. We observed during our studies that the pK_a value of the employed amines seemed to play a critical role.

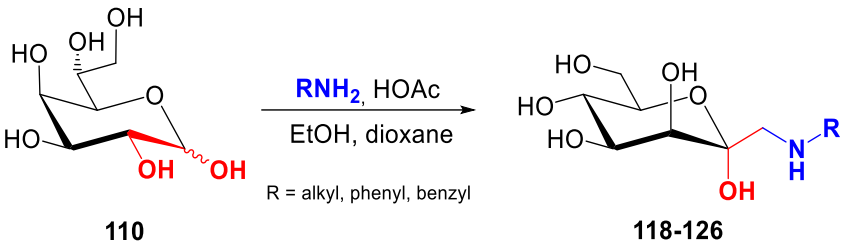
When aniline, with a pK_a value of 4.6, was employed as the amino component in the Amadori rearrangement, the desired product **116** was isolated in only 26% yield. Even the presence of an excess of the amine as well as increased reaction time could not increase the formation of the desired product. Whereas, by applying amines with a pK_a value higher than 7, the yields of the isolated products could be increased. As a conclusion from these results, it seems that amines with a pK_a value range between 8 and 12 are more efficient nucleophiles for this kind of reaction than less basic amines.

Taking advantage of the influence of the pK_a value of the amino component, we investigated the use of 4-aminobenzylamine (**117**) as a bi-directional linker. Treatment of amino component **117** with both aldoheptoses **48** and **110** gave exclusively the single modified Amadori rearrangement products **114** and **118** (Scheme 35), which was determined by HMBC NMR experiments. This regioselective ligation reaction with amine **117** can be rationalized on the basis of the pK_a values of the amino group. As basicity is a curcial parameter for the success of the Amadori rearrangement, 4-aminobenzyl amine with pK_a values of 9.3 for the benzylic amine and 4.6 for the aniline amino group, allows for a selective reaction at the benzylic position. This bi-directional linker enables the conjugation of a carbohydrate moiety *via* the Amadori rearrangement chemoselectively at one terminal amine whereas the second amino function was intended to stay available for further modification reactions.



Scheme 35: pK_a study employing 4-aminobenzylamine in the Amadori rearrangement.

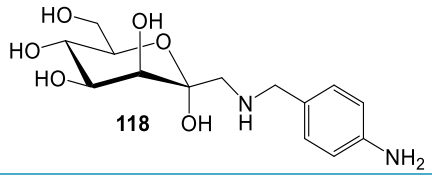
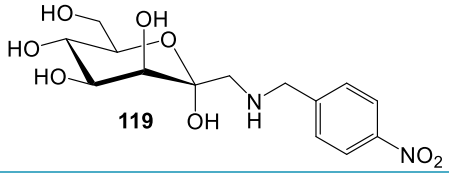
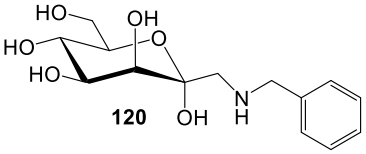
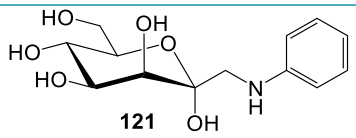
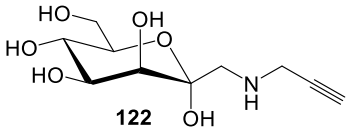
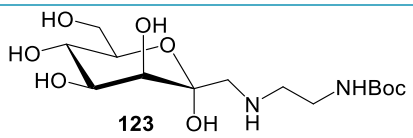
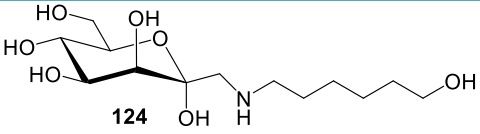
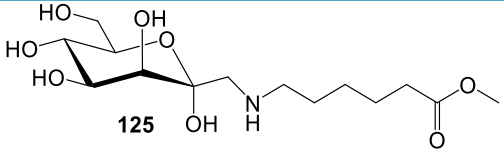
To investigate the scope of sugar moieties, D-*glycero*-D-*galacto*/D-*talo* aldoheptose (**110**), synthesized in six steps from D-mannose[217] (Scheme 33), was used as starting material in the Amadori rearrangement, to obtain D-*manno* configured *C*-glycosyl-type glycoconjugates (Scheme 36). As before, we investigated aliphatic, benzylic as well as aromatic amines for the rearrangement reaction (Table 8) transferring the reaction conditions for the sytheseis of D-*gluco* configured Amadori products.

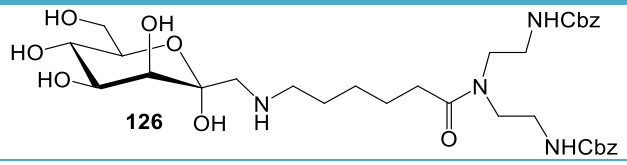


110 **118-126**

Scheme 36: Amadori rearrangment employing D-*glycero*-D-*galacto*/D-*talo* aldoheptose (**110**) for the synthesis of D-*manno* configured products (**118-126**).

Table 8: Synthesized Amadori rearrangement products in the D-*manno* series employing simple amines.

Amadori rearrangement products in D-*manno* series	Yields [%]	pK_a-value of the amine
 **118**	73	9.3
 **119**	57	8.1
 **120**	56	9.3
 **121**	24	4.6
 **122**	42 77[a]	8.2
 **123**	73 93[a]	7.8
 **124**	73	10.6
 **125**	70	10.8

Amadori rearrangement products in D-*manno* series	Yields [%]	pK_a-value of the amine
 **126**	48	10.8

a) Microwave-assisted Amadori rearrangement

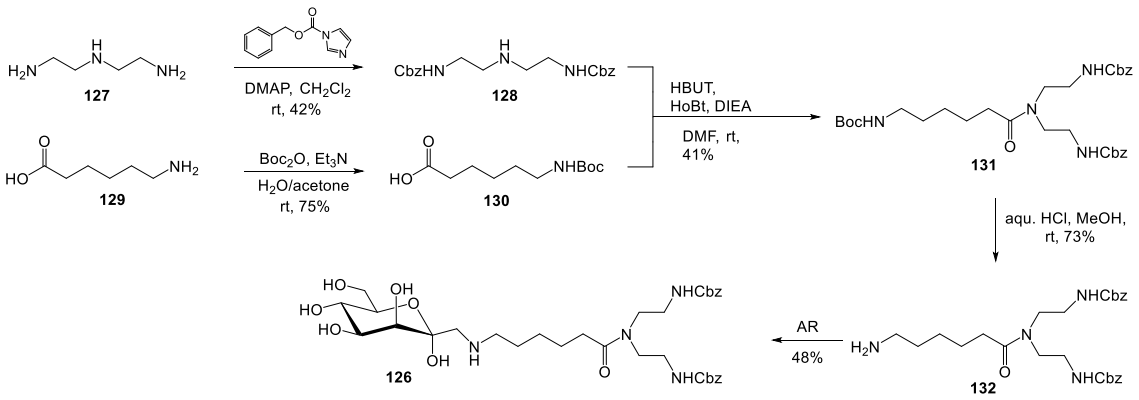
The rearrangement products in the D-*manno* series using different simple amines were isolated in good yields. Also in this case, the pK_a value of the employed amines had an influence on the formation of the desired product and accordingly on the obtained yields.

In context of configuration of the sugar substrate in the Amadori products, we expected that yields in the D-*gluco* series would be significantly higher than in the D-*manno* series, as the hydroxyl groups of the sugar moieties in D-*gluco* configured products are found in the thermodynamically favoured all-equatorial position, whereas in D-*manno* configured products the hydroxyl group at position C-3 is oriented in the less favoured axial orientation. However, no significant differentiation in the obtained yields was observed during our investigations.

Moreover, we wanted to investigate microwave-assisted Amadori rearrangement in order to reduce the reaction time and consequently minimize the possibility of forming a range of side as well as degradation products. Thus, propargylamine as well as *N*-(*tert*-butoxycarbonyl)ethylenediamine and D-*glycero*-D-*galacto*/D-*talo* aldoheptose (**110**) in the presence of acetic acid were stirred at 70 °C and 70 W in the microwave until complete consumption of the starting material was detected by TLC. The reaction time could be shortened in both cases to 5 hours instead of two days. Additionally, using the microwave-assisted reaction, the formation of the desired products could be increased. In case of propargylamine, the rearrangement product **122** was isolated in 77% yield in comparison to 42% when applying the general reaction method (Table 8). Likewise, with *N*-(*tert*-butoxycarbonyl)ethylenediamine, formation of product **123** was enhanced from 73% to 93% yield.

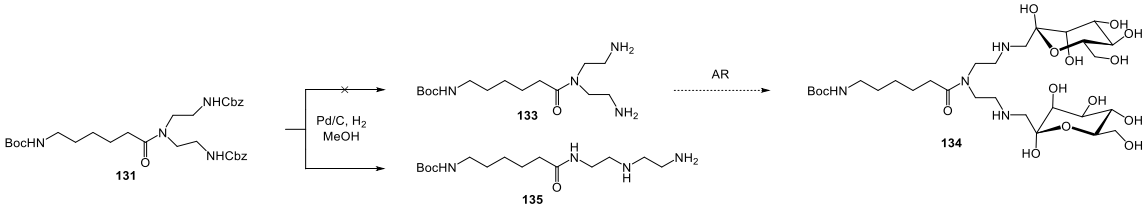
Offering the possibility of orthogonality in the amino spacer of Amadori products, which can be used for further modification reactions, we were interested in the synthesis of compound **126**. Therefore, the two primary amine groups of diethylenetriamine (**127**) were protected with carboxybenzyl protecting groups (**128**),[218] following by the treatment with Boc-6-aminohexanoic acid **130**,[219] using standard coupling conditions,[220] to obtain compound **131** (Scheme 37). After acidic cleavage of the Boc-protecting group, the free amine **132** was obtained

in 73%, which was treated with aldoheptose **110** forming the Amadori rearrangement product **126**.



Scheme 37: Synthesis of amino component **132**.

In order to obtain an amino spacer carrying two unprotected amine groups (Scheme 38) which can be applied for the synthesis of dimeric Amadori product **134**, the caboxybenzyl groups of compound **131** were cleaved hydrogenolytically using palladium on carbon under hydrogen atmosphere (Scheme 38). However, after the removal of the carbamates, the amide-bond migrated from the secondary amine to one of the new generated terminal primary amines forming compound **135**. We supposed that this migration would possible occur also after hydrogenolytic cleavage of compound **126**.



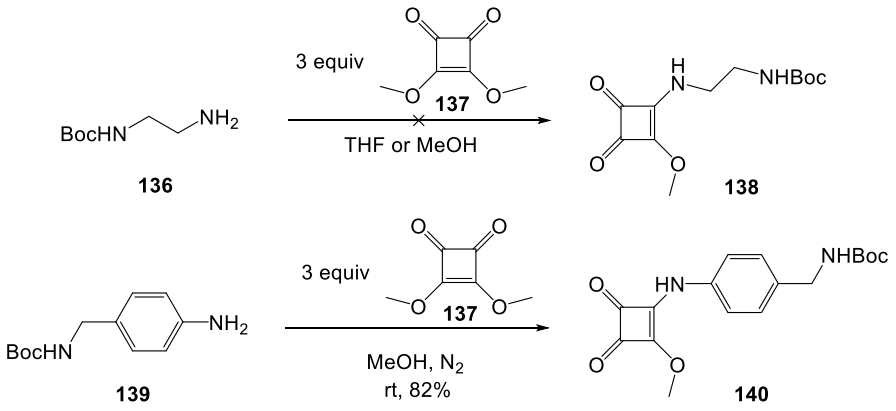
Scheme 38: Hydrogenolytic cleavage of compound **135**.

Amadori rearrangement products which lead to *C*-glycosyl type neoglycoconjugates are of interest for biological investigations because they are believed to bear great potential as therapeutics and as tools for mechanistic studies in biology. This type of glycosidic linkage between the sugar moiety and the amino spacer is not sensitive towards enzymatic or chemical hydrolysis in physiological environments as compared to naturally occurring common *O*- and *N*-glycosidic bonds. Furthermore, D-*manno* configured Amadori rearrangement products are interesting candidates as inhibitors of type-1 fimbirated *E.coli* bacteria (section 2.5). This bacterial lectin, FimH, binds α-mannosides such as simple methyl α-D-mannosides. By

introduction of an aromatic system in the aglycone, the affinity to FimH can be improved because of the π-π-stacking interactions of the aromatic system within the tyrosine gate. Additional interactions exerted by extended aglycone portions can further improve ligand affinity for FimH, such as squaric acid partial structures.[185] Consequently, we were interested in the synthesis of D-*manno* configured rearrangement products carrying a squaric acid moiety in the aglycon part.

Following the literature,[221] *N*-(*tert*-butoxycarbonyl)-1,2-diaminoethane (**136**), obtained by the treatment of ethylenediamine with 1 equiv. di-*tert*-butyl dicarbonate, was reacted with dimethyl squarate (**137**) in dry THF at room temperature overnight (Scheme 39). The solvent was removed under reduced pressure and the fully protected amine should have been isolated after recrystallization from EtOAc/Cy. However, only the staring material (**136**) was obtained. Therefore, another approach[222] was chosen using dry MeOH as solvent. Nevertheless, after a reaction time of 12 hours and further addition of dimethyl squarate no product formation could be observed *via* TLC.

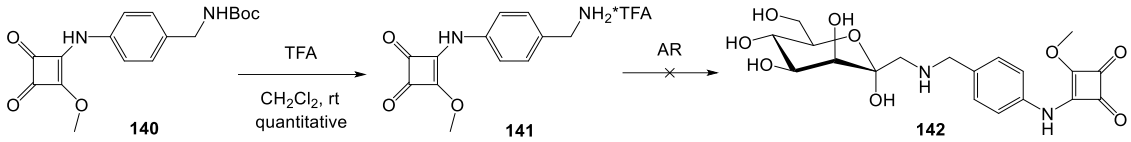
As mentioned above, aromatic systems in the aglycone of D-mannosides can improve the affinity to FimH. Hence, 4-[(*N*-boc)aminomethyl]aniline (**139**) was selected as monoprotected amine for the reaction with dimethyl squarate (**137**) in dry MeOH obtaining compound **140** after silica gel chromatography in 82% as a white solid.



Scheme 39: Synthesis of amino spacer **138** and **140** carrying a squaric acid partial structure.

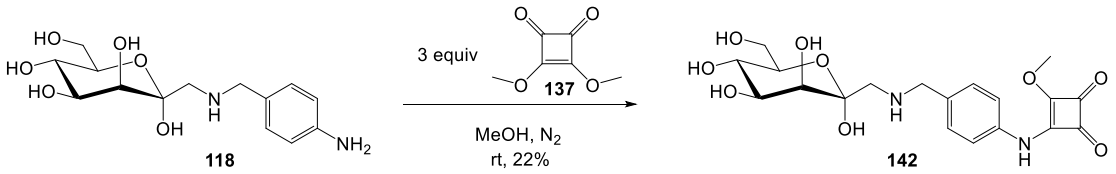
After acid cleavage of the Boc-protecting group with trifluoroacetic acid, the amino component **141** was obtained in quantitative yield, which was then treated with aldoheptose **110** under typical reaction conditions, employing Et_3N for liberation of the free amine (**141**) from the TFA-salt (Scheme 40). However, no conversion of the aldoheptose was observed by TLC. Instead, the

consumption of the amino component was detected forming an insoluble participation, which was not further investigated.



Scheme 40: Synthesis of Amadori rearrangment product **142**.

Therefore, another synthetic approach was investigated to synthesize desired product **142**. Compound **118** was treated with 1.2 equivalent of dimethyl squarate (**137**) forming the desired product **142** in 22% yield (Scheme 41). To increase the yield, prolonged reaction time as well as further addition of dimethyl squarate were investigated. However, these attempts did not lead to the desired increase of product formation. Instead, by extending the reaction time, degradation of the Amadori product was observed. In these cases, the reactions had to be stopped after 3 days at rt to avoid further side product formation and degradation.

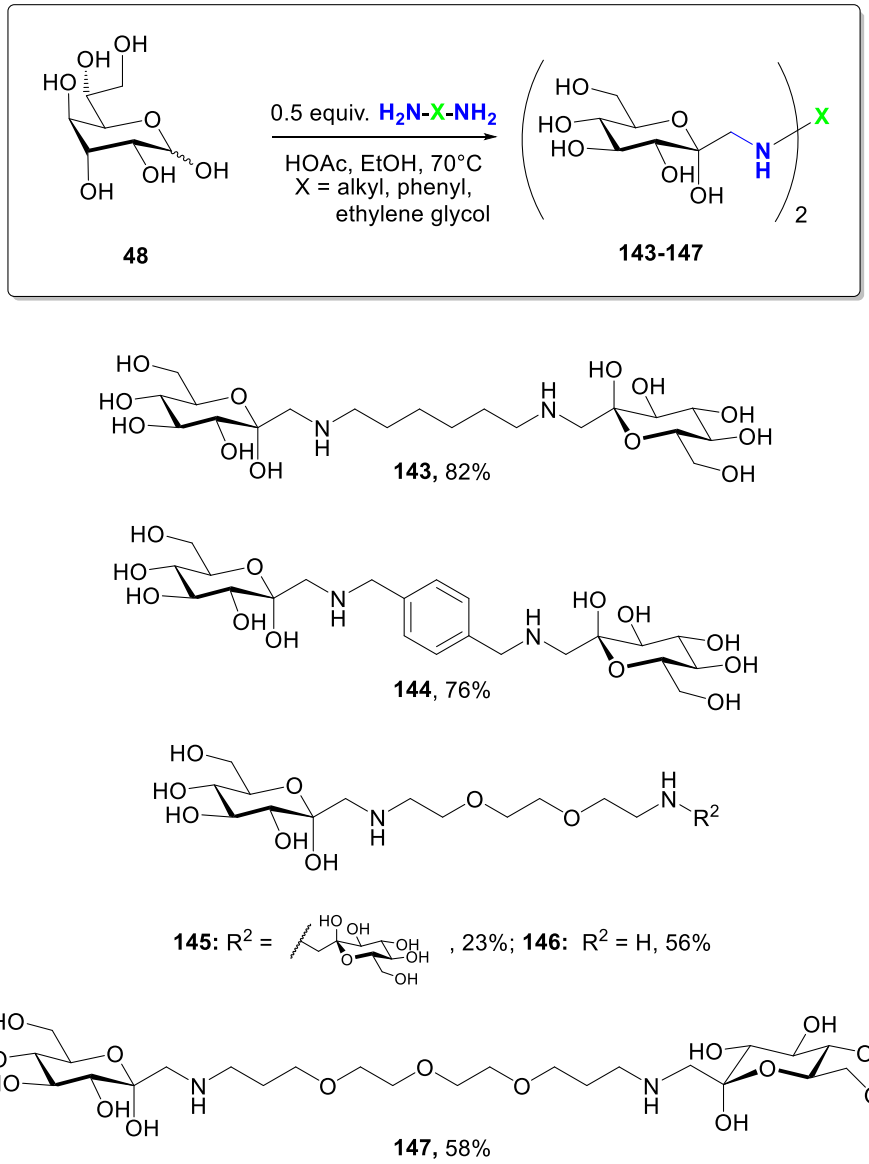


Scheme 41: Synthesis of compound **142**.

3.2.2. Diamines

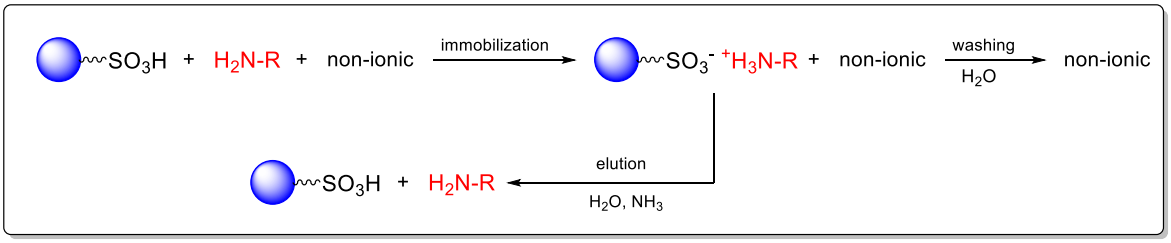
To investigate the scope and limitation of amino components in the Amadori rearrangement, we employed different diamines for the synthesis of D-*gluco* as well as D-*manno* configured products (Scheme 42 and 44).

When applying 1,6-diaminohexane, the Amadori rearrangement, using D-*glycero*-D-*gulo* aldoheptose (**48**) as starting material, was performed with 0.5 equiv. of the diamine in ethanol and 1,4-dioxane as co-solvent in the presence of one equivalent acetic acid, to give, after purification by conventional column chromatography, the double rearrangement product **143** in a yield of 82% in the D-*gluco* series.



Scheme 42: Amadori rearrangement in the D-*gluco* series employing diamines.

The purification of the reaction mixture by silica gel chromatography was tedious, due to the same polarity of the aldoheptose and the corresponding disubstituted rearrangement product. Hence, the crude product was passed through an ion exchange CG-120-II (Na^+) Amberlite resin column using water as eluent in the first step to wash the remaining non-ionic starting materials from the resin. After the application of a water–NH_4OH conc. mixture to the column, the rearrangement product as well as the remaining amine were eluted (Scheme 43 and Figure 43), which were separated in the second step by conventional silica-gel chromatography employing $CHCl_3$/MeOH/NH_4OH as eluent mixture.



Scheme 43: Concept of ion exchange chromatography.

A typical TLC obtained from the separation of the reaction mixture after ion exchange chromatography is shown in Figure 43. On the left side, the elution of the non-ionic aldoheptose is depicted and on the right side the elution of the ionic rearrangement products as well as the remaining amine is shown, which was achieved by employing a water–NH_4OH conc. eluent. The rearrangement product could be separated in a second step by conventional silica gel chromatography.

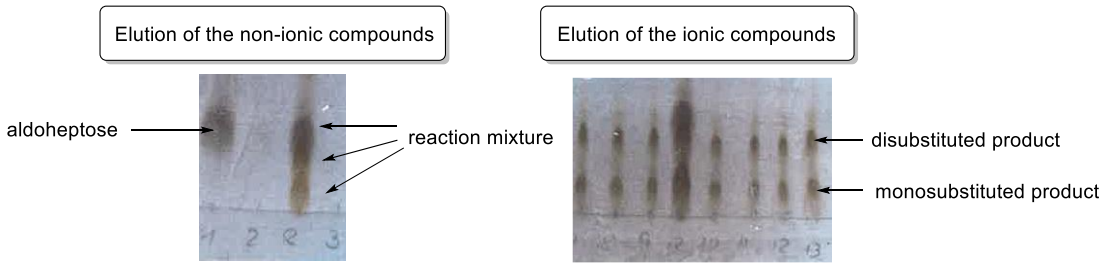
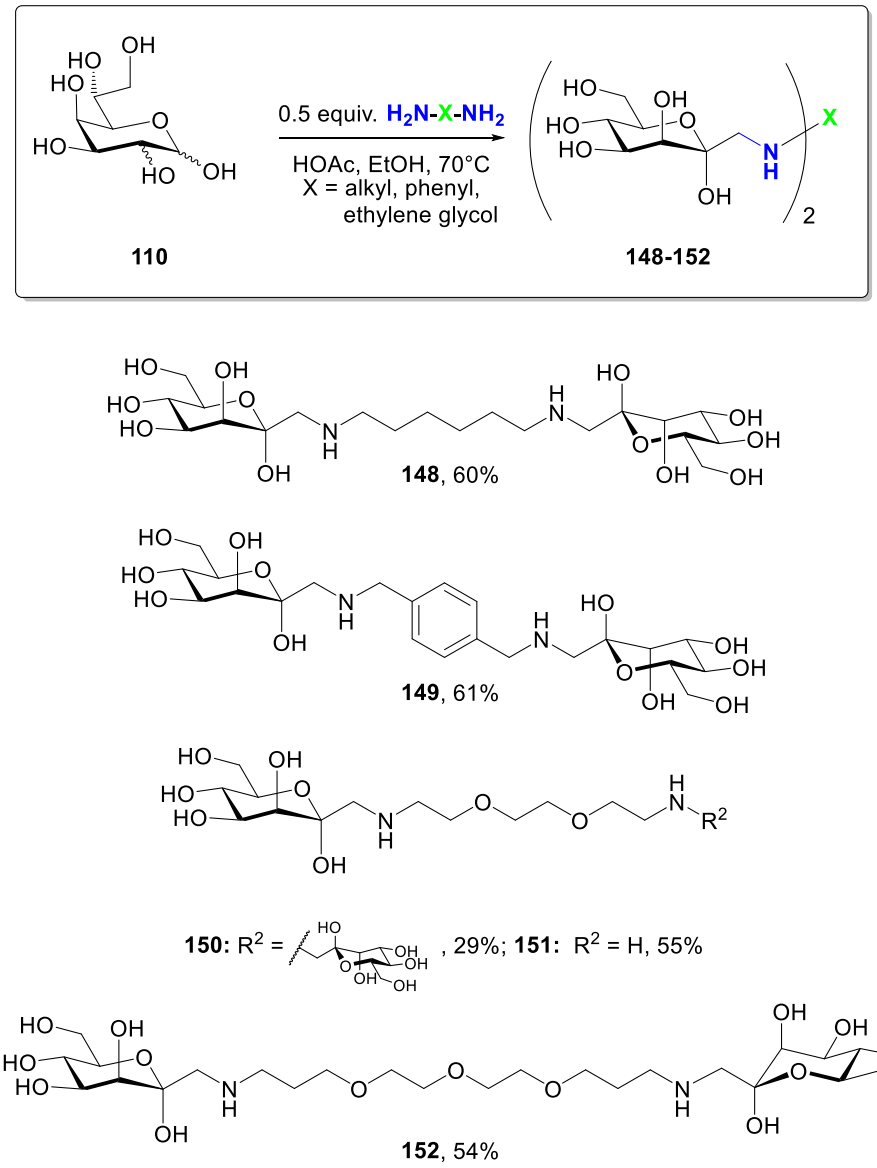


Figure 43: TLC from the separation of the reaction mixture after ion exchange chromatography.

Employing this purification protocol, compound **144** could be isolated in 76% yield, when *p*-xylenyldiamine was used as amino component in the Amadori rearrangement reaction. In case of 2,2´-(ethylenedioxy)bis(ethylamine), the D-*gluco* configured bis-product **145** was isolated in only 23% yield, whereas the corresponding monosubstituted rearrangement product **146** was obtained in a yield of 56%. In this case, the low yield might be explained by the limited availability of the remaining amino group in monomeric compound **146**. Longer reaction time promoted the formation of compounds along the Maillard reaction cascade pathway as well as compound degradation, lowering the yield of the desired disubstituted product. Howerver, employing 4,7,10-trioxa-1,13-tridecanediamine as amino component and an excess of aldoheptose **48** (three equivalents), the Amadori rearrangement gave exclusively the bivalent product **147** in a yield of 58% (Scheme 42). Consequently, these investigations showed that *via* the amount of aldoses employed in the Amadori rearrangement with diamines, the amount of monosubtition versus disubstitution of diamines can be controlled in a quantifiable manner. Furthermore, the

availability of the second amino group, which remains free after the first Amadori rearrangement, increases with chain length of the spacer of the diamine.



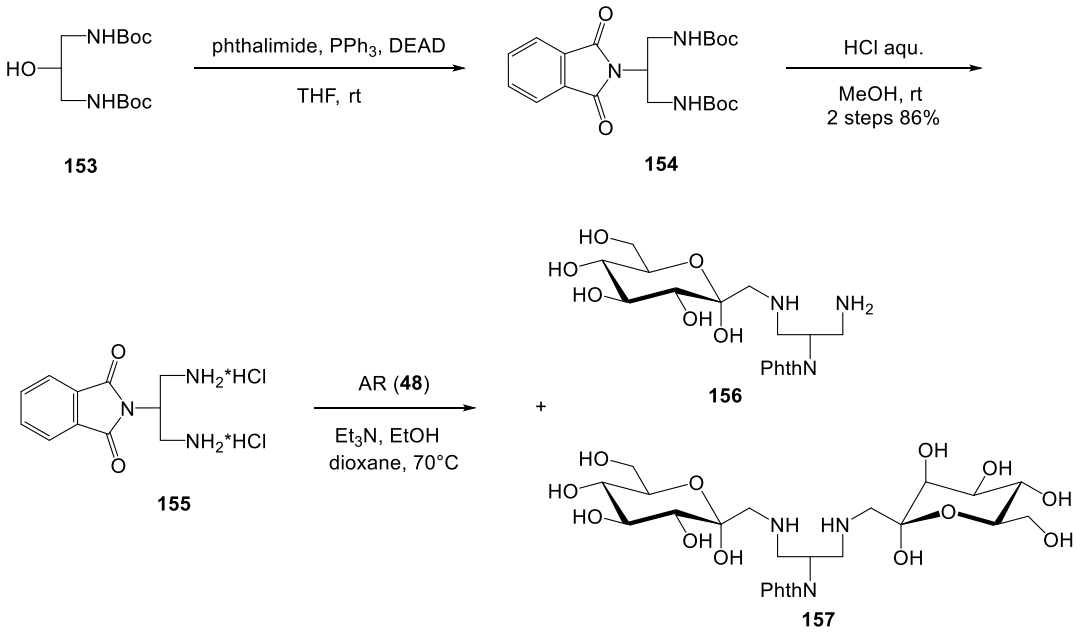
Scheme 44: Amadori rearrangement in the D-manno series employing divalent amines.

The same diamino components as well as the same purification protocol were applied in the synthesis of D-*manno* configured rearrangement products (Scheme 44). When D-*glycero*-D-*galacto*/D-*talo* aldoheptose (**110**) was treated with 1,6-diaminohexane, the bis-compound **148** was obtained in 60% yield. Employing *p*-xylenyldiamine, the Amadori rearrangement led to the desired product **149** in 61% yield. However, using 2,2´-(ethylenedioxy)bis(ethylamine) under the same reaction conditions and in analogy to the reaction with aldoheptose **110**, the disubstituted product **150** was isolated in 29% yield and the corresponding monosubstituted product **151** was obtained in 55% yield. In case of 4,7,10-trioxa-1,13-tridecanediamine as amino component and

employing an excess of sugar substrate, again the double Amadori product **152** was obtained exclusively in 54% yield.

As mentioned previously, due to the favored all-equatorial orientation of the hydroxyl groups in D-*gluco* configured product, we expected that the yields in this series should be slightly higher as in the case of D-*manno* configured products, where the hydroxyl group in position C-3 is found in the less favored axial position. Also in this study we did not observe considerable differences in terms of the isolated yields in both series.

In addition, we also investigated the synthesis of diamino spacers. 1,3-Diamino-2-propanol was treated with 2 equivalents of di-*tert*-butyl dicarbonate leading to diprotected compound **153**,[223] followed by conversion of the alcohol function to the phthalimide protected amine *via* the Mitsunobu reaction.[224] Therefore, the alcohol was treated with triphenylphosphane (PPh_3), diethyl azodicarboxylate (DEAD) and phthalimide to achieve compound **154**. Due to the high excess of phthalimide (3 equivalents) used in this reaction, purification of the crude product turned out to be tedious. After acid cleavage of the Boc protection group with aqueous HCl solution in MeOH, the solvent was removed under reduced pressure. The residue was taken up in CH_2Cl_2 and extracted with H_2O, whereby, the remaining phthalimide from the previous step was dissolved in the organic phase. The aqueous phase was then extracted with EtOAc to remove the hydrazine derivative and compound **155** was obtained in 86% overall yield starting from compound **153**. The amine hydrochloride **155** was treated with Et_3N in EtOH to give the free amines which was then reacted with aldoheptose **48** (2 equivalents) under typical reaction conditions (Scheme 45). NMR measurements showed that monomeric (**156**) as well as dimeric rearrangement (**157**) products were formed in a ratio of 1:1. Due to their same polarity, separation of the two rearrangement products *via* silica gel chromatography could not be achieved.

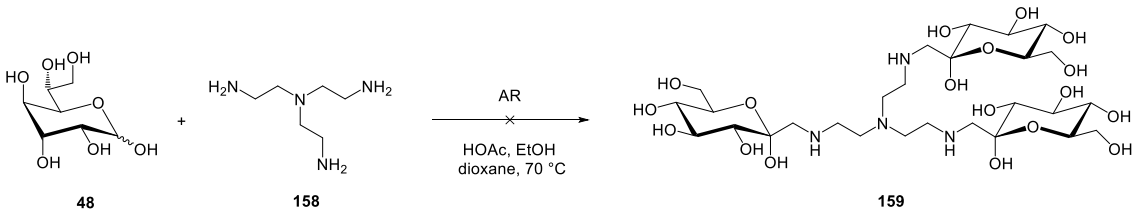


Scheme 45: Synthesis of divalent amino spacer **155** and following Amadori rearrangement employing aldoheptose **48**.

3.2.3. Trivalent amines

Multivalent glycoconjugates which offer well-defined structures are known to have a considerable potential as inhibitors of cell surface protein-carbohydrate interactions,[225] including cell adhesion, host invasion by pathogens, pathogen neutralization by host and numerous cell regulatory signaling pathways.[226] Multivalency is very important for carbohydrate-receptor interactions because individual glycans may bind with low affinity to a single binding site, whereas glycoclusters can create a high-avidity interaction with clustered binding sites, which is also known as the "carbohydrate cluster effect".[227]

Therefore, we were interested in applying the Amadori rearrangement for the synthesis of multivalent neoglycoconjugates, such as trivalent Amadori products. First, tris(2-aminoethyl)amine (**158**) were treated with 3 equivalents of aldoheptose **48** (Scheme 46) under reaction conditions for the Amadori rearrangement, detection of product formation by normal phase TLC as well as reversed phase TLC employing various solvent mixtures was not satisfying (Table 9). In all cases, the detection of the progress of the reaction was not possible *via* TLC because the presumed product spot barely moved from the starting front of the TLC plate. Peracetylation of the reaction mixture in pyridine using acetic anhydride and catalytic amounts of DMAP gave only the peracetylated aldoheptose. Isolation of the desired product failed.



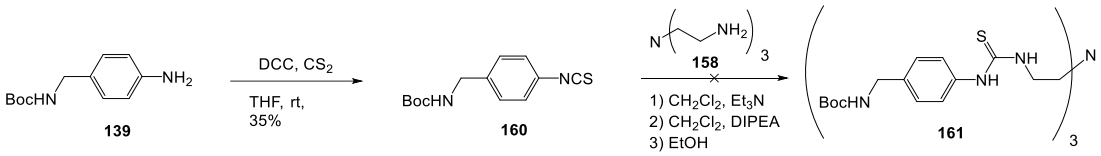
48 158 159

Scheme 46: Amadori rearrangement employing trivalent amine tris(2-aminoethyl)amine (**158**).

Table 9: Investigation of different solvent mixtures for TLC detection of compound **159**.

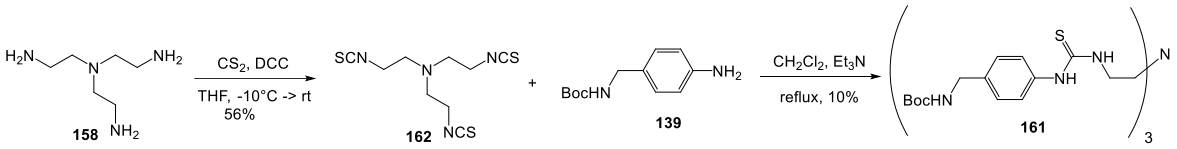
Normal phase TLC	Reversed phase TLC
$CHCl_3$/MeOH/NH_4OH 1:10:4	MeCN/MeOH 1:5
MeOH/NH_4OH 10:3	MeCN/H_2O/NH_4OH 2:6:1
MeCN/H_2O/NH_4OH 1:1:1	H_2O
EtOAc/MeOH/H_2O/NH_4OH 6:2:2:1	
EtOAc/MeOH/H_2O/HOAc 2:2:2:1	
Acetone/MeOH/MeCN/H_2O/NH_4OH 2:4:2:1:1	
Acetone/MeOH/MeCN/H_2O/HOAc 2:8:2:1:1	
NH_4OH/EtOH 4:1	

Next, we synthesized trivalent amino moieties carrying a thioisocyante which can be used for thiurea-bridging as alternative ligation method. This ligation method allows the conjugation with amino-functionalized Amadori rearrangement compounds forming the desired trivalent products. In the first step, 4-[(*N*-boc)aminomethyl]aniline (**139**) was treated with 4 equivalents of *N,N'*-dicyclohexylcarbodiimide (DCC) and an excess of carbon disulfide (CS_2) in THF. The reaction mixture was stirred at room temperature overnight, the solvent was removed under reduced pressure and purified by silica gel chromatography to obtain compound **160** in 35% yield. Compound **160** should be conjugated to tris(2-aminoethyl)amine (**158**) by thiourea-bridging furnishing compound **161** (Scheme 47). This conjugation step was carried out once in CH_2Cl_2 using Et_3N or Hünig´s base (DIPEA)[228] or in EtOH.[229] However, no product formation was detectable. Heating the reaction mixture at reflux failed as well as further addition of isothiocyanate.



Scheme 47: Thiourea-bridging as ligation method for the synthesis of compound **161**.

Another strategy was to generate first tris(2-isocyanatoethyl)amine (**162**)[230] from tris(2-aminoethyl)amine (**158**) by the treatment with CS_2 and DCC in THF. Compound **162** was conjugated with 4-[(*N*-boc)aminomethyl]aniline (**139**) leading to compound **161** (Scheme 48) in very low yields (10%).



Scheme 48: Thiourea-bridging as ligation method for the synthesis of compound **161**.

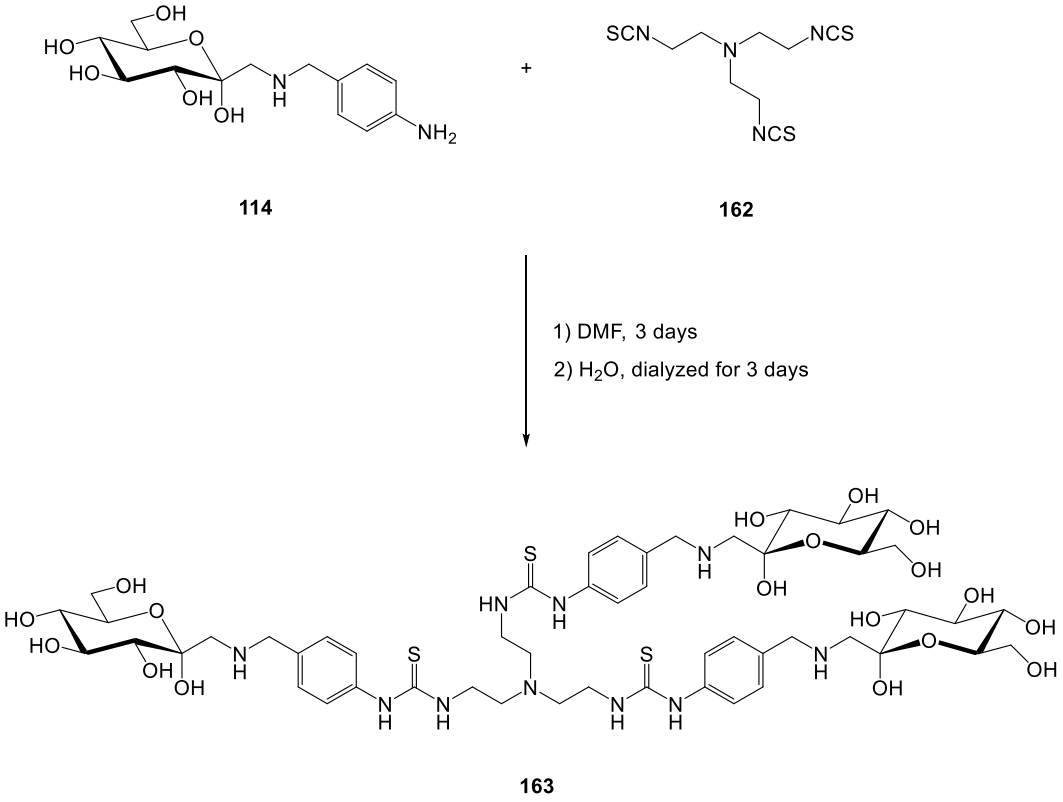
For this reason, we investigated a third synthetic pathway to obtain the desired trivalent Amadori rearrangement product. Amadori product **114** was treated with tris(2-isocyanatoethyl)amine (**162**). For this reaction, several different solvent mixtures as well as basic conditions were evaluated, conditions are listed in Table 10.

Table 10: Reaction conditions for the synthesis of the trivalent Amadori product **163**.

Entry	Solvents	Base	Product formation
1	MeOH/H_2O (1:1)	Et_3N	-
2	CH_2Cl_2/MeOH	$NaCO_3$	-
3	DMF		+
4	DMF	DIPEA	+
5	H_2O		+

Using Et_3N as base and a solvent mixture of MeOH/H_2O (1:1 v/v), no product formation was observed after a reaction time of 3 days. Also, in case of employing CH_2Cl_2/MeOH (1:1 v/v) as solvent mixture and $NaCO_3$ as base, the amino-functionalized rearrangement product **114** did not react with the thioisocyanate (**162**) to compound **163** after a reaction time of 4 days. When DMF was used as solvent without the addition of any base, desired product **163** was isolated in a yield of only 12%. To increase the yield, DIPEA[228, 231] was added to the reaction mixture, however, instead of enhancing the product formation, a polymer was obtained which was insoluble in any solvent. Therefore, following the protocol from Kiessling *et al.*[232] for the

synthesis of multivalent ligands, the D-*gluco* configured amino-functionalized Amadori product **114** was reacted with tris(2-isocyanatoethyl)amine (**162**) in DMF for 3 days. After addition of water, the product was dialyzed (100 molecular weight cutoff (mwco)) for 72 hours, whereby during this purification time, the water was exchanged seven times. By this method, desired trivalent compound **163** was obtained in a yield of 67% (Scheme 49).



Scheme 49: Synthesis of the trivalent Amadori rearrangement product **163** *via* thiourea-bridging.

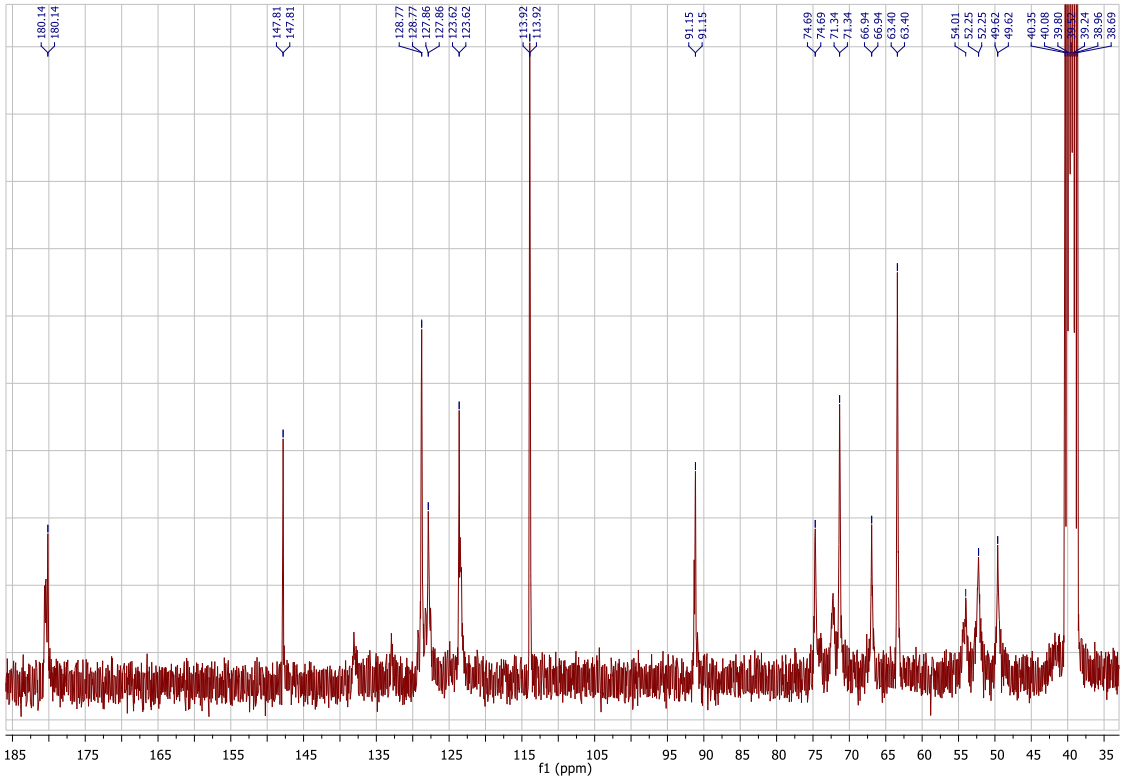
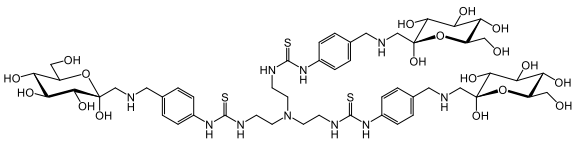


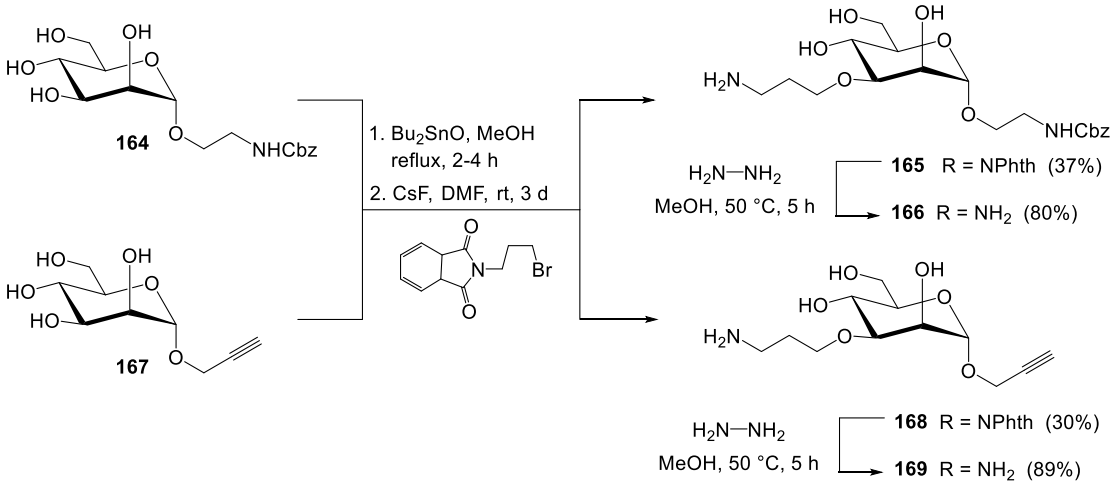
Figure 44: 13C spectrum of compound 163.

Table 11: 13c NMR siganls of compound **163**.

	3 x C-13	180.6 – 180.1 ppm
	3 x phenyl	147.8 – 113.8 ppm
	3 x C-2	91.3 – 91.0 ppm
	3 x C-6, 3 x C-5, 3x C-4, 3x C-3	74.8 – 66.8 ppm
	3x C-7	63.4 - 63.3 ppm
	3 x C-1	54.5 – 54.3
	3 x C-15	52.6 – 52.4 ppm
	3 x C-8	49.7 – 49.5 ppm
	3 x C-14	40.1 – 39.8

3.3. Amino-functionalized carbohydrates

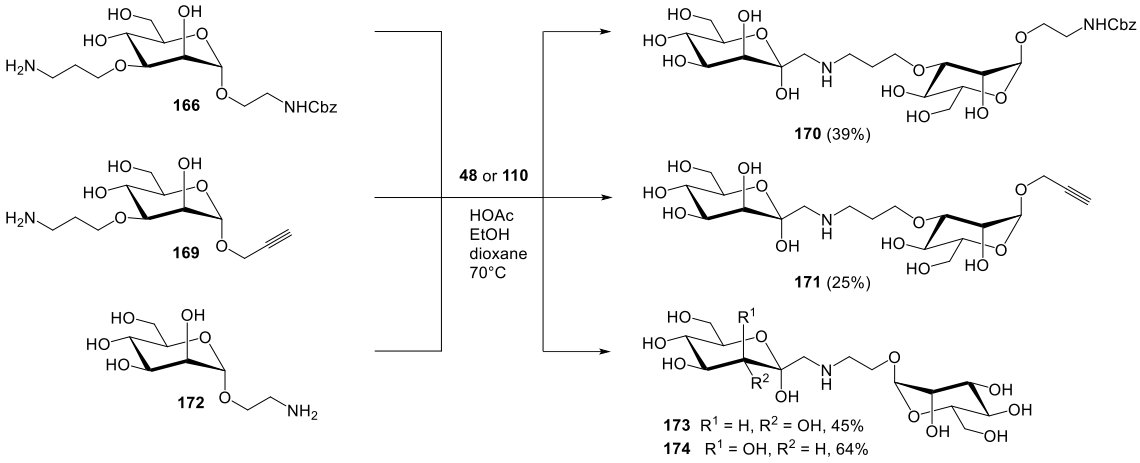
For the synthesis of more complex glycoconjugates using the Amadori rearrangement, amino-functionalized carbohydrates were employed as amino components. Therefore, two different 3-*O*-(aminopropyl)-functionalized mannosides (**166** and **169**) were synthesized by Anne Müller from the Lindhorst group using a regioselective, tin-mediated etherification of glycosides.[233] Thus, mannosides **164**[234] and **167**[235] were treated with bis-dibutyltin oxide, followed by subsequent opening of the intermediate stannylidene acetals with *N*-(3-bromopropyl)phthalimide to give the respective 1,3-functionalized mannosides **165** and **168** in moderate yields. This protocol leads to a direct access of selectively 3-*O*-functionalized mannosides having the advantage that no protecting group chemistry is necessary. After hydrazinolysis,[233] the respective amino compounds **166** and **169** were obtained in 80% and 89% , respectively (Scheme 50).



Scheme 50: Regioselective synthesis of amino-functionalized mannosides **166** and **169**.

Compounds **166** and **169**, carrying a free amino group at position C-3 as well as 2-aminoethyl α-D-mannopyranoside **172**,[188b] carrying the amine in the aglycon part, were treated in the next step with aldoheptoses **48** and **110** to generate more complex glycoconjugates. 2-(Benzyloxycarbonyl)amnioethyl 3-*O*-(3-aminopropyl)-*α*-D-mannopyranoside (**166**) gave Amadori rearrangement compound **170** in 39% yield. Employing propynyl-3-*O*-(3-aminopropyl)-*α*-D-mannopyranoside (**169**) as amino component, the rearrangement product **171** was isolated in 25% yield. Since the reactions had to be stopped after 5 days to avoid side product formation and compound degradation, the yields were rather moderate in these cases. Additionally, the purification of the obtained reaction mixtures was very challenging because of the very high polarity of the remaining starting materials as well as the generated products. Nevertheless, compound **170** and **171** offers orthogonal groups in the anomeric position allowing further

modification reactions. Compound **170** exhibits a Cbz-protected amine group and compound **171** carries a versatile propylene group; two attractive functional groups for further modification reactions. In particular, 2-aminoethyl α-D-mannopyranoside **172**[188b] was treated with D-*glycero*-D-*gulo* aldoheptose (**48**) as well as D-*glycero*-D-*galacto*/D-*talo* aldoheptose (**110**) to obtain the rearrangement products **173** in a yield of 45% and **174** in 64% yield, respectively (Scheme 51).

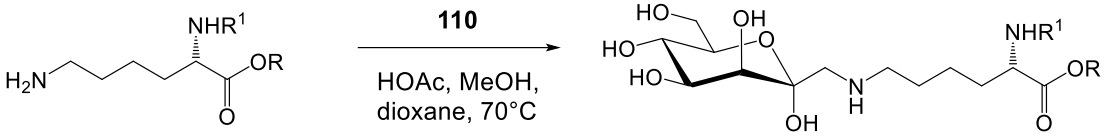


Scheme 51: Synthesis of heterobivalent glycoconjugates employing mannosides **166**, **169** and **172** as amine components in the Amadori rearrangement with aldoheptoses **48** or **110**.

3.4. Amino acids and peptides

Due to the versatile cellular functions of glycoconjugates, synthetic strategies towards the conjugation of carbohydrates to amino acid moieties are of high interest. Most methods employed for this purpose lead to hydrolytically labile *O*- or *N*-glycosidic linkages, whereas the Amdori rearrangement allows the access to stable *C*-glycosyl type glycoconjugates. Thus, we became interested to evaluate the Amadori rearrangement as conjugation/ligation method for the synthesis of amino acid glycoconjugates.

Partly protected lysine derivatives **175**, **177** and **179** were synthesized following standard procedures[236] to be employed as amino components in the Amadori rearrangement (Scheme 52).



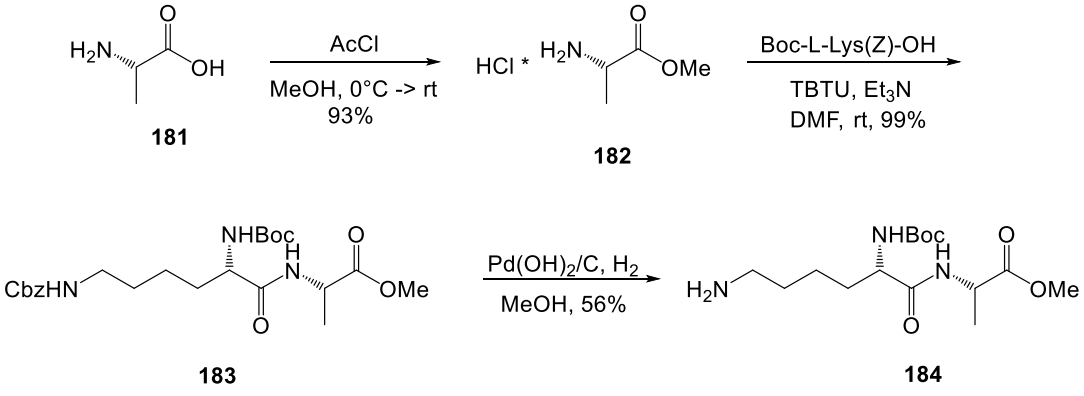
175: R = H, R^1 = Boc
177: R = H, R^1 = Cbz
179: R = Me, R^1 = Boc

176: R = H, R^1 = Boc, 63%
178: R = H, R^1 = Cbz, 65%
180: R = Me, R^1 = Boc, 68%

Scheme 52: Synthesis of Amadori rearrangement products **176**, **178** and **180** employing lysine derivatives **175**, **177** and **179**.

By treatment of N_α-(tert-butoxycarbonyl)-L-lysine (**175**) with D-*glycero*-D-*galacto*/D-*talo* aldoheptose (**110**) under acidic conditions, the corresponding Amadori rearrangement product **176** was obtained in a yield of 63%. Using N_α-(benzyloxycarbonyl)-L-lysine (**177**) as the amino component, the D-*manno* configured product **178** was isolated in 65% yield. Lysine derivative **179**[236-237] was synthesized by treating N_ε-(benzyloxycarbonyl)-N_α-(tert-butoxycarbonyl)-L-lysine with O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU) and Et_3N in methanol to generate the methyl ester followed by hydrogenolytical cleavage of the Cbz-protecting group to give the free amine at the ε-position, which was treated immediately with aldoheptose **110** to obtain the desired rearrangement product **180** in 68% yield (Scheme 52).

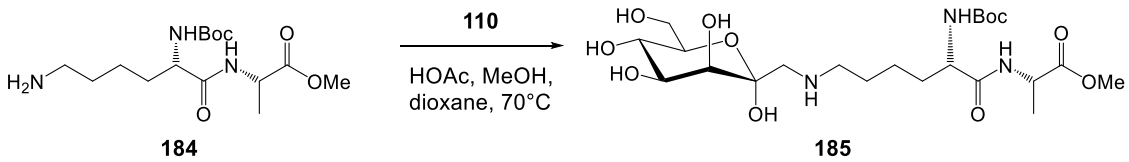
Next, for the synthesis of glycopeptides, dipeptide **184** and tripeptide **188** were utilized in the Amadori rearrangement which were synthesized using standard peptide coupling procedures (Scheme 53 and 55).[236]



Scheme 53: Synthesis of the dipeptide derivative **184**.

The preparation of the L-lysine containing dipeptide building block (**184**) started with the synthesis of L-alanine methyl ester hydrochloride (**182**) obtained from L-alanine (**181**) employing anhydrous HCl in methanol. After activation of Boc-L-Lys(Z)-OH with TBTU and Et_3N, the peptide

coupling with **182** was achieved in 99% yield of Boc-L-Lys(Z)-L-Ala-OMe (**183**). To generate the free amine **184** at the ε-position, the Cbz-protecting group of compound **183** was cleaved using standard deprotection conditions[238] in dry methanol containing a catalytic amount of $Pd(OH)_2/C$. The reaction mixture was stirred overnight under a hydrogen atmosphere at ambient pressure. After filtration of the catalyst and removal of the solvent, compound **184** was isolated in 56% which was immediately treated with aldoheptose **110** to obtain the rearrangement product **185** in 62% yield (Scheme 54). Immediate use of amine **184** is of crucial importance as upon storage decomposition of this compound occurs.



Scheme 54: Synthesis of the Amadori rearrangement **185** using the dipeptide **184** as amino component.

For the synthesis of the desired tripeptide (**188**) which resembles the C-terminal structure of the β-chain of pig-insulin (Figure 45),[239] compound **183**, an intermediate from the synthesis of the dipeptide (**184**), was treated in dry methanol containing anhydrous HCl to cleave the Boc-protective group. The same coupling procedure as mentioned above was applied to compound **186** with Boc-L-proline to generate intermediate **187**. The final step was a hydrogenolytic cleavage of the Cbz-protective group to furnish the desired tripeptide **186** (Scheme 55). To prevent its degradation, the tripeptide was treated immediately after amine liberation (Scheme 56).

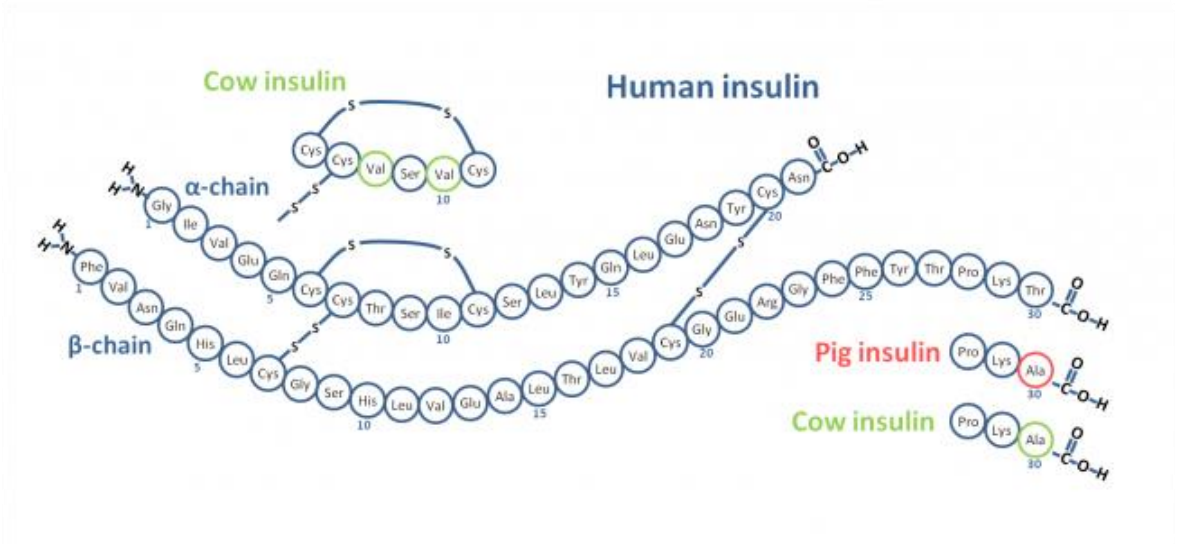
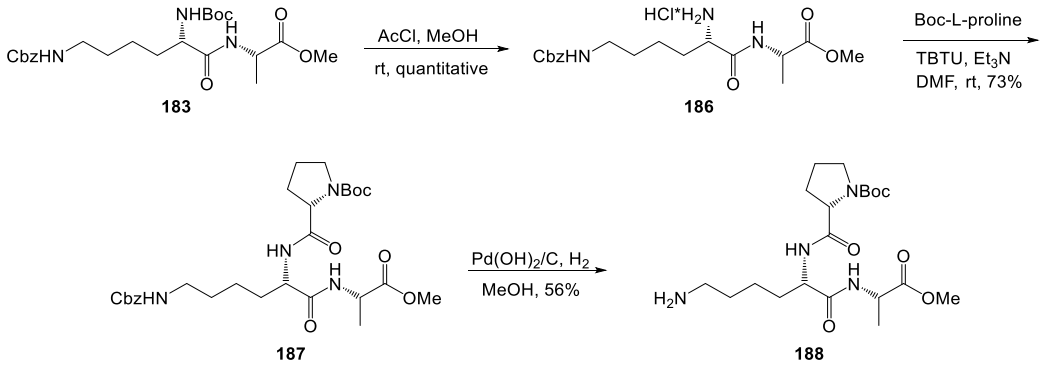
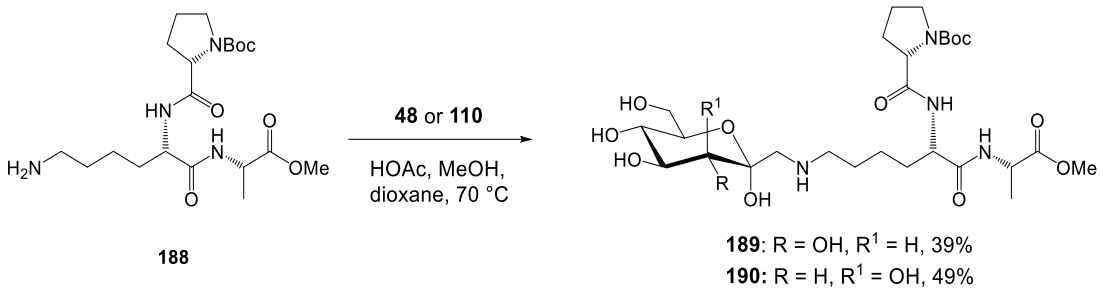


Figure 45: Amino-acid compositions of human, cow and pork insulin.[240]



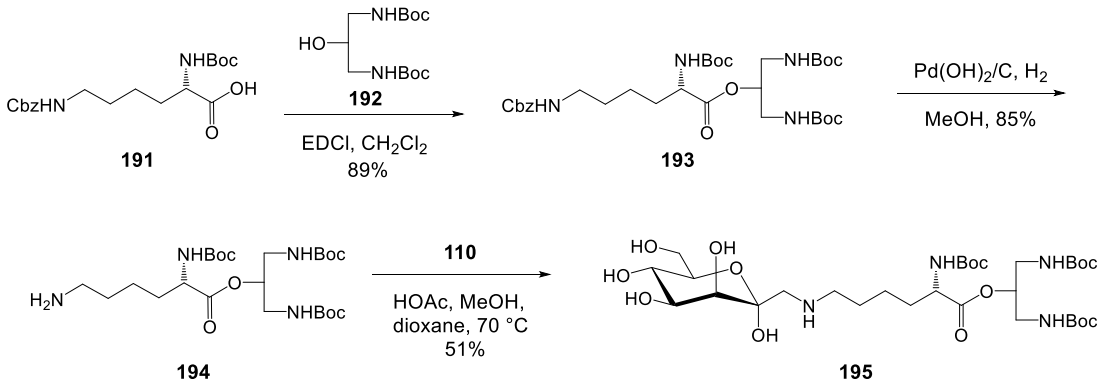
Scheme 55: Synthesis of the tripeptide **188**.

Tripeptide **188** gave in the Amadori rearrangement with D-*glycero*-D-*gulo* aldoheptose (**48**) and D-*glycero*-D-*galacto*/D-*talo* aldoheptose (**110**), desired compounds **189,** (39%) and **190** (49%), respectively (Scheme 56).



Scheme 56: Synthesis of glycopeptides **189** and **190** applying the Amadori rearrangement with tripeptide **188** as amino component.

To enable further modification reactions at the amino functions, component **194** was synthesized as a building block for the synthesis of a more complex lysine-containing Amadori rearrangement product. Thus, the two amino groups of 1,3-diamino-2-propanol (Scheme 57) were initially protected as *tert*-butyldicarbonyldicarbonates (**192**),[241] the secondary alcohol of this biscarbamate was treated with N_{ε}-(benzyloxycarbonyl)-N_{α}-(tert-butoxycarbonyl)-L-lysine (**191**) in presence of EDCI (*N*-(3-dimethylaminopropyl)-*N'*-ethylcabodiimide hydrochloride) as an activator to provide ester (**193**).[242] Hydrogenolytical removal of the Cbz protecting group afforded the free amine **194** which was employed as the amino component in the Amadori rearrangement with aldose **110** to obtain compound **195** in 51% yield. Since this reaction is acid catalyzed, a small amount of the ester was hydrolyzed explaining the moderate yield of the rearrangement product **195** (Scheme 57).

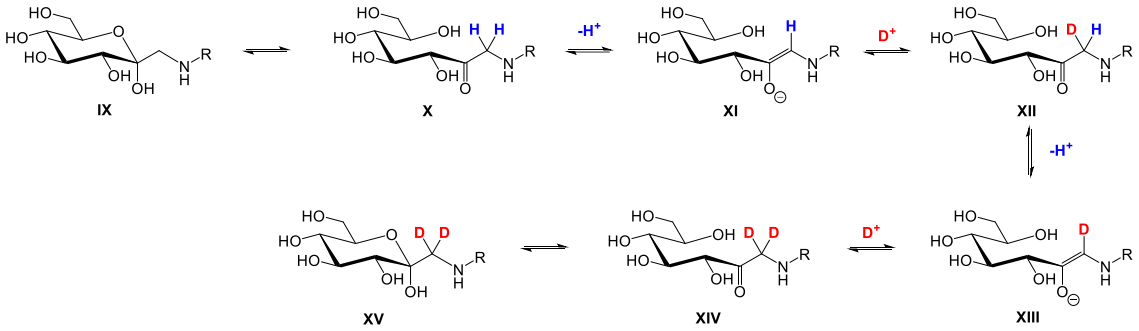


Scheme 57: Synthesis of amino component **194** and following Amadori rearrangement to obtain product **195**.

3.5. H/D exchange

During our investigations of the Amadori rearrangement we observed an H/D exchange at position C-1 of the rearrangement products during NMR studies.

In 1983 Heyns and coworkers[243] observed during the NMR studies of *N*-(1-deoxy-D-fructos-1-yl)-L-amino acids (fructose-amino acids) that protons at position C-1 undergo a slow H/D-exchange. On prolonged storage of the amino acid-fructose Amadori rearrangement compounds in D_2O solution, the signal intensity for H-1 decreased based on the isotopic exchange, which accelerates with increasing pH value (Scheme 58).



Scheme 58: Proposed mechanism of H/D exchange of Amadori products.[244]

This isotopic exchange is initiated by formation of the open chain structure (**X**) of the Amadori rearrangement product which contains the free keto-function (**X** and **XII**). During the reaction an enolate ion is generated (**XI** and **XIII**) and this intermediate facilitates the H/D exchange.[244]

Such an isotopic exchange was observed in our studies during the NMR measurement of the synthesized disubstituted Amadori rearrangement products (**145**, **147** and **150**) in D_2O. As mentioned above, this exchange accelerates with higher pH values. In particular, due to the purification method described for compounds (**143**-**147** and **148**-**152**) employing ion exchange CG-120-II (Na^+) Amberlite resin column, a pH value of about 9 was generated in the product fraction. This basic pH value accelerates the H/D exchange in D_2O solution significantly.

In the course of NMR measurement for compound **145,** the signal according to C-1 in the carbon NMR (Figure 46) splits into a pentet signal and likewise, the signals of both protons at position C-1 were not detectable in the proton NMR (Figure 47) after a few minutes.

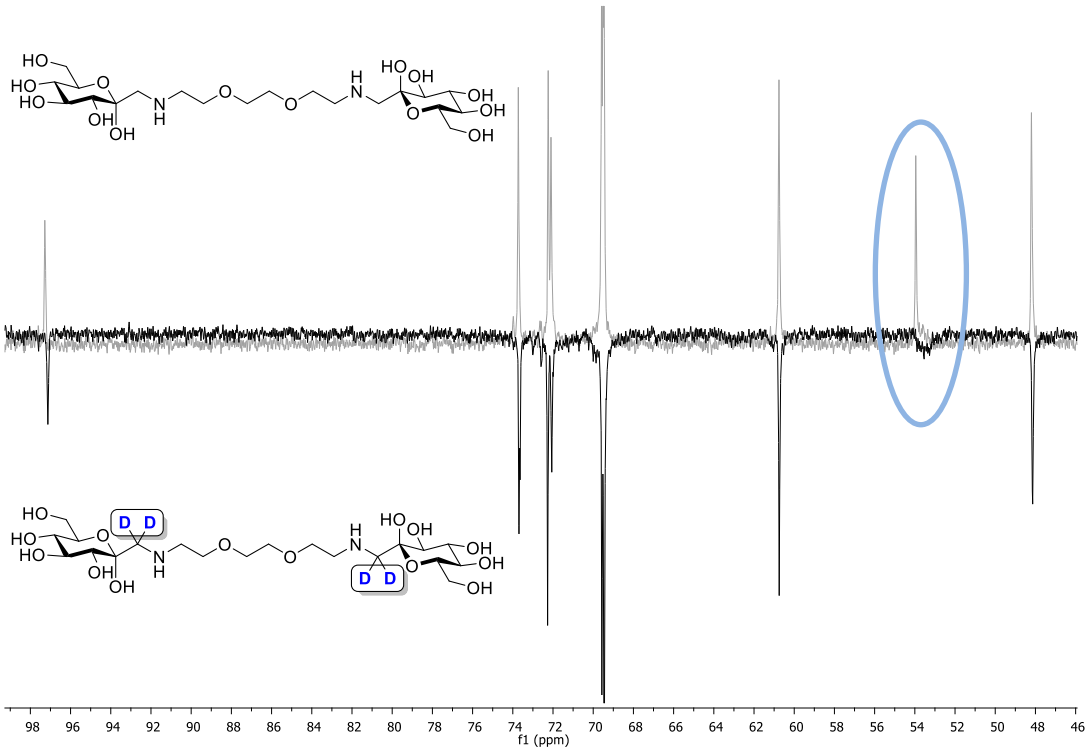


Figure 46: H/D exchange of Amadori product **145**; ^{13}C NMR.

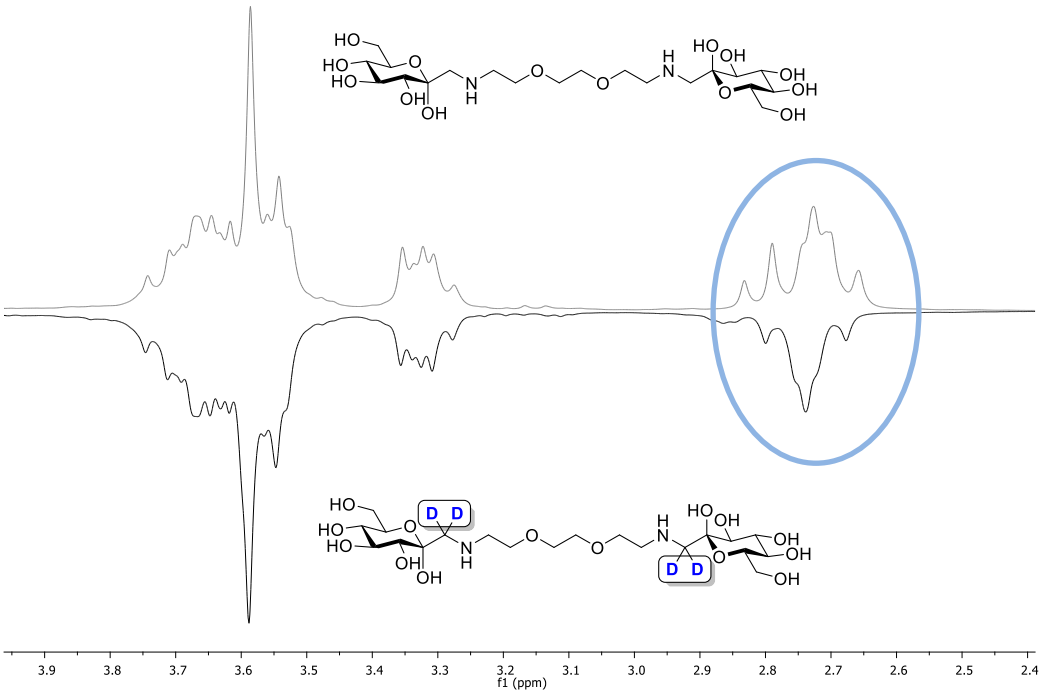


Figure 47: H/D exchange of Amadori product **145**; ^{1}H NMR.

When applying D_2O in the NMR measurements, this phenomenon was noticed with the disubstituted rearrangement products **145, 147** and **150** as well as with the rearrangement

products **170** and **171** using the amino-functionalized carbohydrate moieties, which are illustrated in Figures 48 to 53.

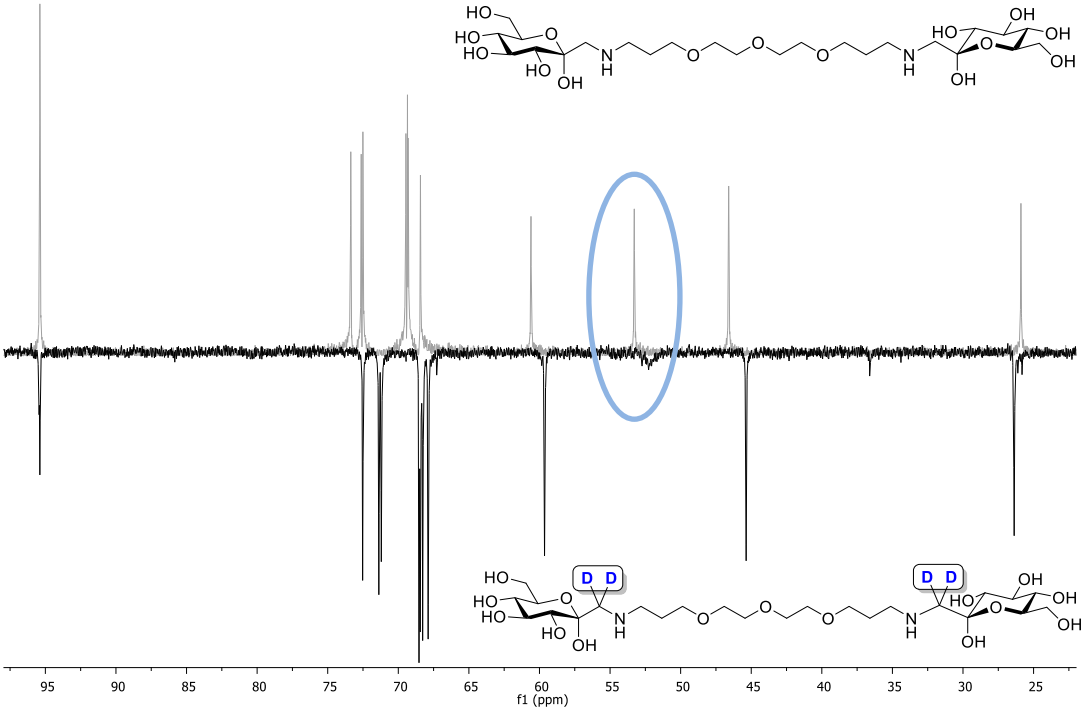


Figure 48: H/D exchange of Amadori product **147**; ^{13}C NMR.

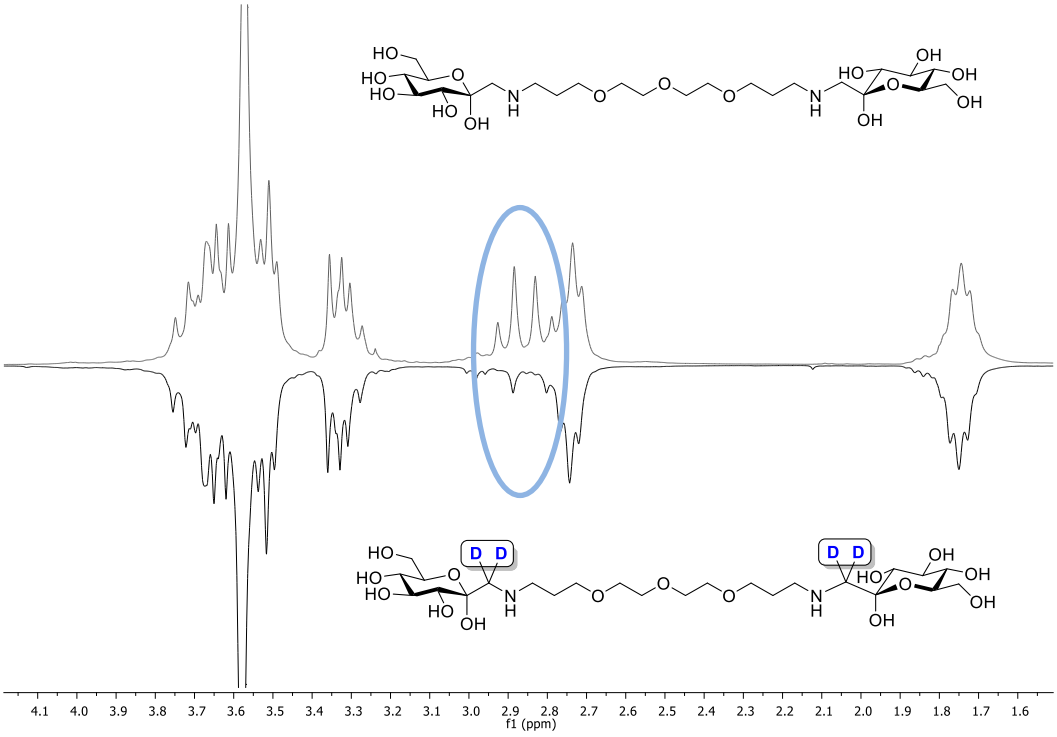


Figure 49: H/D exchange of Amadori product **147**; ^{1}H NMR.

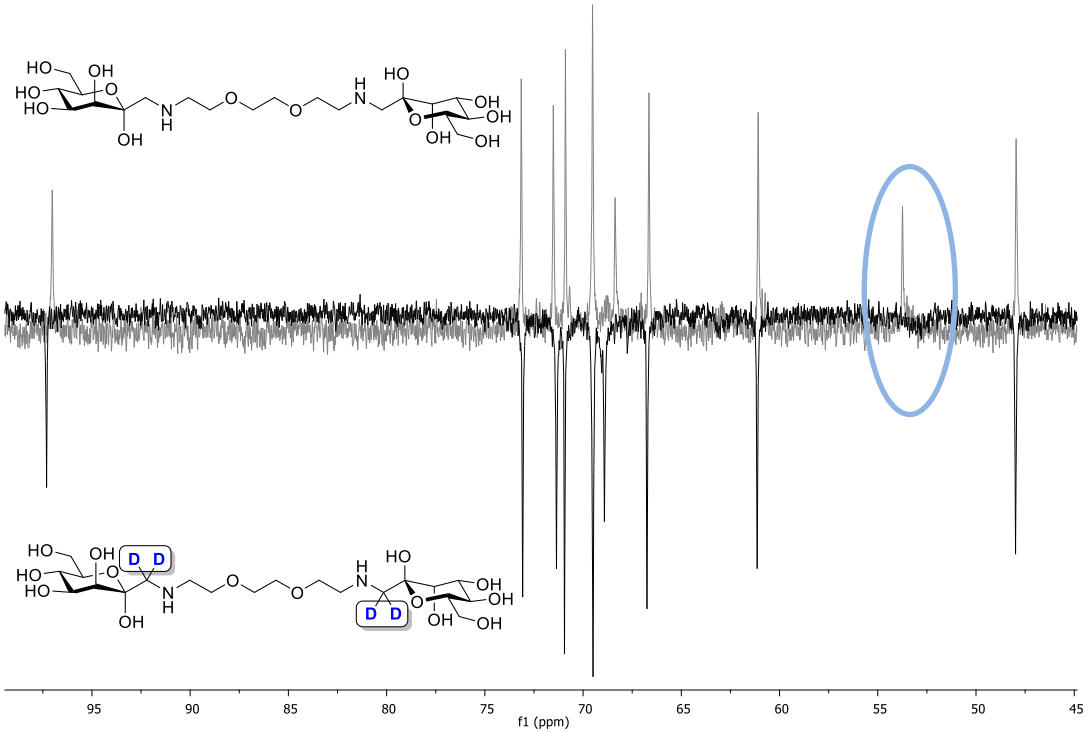


Figure 50: H/D exchange of Amadori product **150**; ^{13}C NMR.

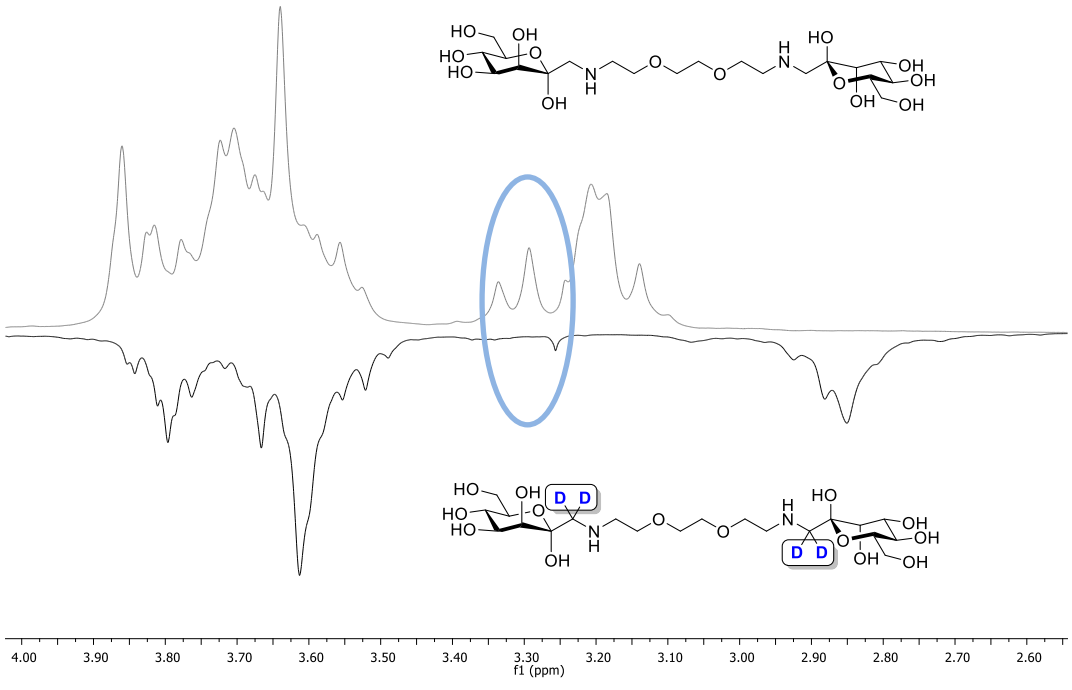


Figure 51: H/D exchange of Amadori product **150**; ^{1}H NMR.

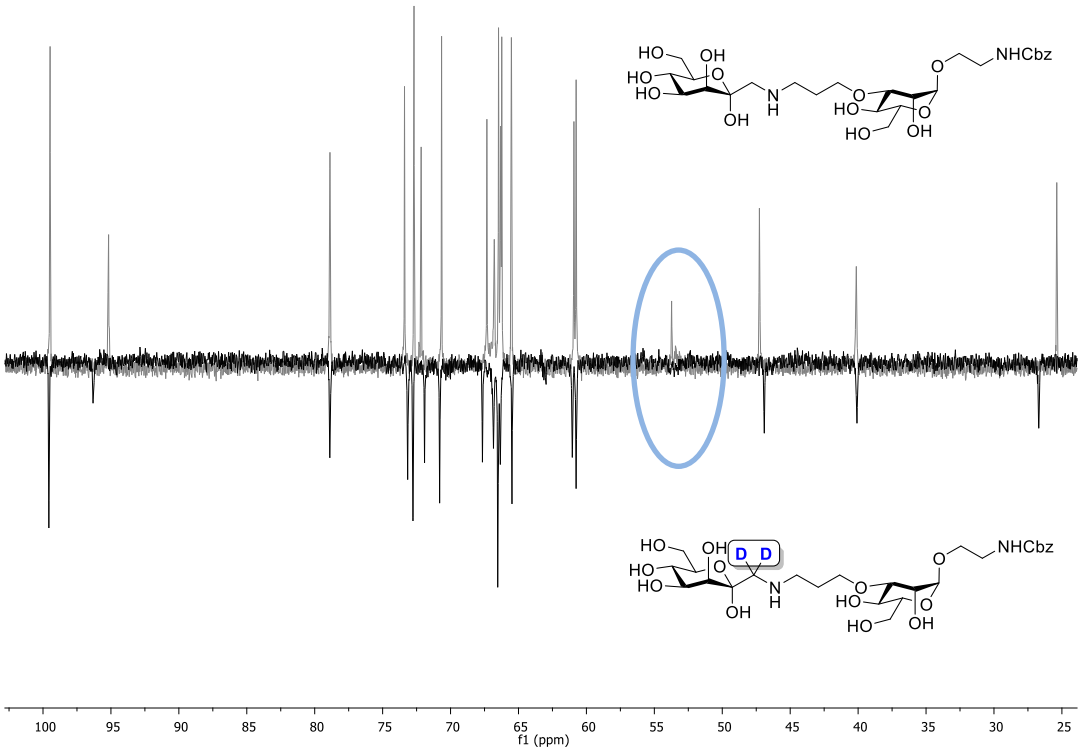


Figure 52: H/D exchange of Amadori product **170**; ^{13}C NMR.

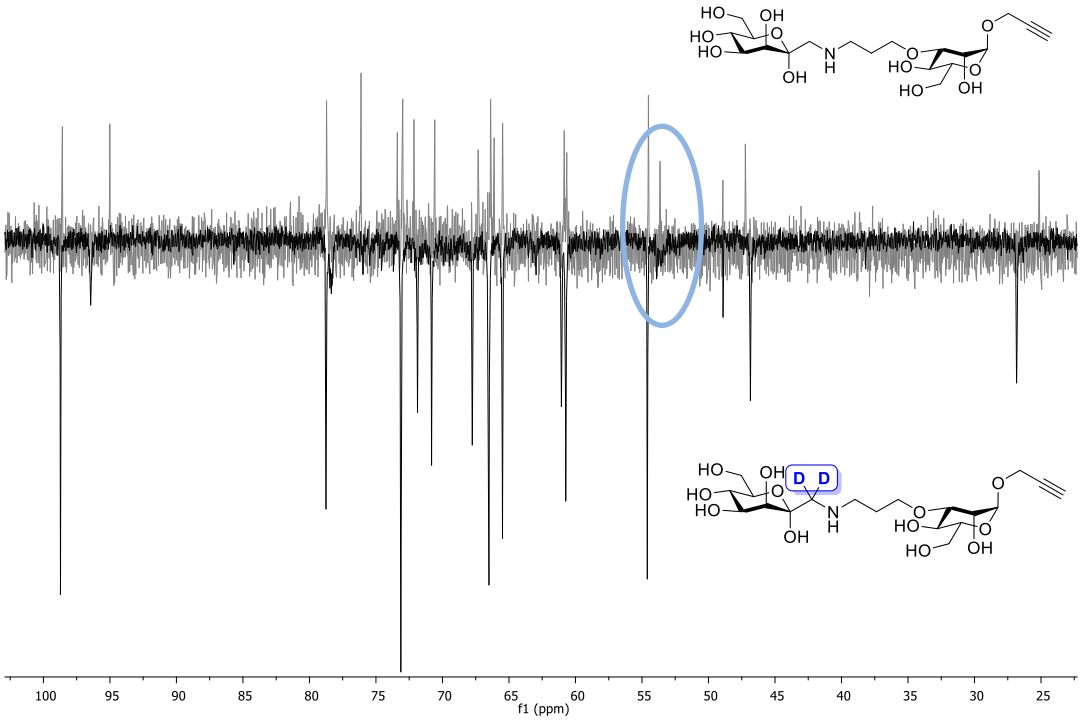


Figure 53: H/D exchange of Amadori product **171**; ^{13}C NMR.

To circumvent the fast isotopic exchange in D_2O solution, the NMR samples of respective rearrangement products were recorded in H_2O/D_2O (9:1 v/v). In this case, an exchange was monitored as well, but significantly slower. Dissolving the products in H_2O dest. and using a D_2O capillary as external reference to lock and shim, no H/D exchange was observed during the NMR measurements.

In order to prove this isotopic exchange, we carried out further NMR experiments. Compound **123** was dissolved in D_2O as well as in an aprotic solvent (DMSO-d_6) and the isotopic exchange was measured over a time period of thirteen days. As shown in Figure 54, the signal intensity for C-1 decreased slowly over a period of 5 days on prolonged storage in D_2O solution which exhibit a pH value of 7.

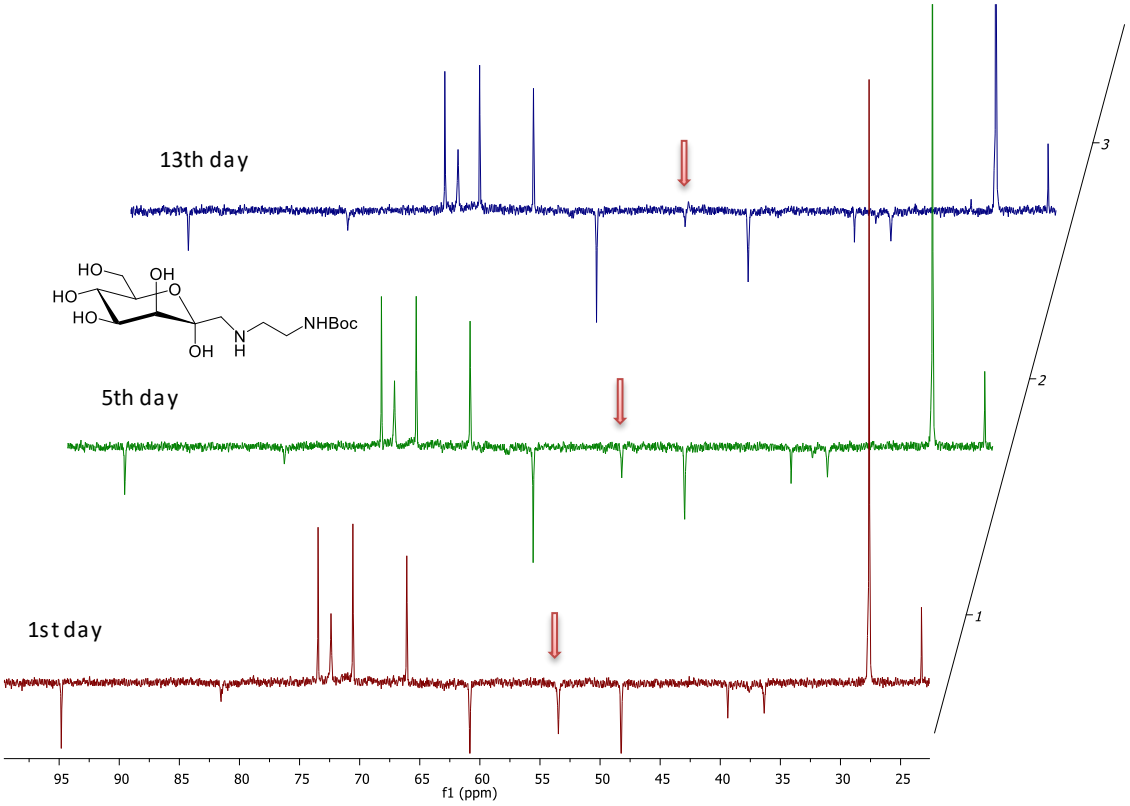


Figure 54: Time sequence of the isotopic exchange of compound **123** in D_2O solution.

In contrast, when DMSO-d_6 was employed as solvent system, as expected, no H/D exchange was observed over the same time period (Figure 55).

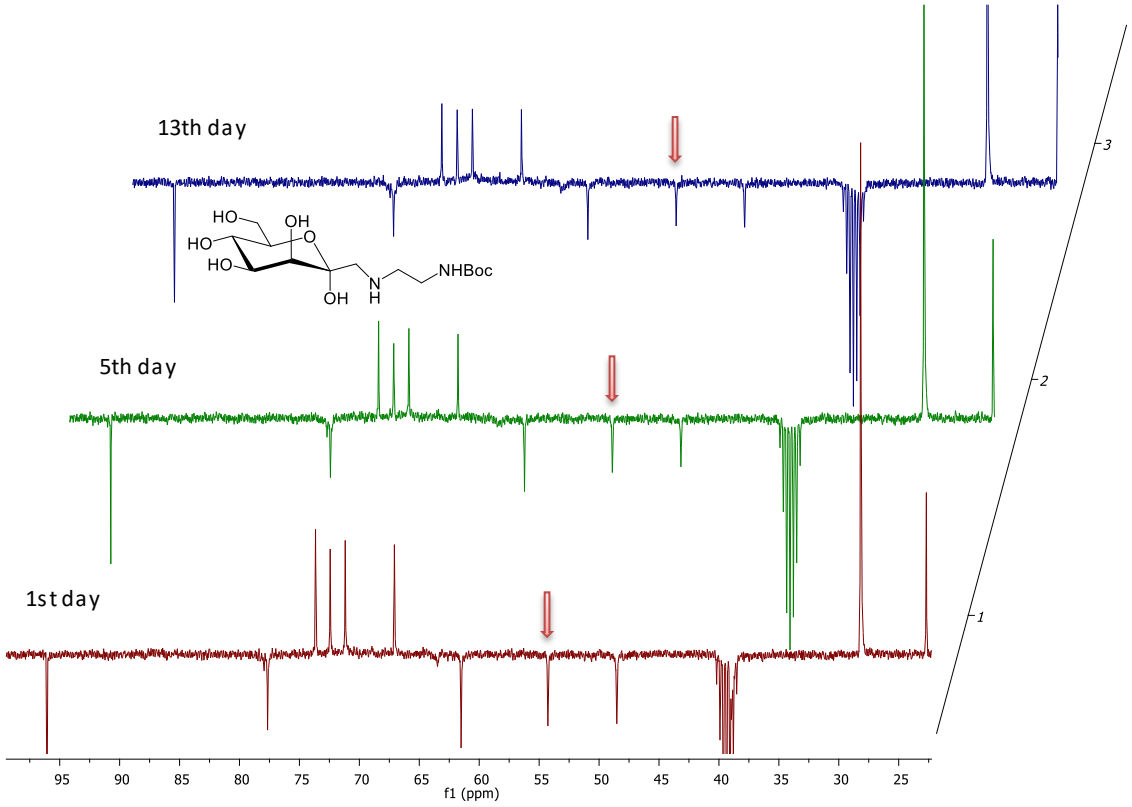


Figure 55: Time sequence of the isotopic exchange of compound **123** in DMSO-d_6 solution.

3.6. Biological evaluation

Two selected D-*manno* configured Amadori rearrangement products were investigated as potential ligands for mannose specific lectins of bacterial, plant as well as human source. Relevant tests were performed at the University of Kiel in the group of Prof. Lindhorst and Prof. Landemarre and co-workers at GLYcoDiag in France.

3.6.1. Bacterial lectin FimH

In context of anti-adhesion therapy, ligands for the bacterial lectin FimH are relevant as therapeutics against bacterial infections.[182] The key step in infection, which are caused by type 1-fimbriated bacteria, is the FimH-mediated adhesion od the bacteria to the glycosylated surface of host cells and hence FimH antagonists that inhibit bacterial adhesion can be precious for treatment of bacterial infection diseases.[181]

It is known that bacterial lectin FimH binds to α-mannosides, such as methyl α-D-mannoside (MeMan **196**) exclusively, because the receptor-binding lectin domain of the protein, named $FimH_L$, accommodates the CRD of FimH and selects out the α-configuration of D-mannose.[173] Furthermore, ligands can improve their affinity to FimH when they exhibit an aromatic aglycon, such as *p*-nitrophenyl α-D-mannoside (**197**) due to π-π-stacking interactions of the aromatic moiety with the so called tyrosine gate (Y48 and Y137) located at the entrance of the carbohydrate binding site.[172, 175] In addition, extending *p*-nitrophenyl α-D-mannoside by an *ortho*-chloro substituent (**90**) in the phenyl ring or squaric acid structures (**93**) (Figure 56), the ligand affinity for FimH can be further increased. [217, 245]

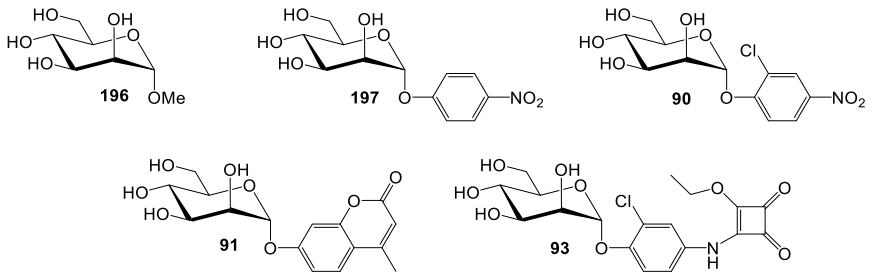


Figure 56: Ligands for the bacterial lectin FimH.

Methyl-α-D-mannoside **196** (MeMan) binds to FimH with an IC_{50} value (the concentration at which 50% of bacterial adhesion is prevented) in the millimolar range.[172] Compared to MeMan (**196**) as reference ligand, the affinity of the *p*NPMan derivative **90** is 717-times improved,[183] that of the methylumbelliferyl mannoside **91** 116 times improved,[184] and the affinity of the squaric ester monoester **93** is 6900 times higher.[185] The RIP value (relative inhibitory potency)

allows for the comparison of different inhibitors even when they were not tested on the same plate.

Two Amadori rearrangement products (**121** and **122**) were evaluated in inhibition-adhesion studies with type 1-fimbriated fluorescent *E. coli*.[246] Thus, these mannosides were tested as inhibitors of FimH-mediated bacterial adhesion to mannan employing a microtiter plate format and GFP-transfected *E. coli* (pPKL1162). To deliver sigmoidal inhibition curves from which IC_{50} values (the concentration at which 50% of bacterial adhesion is prevented) for both inhibitors were deduced, a serial dilution of compounds **121** and **122** in buffer were used. In order to correlate the inhibitory potency of **121** and **122** to that of MeMan (**196**), all assays were performed with MeMan tested in parallel on the same plate. From the deduced IC_{50} values, the relative inhibitory potencies (RIP values; with inhibitory potency of MeMan ≡ 1) can be calculated which allows for the comparison of different inhibitors even when they were not tested on the same plate.[217]

Using a published assay,[246] black 96-well microtiter plates were treated with a solution of mannan from *Saccharomyces cerevisiae* and desiccated overnight at 37 °C. After washing with PBST, the wells were blocked with PVA for 4 h at 4 °C. After washing the plates with PBST and PBS, solutions of Amadori compounds **121** and **122** as well as MeMan (**196**) were prepared (200 mM in PBS) and serial dilutions of each solution was added to the mannan-coated plates. Subsequently, the bacterial suspension (OD_{600} = 0.4, 50 µL/well) was added and the plates were incubated for 1 h at 37 °C and 100 rpm. After washing with PBS, the fluorescence intensity (485 nm/535 nm) was determined.

Each inhibitor was tested at least in triplicate and in parallel with the standard inhibitor MeMan (**196**) on the same plate. The sigmoidal concentration-response curves were fitted by non-linear regression. Error bars are standard deviations from multiple testing results on one plate (Figure 57 and 58).

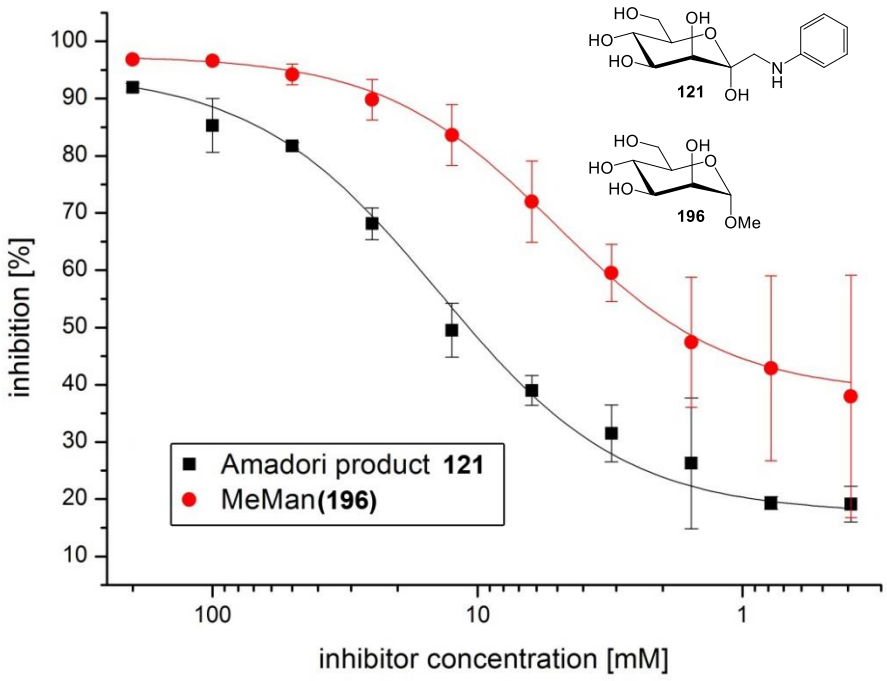


Figure 57: Inhibition curve obtained with Amadori rearrangement product **121** from inhibition of type 1 fimbriae-mediated bacterial adhesion to mannan.[217]

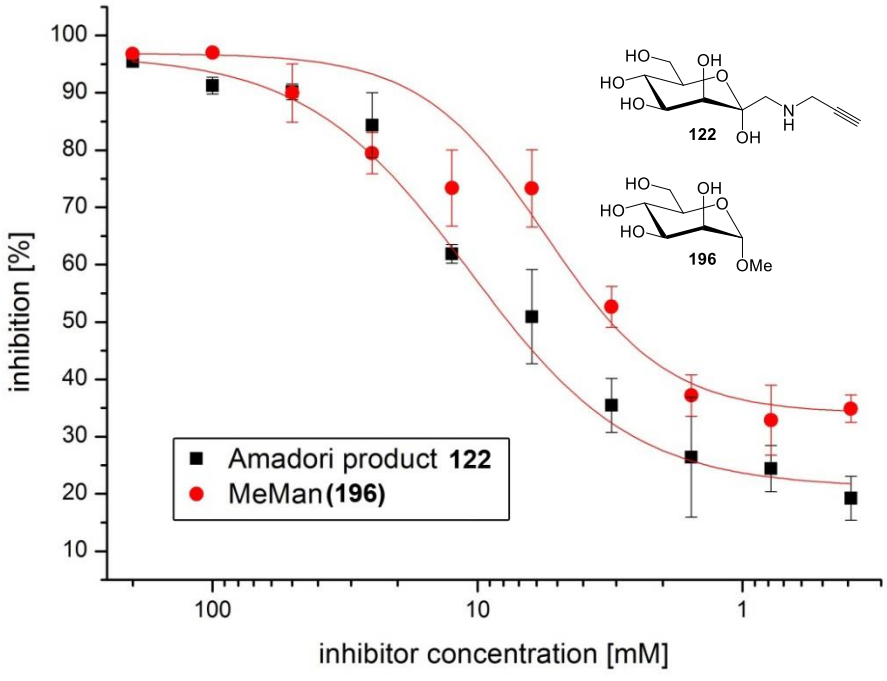


Figure 58: Inhibition curve obtained with Amadori rearrangement product **122** from inhibition of type 1 fimbriae-mediated bacterial adhesion to mannan.[217]

From the calculated RIP values listed in Table 10, it could be concluded that both Amadori products, **121** and **122,** show weaker inhibition to FimH than the internal standard MeMan. Counterintuitively, these results showed that the propynyl derivative **122** exhibited a slightly better binding compared to compound **121**, which carries an aromatic system.

Table 12: Inhibition of bacterial adhesion (*E. coli*) to manna–coated surface. The inhibitory potencies of the Amadori products were compares to the standard inhibitor MeMan.

	Compound 121	Compound 122
IC_{50} ± SD (mM)	10.811 ± 1.470	7.625 ± 1.146
RIP (MeMan)	0.16	0.41

In order to understand the results from the inhibition-adhesion assays, we investigated molecular docking studies, carried out by Insa Stamer form the Lindhorst group. The complexation of the standard inhibitor MeMan (**196**) in the carbohydrate recognition domain of FimH has been described in chapter 1.4 and is depicted in a cartoon fashion in Figure 40. The methoxy group of the glycoside located in the α-orientation of the aglycone is pointing towards the binding site, whereas all hydroxyl groups of the sugar ring are complexed within the FimH carbohydrate binding domain. A conserved water molecule inside this carbohydrate binding site can further support the complexation of mannoside ligands because this water molecule can interact predominantly with the axial oriented 2-OH group of the sugar ring. Comparing the complexation of MeMan (**196**) with the structural difference of the D-*manno* configured Amadori rearrangement products **121** and **122**, the axial methoxy group in MeMan can be correlated with the equally axial oriented anomeric hydroxyl group of the Amadori products. In addition, the (N-aryl/alkyl amino) methylene groups in **121** and **122** are located in the β-face and hence can cause a steric clash in the binding pocket due to their bulkiness (Figure 59, route a).[217]

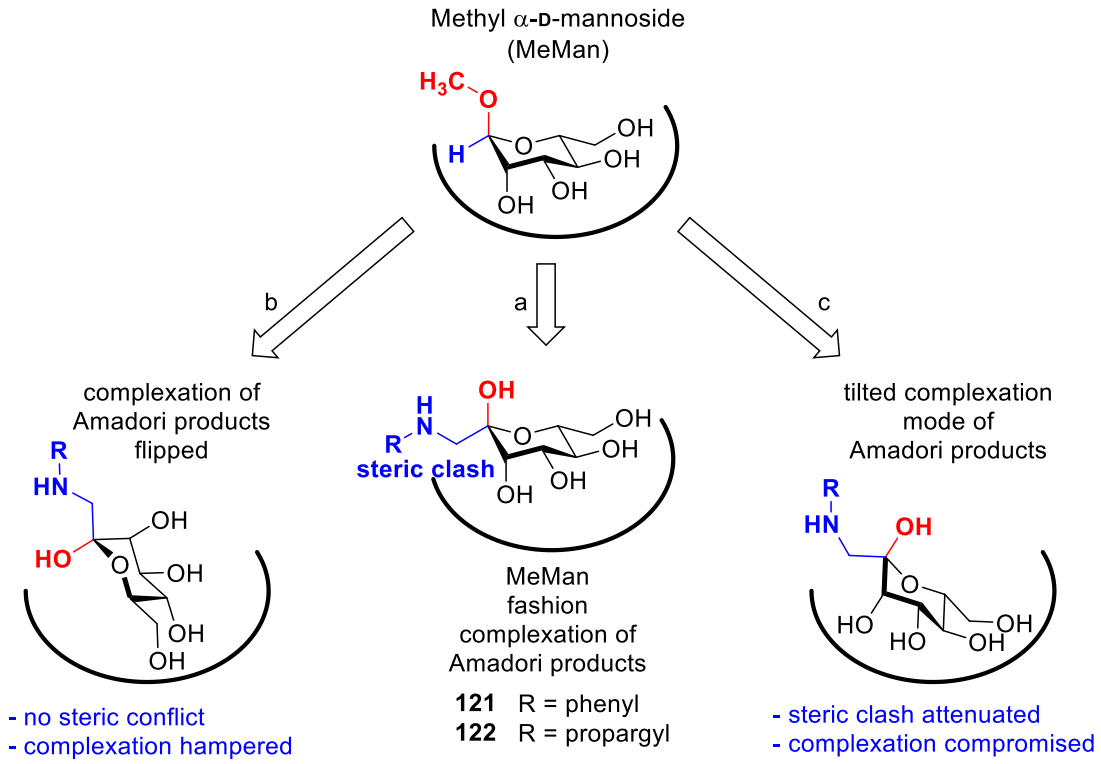


Figure 59: Cartoon illustrating ligand binding by the bacterial lectin FimH. Complexation of D-manno configured Amadori rearrangement products inside the carbohydrate recognition domain of FimH in analogy to MeMan.[217]

To circumvent the steric conflict of the bulky aminomethyl substituent, this group should be pointing outwards of the sugar binding site achieved by flipping of the Amadori products (Figure 59, route b). However, in this type of complexation, proper complexation of the sugar ring will be hampered because the ring hydroxyl groups are not able to accomplish the hydrogen bonding due to the considerable alteration of its 3D pattern. In another mode, the Amadori product could be tilted, as indicated in Figure 59 (route c), that such a complexation mode would allow the possibility that such compound binds accordingly within the FimH carbohydrate binding site.

To visualise the complexation mode of the Amadori products inside the binding pocket of FimH, flexible ligand docking studies were performed using the program Glide[247] as implemented in the Schrödinger program package, which was performed by Insa Stamer from the Lindhorst group. For these docking studies, the so-called open gate crystal structure of FimH was used[174] in which the tyrosine gate formed by the side chains of Y48 and Y137 has an open conformation. Before these docking studies were implemented, the energies of the Amadori rearrangement products were minimised utilizing the program MacroModel.[248] With this method, 20 different conformers for **121** and 23 different conformers for **122** were achieved with ConfGen[249] by using default settings. Next, holding the FimH carbohydrate recognition domain (CRD) fixed, the diverse conformers of the Amadori products were docked, whereas under the influence of the

force field conformational changes were allowed for the docking ligands. The resulting docking scores were calculated with the SP (single precision) scoring function and correlated with the binding affinity of the ligand for the FimH CRD, whereby more negative scores portend higher binding affinity then less negative values (Table 13).[217]

Table 13: Docking score values of the most stable conformers complexes by FimH (open gate structure PDB 1KLF).

Compound	Scoring value
MeMan (196)	-6.6
Compound 121	-5.7
Compound 122	-4.2

Comparison of the scoring values of the standard inhibitor MeMan (**196**) with the calculated scoring values for the Amadori products, **121** and **122,** which have similar scores, indicates that the values are found in the same range of that for MeMan, nevertheless a weaker complexation, was predicted for the Amadori compounds. Interestingly, the scores for both compounds are very close, as we had expected that compound **121** exhibits a higher affinity for FimH due to the possibility of π-π-stacking interactions between the phenyl substituent in **122** and the tyrosine gate at the entrance of FimH carbohydrate binding site, which seems not to be the case.[217]

Taking a closer look at the docking results by comparing top scoring conformations of the three ligands when inspected from the top phase of the CRD, no difference between complexation of the Amadori products and MeMan can be seen. However, by closer considerations, the Amadori products are slanted compared to MeMan regarding the complexation from the side view. Furthermore, they seemed to be lifted from the binding site (Figure 60B and C).

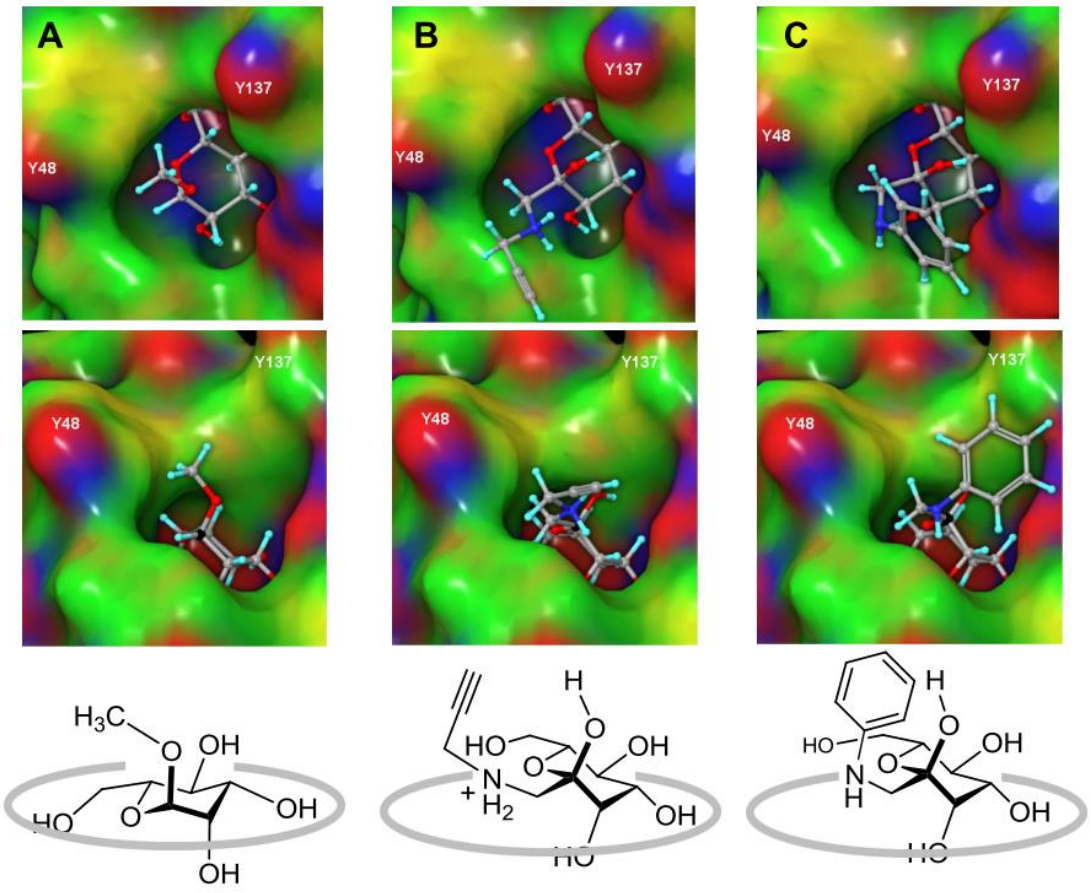


Figure 60: Partial charge coloured Connolly descriptions[250] (negative partial charges coloured in red, positive in blue) of mannoside MeMan (**196**) (A) and the Amadori products **122** (B) and **121** (C) as complexed within the CRD of FimH (PDB 1KLF, open gate structure).[217]

The top row shows the complexation of the three ligands looking from above the CRD. From this perspective, it seems that all ligands are similarly complexed. Whereas, the side view depicted in the middle row shows that the Amadori products are tilted and when the respective anomeric centres are taken as reference, they are lifted from the binding site, 0.7 Å for compound **121** and 0.5 Å for compound **122** compared to MeMan which are illustrated in bottom row as cartoons. This slanted complexation may reduce the all over affinity of the ligands for FimH as well as affect the π-π-interaction between the tyrosine gate of FimH and the phenyl moiety of compound **121**.[217]

Furthermore, comparison of the hydrogen bonding of MeMan and compound **122** showed that the average lengths of H-bonds established with **122** is higher than for MeMan and thus they are also weaker. Additionally, the Amadori products are not able to interact with the water molecule which is conserved in the FimH binding site (Figure 61). In the top graphics, the results of the simulated 3D-arrangement of amino acid residues of FimH CRD are illustrated. The

bottom cartoons are deduced from the docking results showing the predicted hydrogen bond network between amino acid side chains and the sugar hydroxyl groups (not to scale). [217]

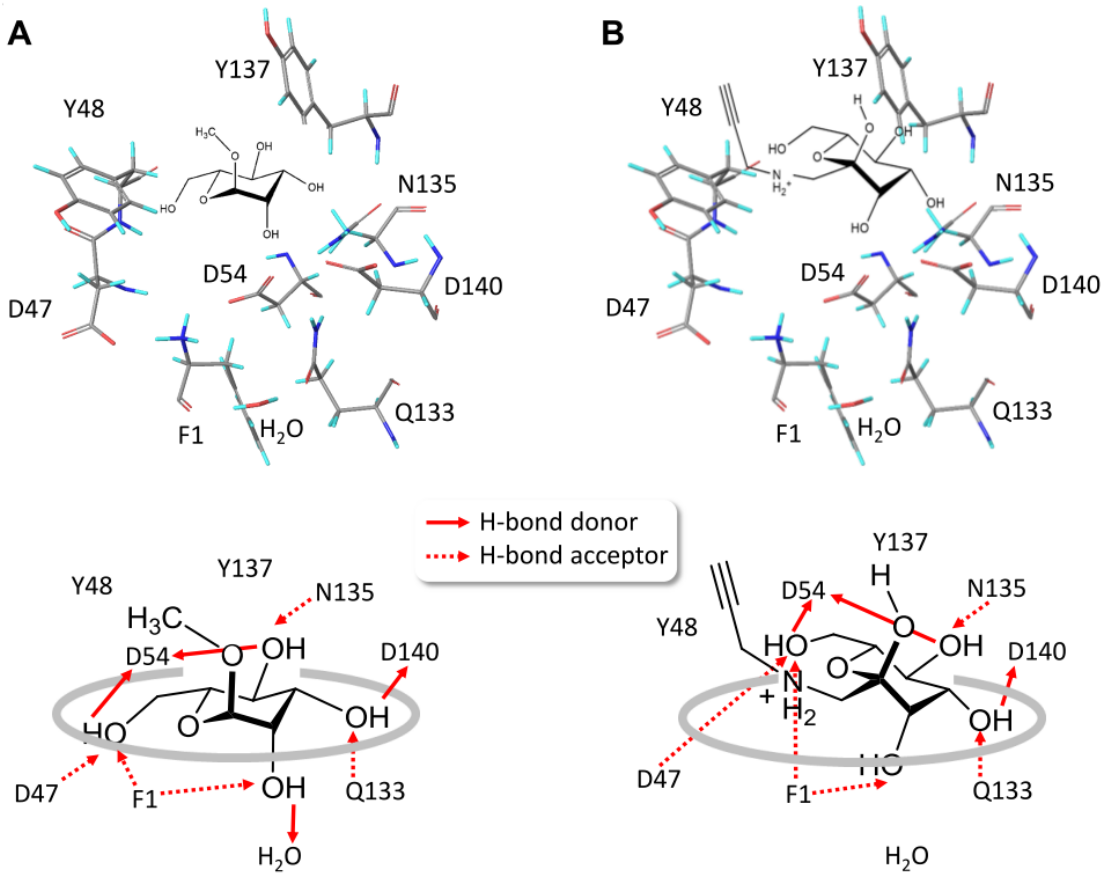


Figure 61: Comparison of mannosides as complexed within the CRD of FimH (PDB 1KLF). A: MeMan (**196**); B: Amadori product **122**.[217]

Next, we are interested in cellular lectins and cell surface carbohydrate ligands, which mediate and regulate cell-cell adhesion. Because cells mainly carry a sugar coating formed by glycoproteins or glycolipids which are involved in many biological processes, the understanding of these mechanisms is essential. One opportunity for the investigation of carbohydrate-specific cell adhesion is the use of microarrays which allows screenings of large libraries of compounds whereby only small quantities of materials are required.[251]

Thus, synthetic glycosylated surfaces, so-called glycoarrays, have become precious tools to study details of carbohydrate-specific cell adhesion in a supermolecular setting investigating carbohydrate specificity of lectins and cell adhesion, respectively. [252]

Therefore, the synthesis of sugar arrays *in situ* on microtiter plates and subsequent bacterial-adhesion assays using GFP-tagged *E.coli* bacteria were investigated (Figure 62). 1,3-Dipolar cycloaddition between azides and alkynes, also known as “click”-chemistry,[69, 253] were chosen,

as they are normally simple, reagent-free and very selective. As alkynes Amadori rearrangement products **122** as well as propargyl α-D-mannopyranoside (**198**) as internal standard were used; the azide counterpart was attached to the microtiter plate. Hence, a black 96-well microtiter plate, which was functionalized with an active ester, was modified with *O*-(2-aminoethyl)-*O'*-(2-azidoethyl)pentaethylene (100 µL/well). Solutions of compound **122** (10mM in MeOH) as well as propargyl α-D-mannopyranoside **198** (10mM in MeOH) were prepared and added to the microtiter plate (50 µL/well), followed by addition of CuI (10mM in MeOH) as well as DIPEA (13mM in MeOH). After serial dilution, the plate was shaken for 8 hours at room temperature and 300 rpm. After washing the plate with PBS, the bacterial suspension (OD_{600} = 0.4, 50 µL/well) was added and the plates were incubated for 1 h at 37 °C and 100 rpm. After washing, the fluorescence intensity (485 nm/535 nm) was determined.

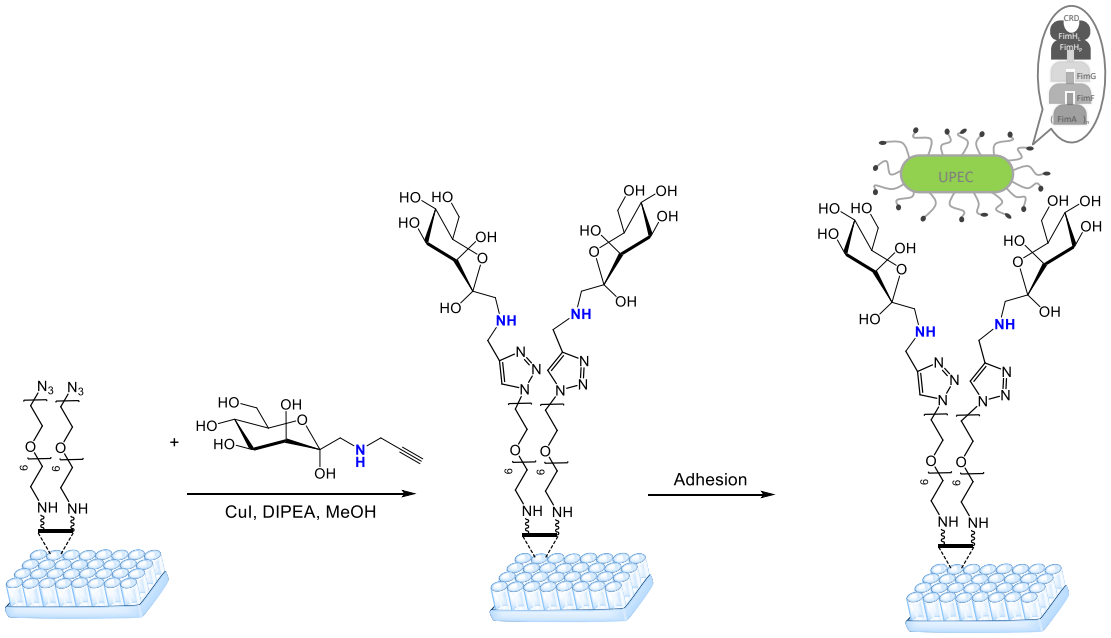


Figure 62: "Click"-chemistry on microtiter plate and subsequent adhesion assay using GFP-tagged *E.coli* bacteria.

To establish that bacterial lectin FimH adheres to the mannosides and not binds nonspecifically to the microtiter plate, Amadori rearrangement products **122** as well as propargyl α-D-mannopyranoside **198** were applied using serial dilution compared to the azido modified microtiter plate spots (blue triangle), which was tested on the same microtiter plate. The resulting adhesion curve is depicted in Figure 63.

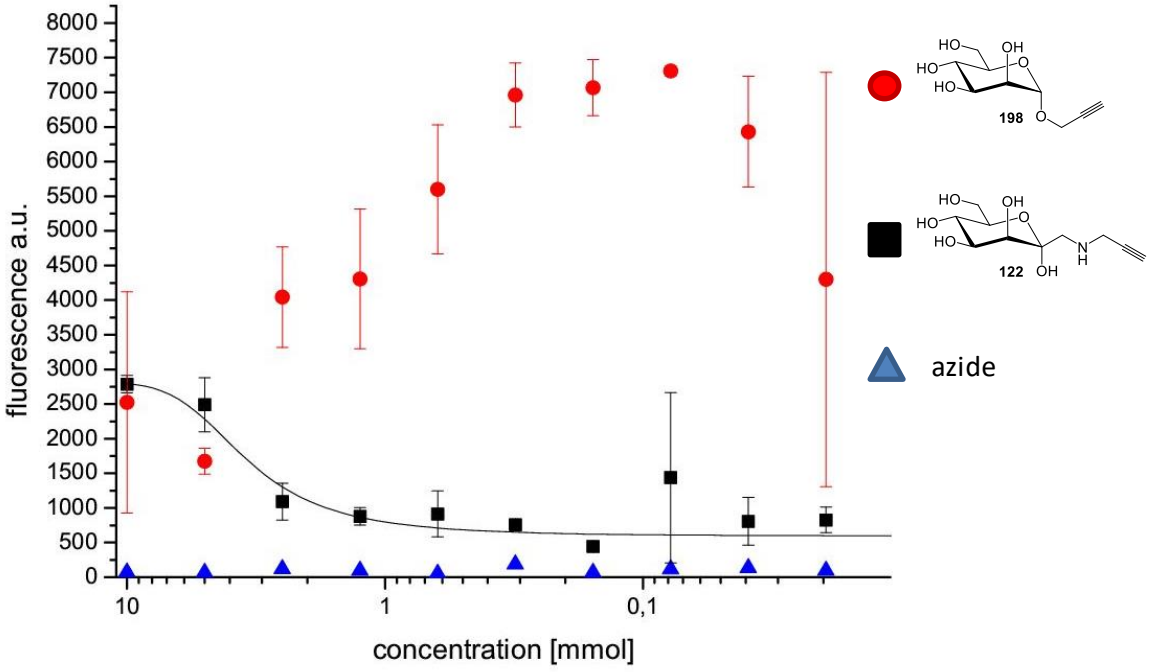


Figure 63: Bacterial adhesion of GFP-tagged *E.coli* bacteria to glycoarray of potential inhibitor on a polystyrene microtiter plate.

Propargyl α-D-mannopyranoside **198** and Amadori product **122** mediate binding to type 1 fimbriated *E. coli*, whereas propargyl α-D-mannopyranoside **198** is clearly more adhesive at a lower concentration than the Amadori product **122,** which shows weak adhesiveness at higher concentrations. This finding correlated with the results obtained from the adhesion-inhibition assays (Table 12) to the known weak FimH affinity of Amadori products **121** and **122**. This results confirms that because of the bulky alkyl/aryl aminomethyl group oriented in the β-face of the sugar ring, the Amadori products are complexed by FimH in a tilted fashion leading to a lifted position from the bottom of the carbohydrate binding site which resulted in compromised H-bonding and weak affinity.

6.2.2. Evaluation of mannoside specific lectins from various sources

Herein, we were interested to investigate the interaction of Amadori rearrangement product **121** with seven different mannoside specific natural or recombinant lectins of various sources, which are listed in Table 12. These biological evaluation was performed by Prof. Landemarre and co-workers at GLYcoDiag in France.[254]

In these studies, the concentration corresponding to 50% of inhibition (IC_{50} value) was determined and the analysis was based on a competition assay between an inhibitor and a specific tracer (labeled molecule) known to be well recognized by the corresponding lectin. On each lectin, previously linked on the bottom of a well (96 wells plate), a mix of the tracer (fixed concentration) and the inhibitor (range of concentrations) was deposed and incubated 2 hours at room temperature. After washing and fluorescence read out, the obtained signal intensity was inversely correlated with the capacity of the tested ligand to be recognized by the lectin in completion with the fluorescent tracer ligand. Consequently, the higher the measured fluorescence intensity, the weaker is the tested ligand as lectin ligand.

Table 14: Different mannose sepcific lectins for the analysis of potential inhibition of Amadori rearrangement product **121** (green: plant lectins; blue: recombinant bacterial lectin; red: human recombinant lectins).

Inhibitors (sugars)						
Short Name	**Common Name**	**Glc**	**Man**	**GlcNAc**	**Fuc**	**Glycan structures specifity**
Con A[255]	*Canavalia ensiformis*	✓	✓	✓		Man > Glc; branched mannoses
VEA[256]	*Vicia ervilia*	✓	✓			Man>trehalose>Glc
HHL, HHA[257]	*Hippeastrum hybrid*		✓			Terminal and internal mannoses, α-3 or 6 linked mannosyl units, bind mannopentaose
GNL/GNA[258]	*Galanthus nivalis*		✓			Terminal mannoses, Manα3Man ; α-2-macroglobulin ; bind mannopentaose
BC2L-A[259]	*Burkholderia cenocepacia lectin A*		✓			Manα1-2, Manα1-3, Manα1-6, dimanoside,
Langerin[199, 260]	*Langerin ECD (Extra Cellular Domain)*		✓	✓	✓	Mycobacteria, M. leprae glycolipid arabinomycolate
DC-SIGN[203, 261]	*DC-SIGN ECD (Extra Cellular Domain)*		✓			High mannose

In these competition assays, various tracers were used in a fixed concentration for each lectin (Table 15), whereas a range of concentration of the inhibitor (compound **121**) was chosen.

Table 15: Tracers used for each lectin.

Lectin	Tracer	Tracer Concentration [μM]
ConA	αMan-BSA	0.28
VEA	Lactoferrin	0.25
HHA	Asialofetuin	0.42
GNA	Thyroglobin	0.13
BC2L-A	αMan-BSA	0.07
Langerin	αMan-BSA	0.03
DC-SIGN	αMan-BSA	0.07

The IC_{50} values for both ligands, the Amadori rearrangement product **121** as well as for the control mannoside MeMan (**196**), for the different lectins were deduced form the obtained inhibition curves and are depicted in Figures 64 to 66.

During this analysis, it was found that Amadori product **121** was able to inhibit the interaction of mannosylated neoglycoproteins in quantitative manner, which means that this compound was recognized by mannose-specific lectins. By comparison of the estimated IC_{50} values (Table 16) for the standard inhibitor MeMan **196** and compound **121** it was determined that inhibition mediated by Amadori product **121** allowed the differentiation of lectins sub-specificities of recognition among mannose-specific lectin. Employing plant lectins ConA and VEA, which are inhibited by mannose or glucose, the inhibition mediated by compound **121** was not better than for the standard inhibitor MeMan. However, lectins which are inhibited by mannose only, such as GNA, HHA and BC2L-A, were able to recognize compound **121** better than MeMan. The larger difference between the IC_{50} value of compound **121** and those of MeMan (Table 16) was obtained with the lectin HHA/HHL. The determined IC_{50} value for compound **121** amounted to 3mM, whereas the standard inhibitor MeMan exhibited an IC_{50} value of 200mM. This result showed a specific CRD recognition process of compound **121** when employed as ligand for selected mannose-specific lectins, which could be exploited to target some relevant glycobiological interactions.

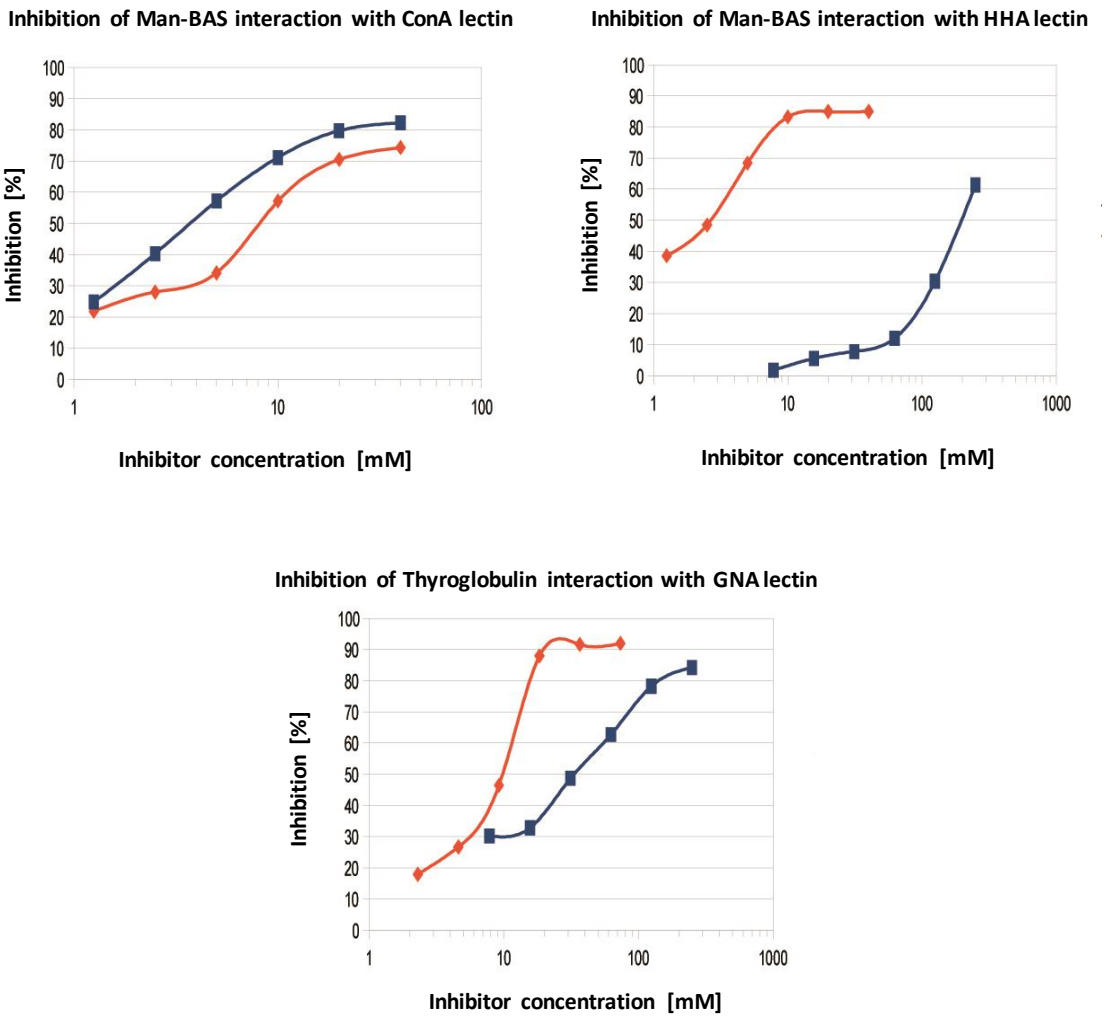


Figure 64: Inhibition of tracers recognition with three plant lectins (Con A, HHA and GNA) by either MeMan (blue curve) or Amadori rearrangement product **121** (red curve).

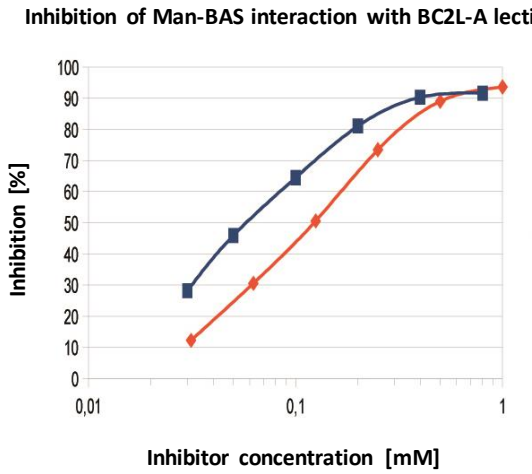


Figure 65: Inhibition of tracers recognition with recombinant plant lectin BC2L-A by either MeMan (blue curve) or Amadori rearrangement product **121** (red curve).

Furthermore, inhibition experiments were also carried out on two human recombinant *C*-type lectins, namely DC-SIGN and Langerin, which have roles in recognition of many pathogens including viruses HIV, HPV, SARS or bacteria *Mycobacterium tuberculosis* of *Helicobacter pylori*. In case of DC-SIGN no difference concerning the inhibition was observed between compound **121** and MeMan. Both molecules have the same IC_{50} values (10 mM) on DC-SIGN. However, compound **121** inhibits human recombinant lectin Langerin ten times better than MeMan (Table 16). This *C*-type lectin, expressed by Langerhans cell in ephithelia, is the first barrier that pathogen encounter and therefore, further studies concerning the recognition mechanism employing Amadori rearrangement products like compound **121** could be relevant for the design of new drugs strategies in terms of decrease pathogens colonization.

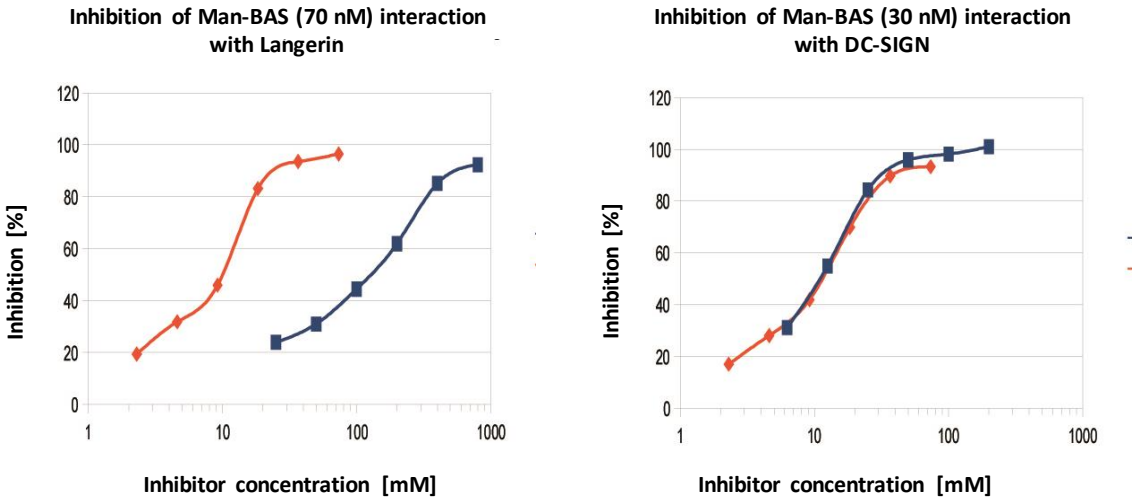


Figure 66: Inhibition of tracers recognition with two human recombinant lectins (Langerin and DC-SIGN) by either MeMan (blue curve) or Amadori rearrangement product **121** (red curve).

Table 16: Estimated IC_{50} values of Amadori product **121** and MeMan.

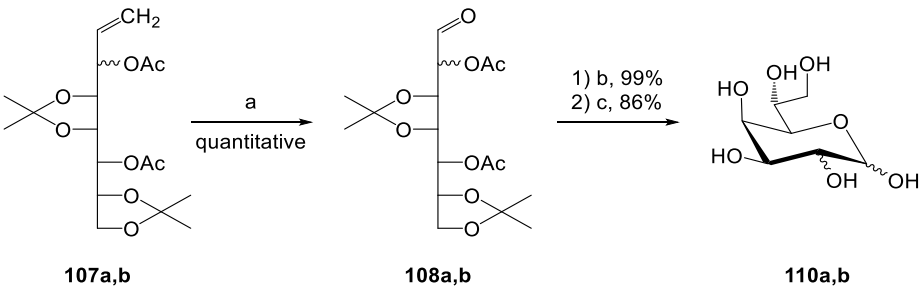
Lectin	Estimated IC_{50} value [mM] with compound 121	Estimated IC_{50} value [mM] with MeMan	Relative Inhibitory Potency[a]
ConA	8	4	0.5
VEA	no	10	n.d.
HHA	3	200	66.7
GNA/	10	30	3
BC2L-A	0.05	0.015	3
Langerin	15	150	10
DC-SIGN	10	10	1

[a] Relative Inhibitory potency: calculated accoriding to estimated IC_{50} value of MeMan

1. CONCLUSION

In this PhD thesis the scope and limitations of the Amadori rearrangement as a new and straight forward glycoconjugation method were investigated, which enables the synthesis of *C*-glycosyl type neoglycoconjugated without the need for protecting group manipulation.

In order to obtain D-*manno* configured rearrangement products, a new protocol for the synthesis of the appropriate aldoheptose was developed in which the use of hazardous hydrogen cyanide was circumvented. Starting from oct-1-enitol derivatives **107a** and **107b**, obtained from Grignard elongation, introduction of an aldehyde function at position C-1 *via* simple ozonolysis provided the protected D-*glycero*-D-*galacto*- and D-*glycero*-D-*talo*-configured heptoses **108a** and **108b**. Subsequent removal of the protecting groups afforded the desired D-*glycero*-D-*galacto*/D-*talo* heptopyranoses (**110a,b**) in good yields.



107a,b **108a,b** **110a,b**

Scheme 59: Synthesis of D-*glycero*-D-*galacto*/D-*talo*-heptopyranose **110a** and **110b**: a) O_3, NaOAc, Me_2S, CH_2Cl_2/MeOH, –50 °C, b) NaOMe, MeOH; c) ion exchange resin IR 120 H^+, H_2O/MeCN.

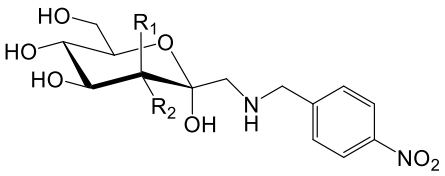
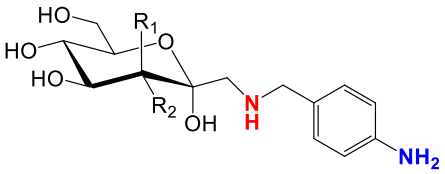
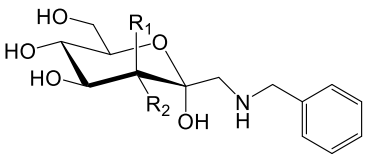
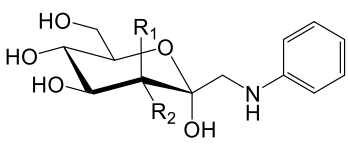
By reaction of the respective aldoheptoses, non-natural *C*-glycosyl type glycoconjugates presenting the sugar moitif in D-*gluco* and D-*manno* configuration are available. To optimize the reaction conditions, commercially available D-*glycero*-D-*gulo* aldoheptose (**48**) was employed in the Amadori rearrangement which gave excess to D-*gluco* configured products, whereas D-*glycero*-D-*galacto*/D-*talo* aldoheptose (**110**) led to D-*manno* configured products.

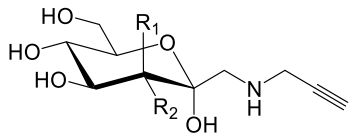
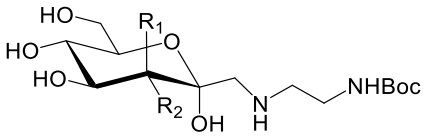
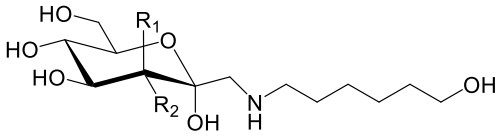
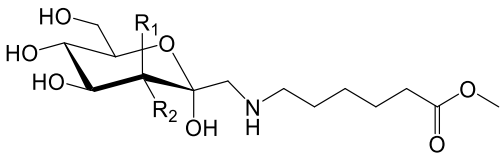
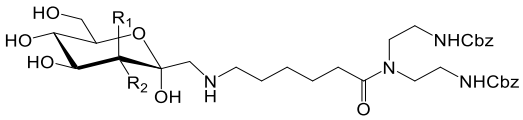
First, various simple amines, like aliphatic as well as benzylic amines, were investigated in the Amadori rearrangement generating a wide range of new *C*-glycosyl type glycoconjugates. During these studies we found that the pK_a value as a parameter for the nucleophilicity of the employed amino components seem to play a crucial role during the reaction. Comparing the pK_a values of the applied amines (Table 15), we concluded that amines with a pK_a range between 8 and 12 are more efficient nucleophiles for the Amadori rearrangement, leading to higher yields of the desired products than less basic amines. For instance, employing aniline with a pK_a value of 4.6 as amino component, the rearrangement products were isolated in only 26% in the *D-gluco* series and 24% in the *D-manno* series. Whereas, in case of

4-nitrobenzylamine, which exhibits a pK_a of 8.1, the corresponding Amadori rearrangement were isolated with a yield of 64% (D-*gluco*) and 57% (D-*manno*). Furthermore, microwave-assisted Amadori rearrangement was investigated using propargylamine as well as *N*-(*tert*-butoxycarbonyl)ethylenediamine as amino components. With this mircrowave-assisted method not only reaction times could be decreased but also product formation could be increased significantly.

Considering the pK_a value of the amines, the regioselectivity of the Amadori rearrangement employing unsymmetrical diamines can be controlled. In case of 4-aminobenzylamine with pK_a values of 9.3 for the benzylic amine and 4.6 for the aniline amino group, the Amadori rearrangement took place selectively at the benzylic position (compounds **114** and **118**, Table 15), whereas the aromatic amino group remained untouched.

Table 17: Amadori rearrangement products using simple amines.

Amadori rearrangement products	Yields	pK_a value of the amino component
	**113**: R_1=H, R_2=OH; 64% **119**: R_1=OH, R_2=H; 57%	8.1
	**114**: R_1=H, R_2=OH; 73% **118**: R_1=OH, R_2=H; 73%	**9.3** **4.6**
	**115**: R_1=H, R_2=OH; 67% **120**: R_1=OH, R_2=H; 56%	9.3
	**116**: R_1=H, R_2=OH; 26% **121**: R_1=OH, R_2=H; 24%	4.6

Amadori rearrangement products	Yields	pK_a value of the amino component
	**111**: R_1=H, R_2=OH; 81% **122**: R_1=OH, R_2=H; 77%	8.2
	**112**: R_1=H, R_2=OH; 98% **123**: R_1=OH, R_2=H; 93%	7.8
	**123**: R_1=OH, R_2=H; 73%	10.6
	**125**: R_1=OH, R_2=H; 70%	10.8
	**126**: R_1=OH, R_2=H; 48%	10.8

Investigations towards symmetrical diamino components in the Amadori rearrangement (Figure 65) indicated that formation of mono-substituted versus disubstituted Amadori products can be controlled *via* the amount of sugar substrate employed. In case of 2,2´-(ethylenedioxy)bis(ethylamine), the disubstituted products **145** and **150** were obtained in 23% and 29% yield, from the aldoheptose **48** and **110**, respectively. Whereas, the corresponding monomeric compounds were isolated in 56% and 55% yield, respectively. On the other hand, when 4,7,10-trioxa-1,13-tridecanediamine was employed as the amino component and an excess of the corresponding aldoheptose (3 equivalents) was used, the Amadori rearrangement exclusively gave the double Amadori products **147** (58%) and **152** (54%).

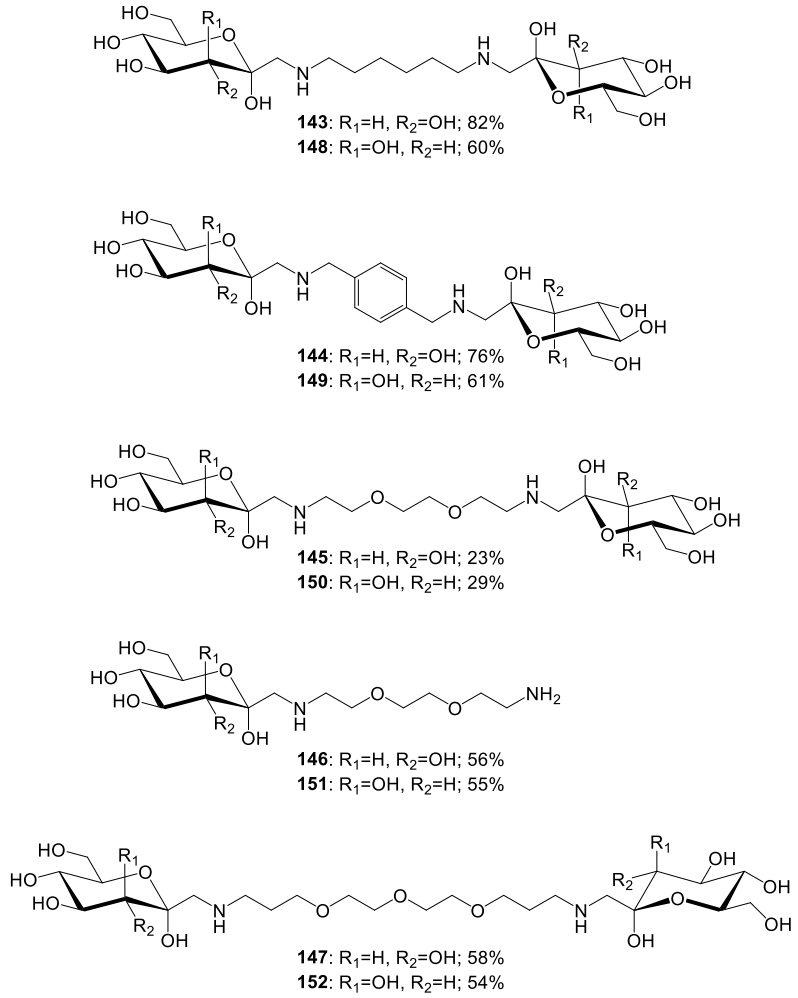


Figure 67: Synthesis of disubstituted Amadori rearrangement products in the D-*gluco* as well as in the D-*manno* series.

In order to perform a triple Amadori rearrangement (Figure 66), tris(2-isocyanatoethyl)amine (**162**) was treated with 1-(*N*-4-aminobenzyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulose (**114**) to generate triple Amadori rearrangement product **163** by thiourea-bridging.

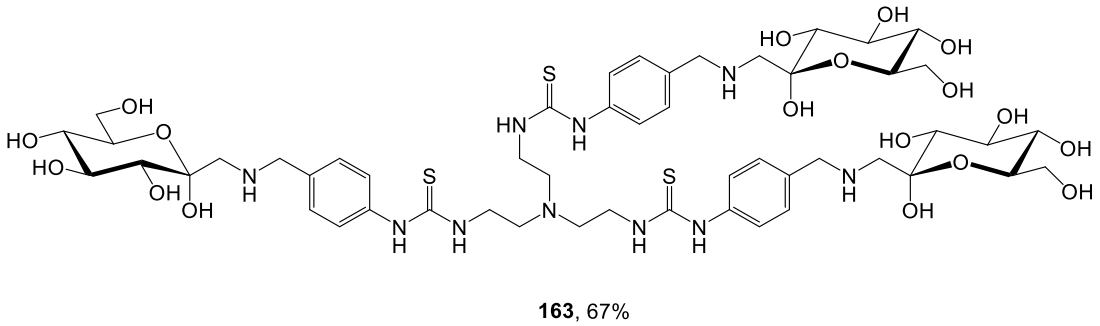


Figure 68: Amadori rearramgement employing a trivalent amine.

For the synthesis of more complex glycoconjugates, amino-functionalized glycosides were employed in the Amadori rearrangement (Figure 67). These glycoconjugates additionally offer

orthogonal groups at the anomeric position of the amino-functionalized glycosides for further modifications, like a masked amino function (compound **170**) or a versatile propargyl group (compound **171**).

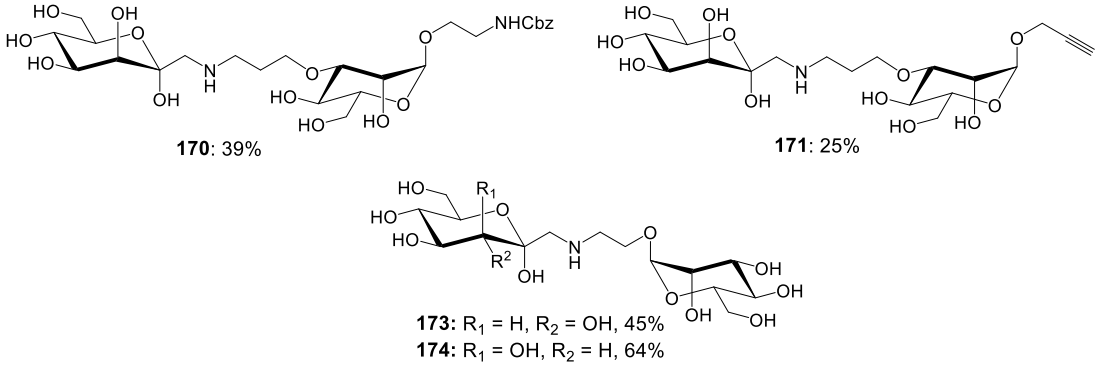


Figure 69: Amadori rearrangement employing amino-functionalized glycosides.

Obtaining *C*-glycosyl type glycopeptide mimetics *via* the Amadori rearrangement, we have successfully developed a protocol for the conjugation of the respective sugar moiety to lysine derivatives as well as lysine-containing di-and tripeptides, which can be used as building blocks for glycopeptide and glycoprotein synthesis (Figure 68).

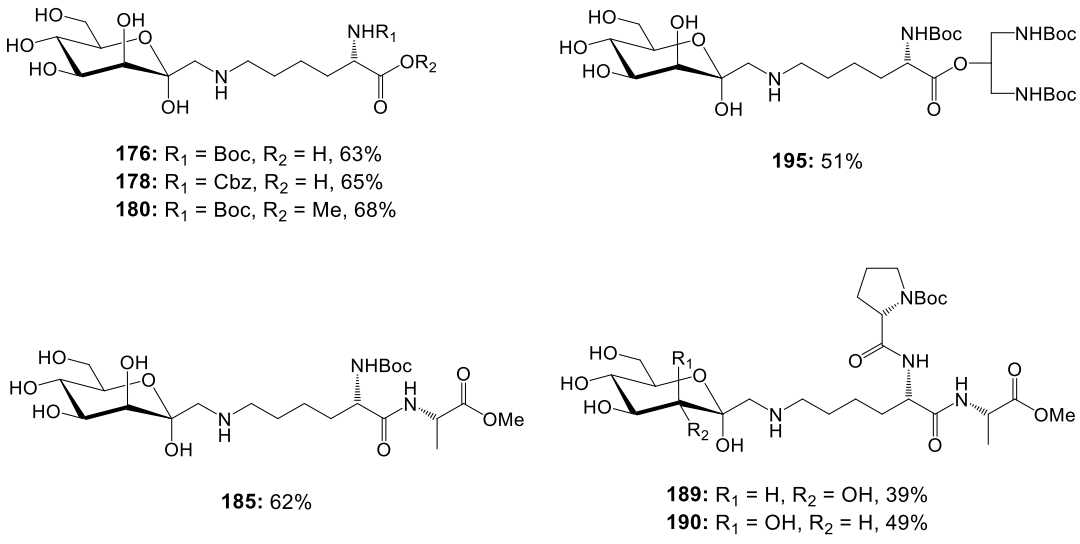


Figure 70: Amadori rearrangement eploying lysine derivatives.

During our investigations, by applying different amines in the Amadori rearrangement we observed that signals of protons at the position C-1 in the Amadori products decreased on prolonged storage of solution in D_2O. This is due to an H/D exchange which accelerates significantly with increasing basic pH values.

Biological evaluation of Amadori rearrangement products **121** and **122** as ligands for the α-D-mannose-specific type 1-fimbrial bacterial lectin FimH by the means of molecular docking and bacterial adhesion studies showed that Amadori rearrangement products exhibited a weaker interaction of FimH-mediated bacterial adhesion than MeMan (**196**). This is due to the rather bulky β-positioned alkyl/aryl aminomethyl group at the anomeric center which considerably hampers complexation within the carbohydrate binding site of the lectin FimH and therefore, limited the activity towards inhibition of FimH-mediated bacterial adhesion.

Competition assays employing different plant, recombinant bacterial as well as human lectins with Amadori product **121** showed a notable inhibition with the plant lectin HHA/HHL as well as with the human recombinant lectin Langerin, which means that this compound was able to inhibit the interaction of mannosylated neoglycoproteins in a quantitative manner. In case of plant lectin HHA and human recombinant lectin Langerin the determine IC_{50} values are lower than for MeMan and therefore the Amadori product is a suitable inhibitor for these kinds of lectins.

In summary, we demonstrated that the Amadori rearrangement is an attractive and straight forward ligation method to conjugate unprotected sugars with different amines leading to *C*-glycosyl type glycoconjugate mimetics without the need for protecting-group manipulation. Although, yields are found in some cases in a preparatively moderate range and product purification turned out to be demanding, this new conjugation method allowed the access to *C*-glycosyl type glycoconjugates, which can be can be used as versatile building blocks for further applications.

2. EXPERIMENTAL SECTION

2.1. General method and materials

All syntheses were carried out under air, except explicitly noted. Experiments with air- or moisture sensitive materials were performed under inert atmosphere using a dual vacuum/nitrogen line and standard Schlenk techniques. Flasks needed for the Grignard reaction were heated in vacuum and ventilated with inert gas. The addition of reagents occurred by application of Schlenk techniques in N_2-counter flow.

Unless otherwise stated, all chemicals were purchase commercially and uses without further purification.

2.1.1. Thin-layer chromatography

Thin layer chromatography was performed on precoated silica gel plates on aluminum 60 F254 (E. Merck 5554). Signal detection was effected by UV light (254 nm). For staining one of the below-mentioned solutions was employed followed by heating with a heat gun at ~180 °C.

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

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

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

2.1.2. Flash chromatography

The purification of the synthesized compounds was performed on silica gel 60 (0.035-0.070 mm, 60 A, Acros Organics 24036) using distilled solvents. The solvent mixtures for each compound are quoted in the procedure.

2.1.3. Ion exchange chromatography

To seperate compounds with nearly the same polarity an ion exchange chromatography was performed on a strong cation exchanger (Amberlite ® CG-120-II, Na^+ form, 200-400 mesh, Fluka 06449) using water and a water/NH_4OH conc. mixture.

2.1.4. Optical rotation

Optical rotations were measured with a Perkin-Elmer 341 polarimeter (sodium D-line: 589 nm, length of cell: 1 dm, temp.: 20 °C) in the solvents indicated in the procedure.

2.1.5. Mass spectrometry

MALDI-TOF Mass Spectrometry was performed on a Micromass TofSpec 2E Time-of-Flight Mass Spektrometer.

2.1.6. Nuclear magnetic resonance spectrometry

NMR spectra were recorded on Bruker Ultrashield spectrometers with autosampler at 300.36 (^1H) and 75.53 (^{13}C) MHz, respectively. For a higher resolution NMR spectra were recorded on VARIAN INOVA 500 MHz at 500.619 (^1H) and 125.894 (^{13}C) MHz, respectively. Chemical shifts are reported relative to internal tetramethylsilane ($\delta = 0.00$ ppm) or D_2O ($\delta = 4.79$ ppm). All spectra were measured at room temperature and data reported as follows:

Table 18: Abbreviations used for reporting NMR signals.

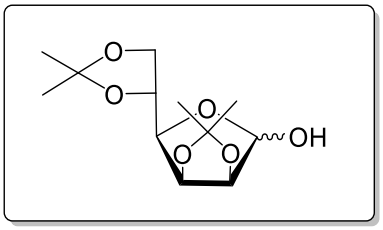
s	singlet
d	doublet
t	triplet
q	quadruplet
bs	broad signal
dd	douplet of douplets
ddd	douplet of douplet of douplets
m	multipet
n.r.	not resolved

Full assignment of the peaks was achieved with the aid of 2D NMR techniques (^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC).

2.2. Experimental procedures

2,3:5,6-Di-*O*-isopropylidene-D-mannose (105)[219, 262]

D-mannose (11 g, 61 mmol) was suspended in acetone (300 mL), H_2SO_4 conc. (7 mL) was added and the reaction mixture was stirred for 1 hour at ambient temperature. The reaction mixture was neutralised with $NaCO_3$, filtered off and the solvent was removed under reduced pressure. The residue was taken up in CH_2Cl_2, washed consequently with H_2O dest., dried (Na_2SO_4) and filtered. Removal of the solvent under reduced pressure and recrystallization from CH_2Cl_2/C gave **105** (13 g, 50 mmol) as a white solid in 82% yield. The NMR data is in accordance with literature.[263]



$C_{12}H_{20}O_6$
Mw = 260.29 [g/mol]
Rf: 0.63 (C/EtOAc 1:1)
Detection: VAN
Purification: Recrystallization from CH_2Cl_2/C
Yield: 82 %

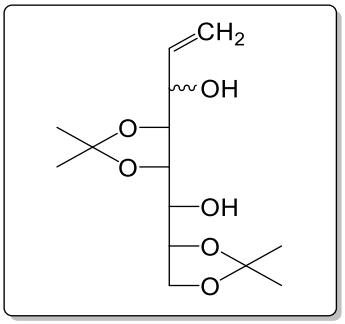
^{1}H-NMR (300 MHz, $CDCl_3$) δ = 5.37 (d, 1H, H-1), 4.80 (dd, 1H, H-3), 4.61 (d, 1H, H-2), 4.39 (dd, 1H, H-5), 4.18 (dd, 1H, H-4), 4.11-4.00 (m, 1H, H-6), 3.06 (d, 1H, OH), 1.46 (s, 3H, CH_3), 1.45 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.32 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 112.8, 109.2 (2C, C-7, C-8), 101.4 (C-1), 85.6 (C-2), 80.4 (C-4), 79.8 (C-3), 73.4 (C-5), 66.7 (C-6), 26.9, 25.9, 25.3, 24.6 (4C, 4x CH3).

1,2-Dideoxy-4,5:7,8-di-*O*-isopropylidene-D-*glycero*-D-*galacto*/D-*talo*-oct-l-enitol (106a and 106b)[212]

2,3:5,6-Di-*O*-isopropylidene-D-mannose (**105**) (4 g, 15.4 mmol) was dissolved in dry THF (40 mL) and the solution was cooled to 0°C. Vinylmagnesium bromide solution (1 M in THF, 46 mL, 46.1 mmol, 3 Equ.) was added dropwise. After stirring at 0 °C for 15 min, the reaction mixture was allowed to come to rt and stirred for further 12 hours. The reaction was quenched with H_2O, extracted with CH_2Cl_2 and washed consequently with 6% aqueous HCl and satd aqueous sodium bicarbonate, dired (Na_2SO_4) and filtered. Removal of the solvent under reduces pressure gave

crude **106a** and **106b** (4.42 g, 15.3 mmol, 99 %) as a mixture of both disastereomeres. The NMR data is in accordance with literature.[212a]



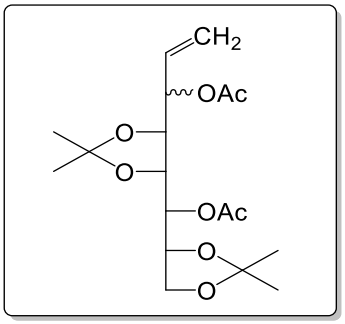
$C_{14}H_{24}O_6$
Mw = 288.34 [g/mol]
Rf: 0.62 (C/EtOAc 1:1)
Detection: VAN
Purification: crude
Yield: 99 %

^{1}H-NMR (300 MHz, $CDCl_3$) δ = 5.96 (ddd, 1H, H-2), 5.45 (d, 1H, H-1), 5.28 (d, 1H, H-1'), 4.46-4.35 (m, 2H, H-6, H-5), 4.22 (dd, 1H, H-4), 4.16-3.99 (m, 3H, H-8, H-7), 3.74-3.61 (m, 1H, H-3), 1.57 (s, 3H, CH_3), 1.40 (s, 3H, CH_3), 1.36 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 137.4 (C-2), 117.5 (C-1), 109.5, 108.7 (2C, C-8, C-9), 79.4 (C-4), 76.2 (C-5), 75.9 (C-7), 70.7 (C-6), 70.5 (C-3), 67.5 (C-8), 27.0, 26.5, 25.4, 24.7 (4C, 4x CH_3).

3,6-Di-*O*-acetyl-4,5:7,8-di-*O*-isopropylidene-1-octene-D-*glycero*-D-*glalacto*/D-*talo*-3,4,5,6,7,8-hexol (107a and 107b)[213]

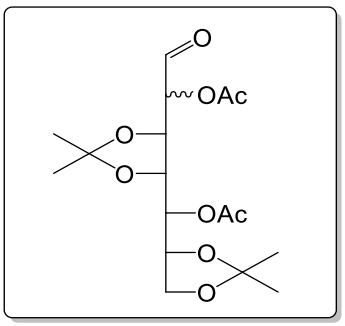
To a solution of a C-3 diastereomeric mixture of isopropylidene protected oct-1-enitol **106a** and **106b** (4.42 g, 15.3 mmol) in pyridine (20 mL) was treated with acetic anhydride (5.8 mL, 61.3 mmol, 4 equ.) at 0°C. After stirring at 0 °C for 10 min, the reaction mixture was allowed to come to rt and stirred for further 1 hour. The reaction was quenched with MeOH, the solvents were removed under reduced pressure. The residue was taken up in CH_2Cl_2 and washed consequently with 6% aqueous HCl and satd aqueous sodium bicarbonate, dried (Na_2SO_4) and filtered. Removal of the solvent under reduces pressure gave crude **107a** and **107b** (5.64 g, 15.1 mmol, 99 %).



$C_{18}H_{28}O_6$
Mw = 372.41 [g/mol]
Rf: 0.52 (C/EtOAc 2:1)
Detection: VAN
Purification: crude
Yield: 99 %

2,5-Di-*O*-acetyl-3,4:6,7-di-*O*-isopropylidene-D-*glycero*-D-*galacto*/D-*talo*-heptopyranose (108a and 108b)[214] (route a)

To a solution of $NaIO_4$ in H_2O a 5% solution of compound **107a** and **107b** (100 mg, 0.268 mmol) in a Et_2O (2 mL) was added and stirred for 10 min at rt. A catalytic amount of OsO_4 was added and the reaction mixture was stirred vigorous. The reaction mixture was extracted with CH_2Cl_2, dried (Na_2SO_4) and filtered. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (C/EtOAc, 6;1 v/v) to obtain compounds **108a** and **108b** (20 mg, 0.053 mmol) in 20% yield. The NMR data confirmed signals in the expected regions.

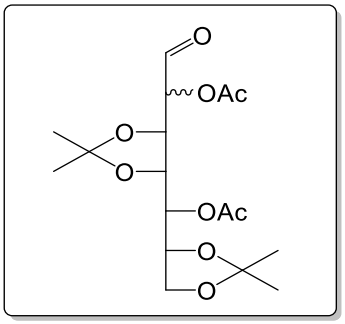


$C_{17}H_{26}O_9$
Mw = 374.39 [g/mol]
Rf: 0.54 (C/EtOAc 1:1)
Detection: VAN
Purification: crude

2,5-Di-*O*-acetyl-3,4:6,7-di-*O*-isopropylidene-D-*glycero*-D-*galacto*/*D*-*talo*-heptopyranose (108a and 108b)[217] (route b)

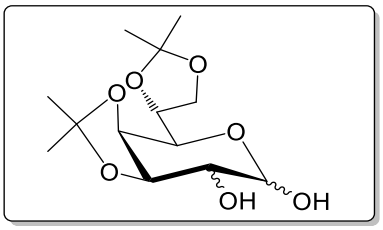
To a solution of a C-3 diastereomeric mixture of protected oct-1-enitol derivative **107a** and **107b** (4.0 g, 11 mmol) in a solvent mixture of CH_2Cl_2/MeOH (80 mL, 1/1 v/v), NaOAc (2.4 g, 30 mmol, 2.8 eq) was added. This reaction mixture was treated with ozone at -50 °C for 6 hours. After TLC (C/EtOAc, 1/1 v/v) confirmed complete consumption of the starting material, nitrogen was bubbled through the reaction mixture for 15 min and the solution was allowed to reach rt, followed by addition of Me_2S (8.0 mL, 0.11 mol, 10 eq.) and stirring at rt for 45 min. The solvents were removed under reduced pressure and the obtained C-2 diastereomeric mixture of

protected aldoheptoses **108a** and **108b** was used for the next step without further purification. The NMR data of the crude material confirmed signals in the expected regions.



$C_{17}H_{26}O_9$
Mw = 374.39 [g/mol]
Rf: 0.54 (C/EtOAc 1:1)
Detection: VAN
Purification: crude

3,4:6,7-Di-O-isopropylidene-D-*glycero*-D-*galatco*/D-*talo*-heptopyranose (109a and 109b)[216]
To a solution of a C-2-epimeric mixture of compound **108a** and **108b** (8.55 g, containing Me_2S) in MeOH (70 mL), a solution of NaOMe (1.0 M in MeOH) was added dropwise at rt until the pH of 10 was reached and the reaction mixture was stirred for 2 hours. The reaction mixture was neutralized by addition of ion exchange resin (Amberlit IR 120 H^+, washed with MeOH). The resin was filtered off, the filtrate was concentrated under reduced pressure and the crude product was purified by silica gel chromatography (C/EtOAc 4:1 v/v) to obtain **109a** and **109b** (3.08 g, 10.6 mmol) in an yield of 99% starting from compounds **107a** and **107b**. The NMR data are in accordance with literature.[264]



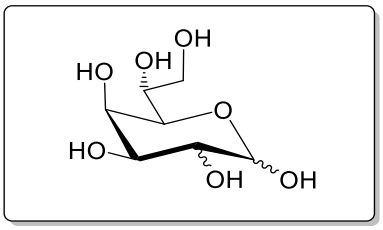
$C_{13}H_{22}O_7$
Mw = 3712.41 [g/mol]
Rf: 0.46 (C/EtOAc 1:2)
Detection: VAN
Purification: crude
Yield: 99 % overall from compound 3

^{1}H-NMR (300 MHz, $CDCl_3$) δ = 4.95 (d, 1H, H-1), 4.55 (dd, 1H, H-3), 4.40 (dd, 1H, H-4), 4.23-4.14 (m, 1H, H-6), 4.06 (dd, 1H, H-7), 3.96 (dd, 1H, H-7'), 3.63 (dd, 1H, H-2), 3.59 (dd, 1H, H-5), 1,45 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.35 (s, 3H, CH_3), 1.33 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 111.0, 110.4 (2C, C-8, C-9), 95.3 (C-1), 76.1 (C-3), 75.7 (C-4), 74.9 (C-6), 71.9 (C-5), 71.1 (C-2), 68.2 (C-7), 27.1, 26.3, 25.5, 25.1 (4C, 4x CH_3).

D-*glycero*-D-*galacto*/D-*talo*-heptopyranose (110a and 110b)

To a solution of 3,4:6,7-di-O-isopropylidene-protected heptose **109a** and **109b** (2.50 g, 8.61 mmol) in a mixture of MeCN/H_2O (50 ML, 1:1 v/v), acidic ion exchange resin (Amberlit IR 120 H+, washed with H_2O) was added until a pH of 2 was reached and the reaction mixture was stirred at 40 °C for 1 hour. The resin was filtered off and the filtrate was concentrated under reduced pressure. Silica gel chromatography ($CHCl_3$/MeOH 10:1 v/v) gave compound **110a** and **110b** (1.55 g, 7.39 mmol) in a yield of 86 %. The NMR data is in accordance with literature.[153]



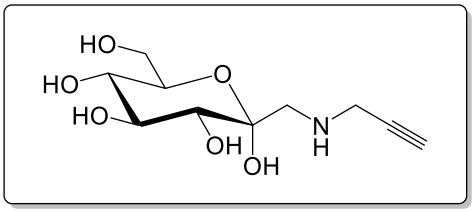
$C_7H_{14}O_7$
Mw = 210,18 [g/mol]
Rf: 0.39 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: VAN, CAM
Purification: SGC $CHCl_3$/MeOH 10:1
Yield: 86 %

^{1}H-NMR (300 MHz, D2O) δ = 5.35 (d, 1H, H-1α), 5.30-5.19 (m,1H, H-1α), 4.57 (d, 1H, H-1β), 4.47-4.29 (m, 1H, H-1β), 4.24-3.4 (H-7, H-7', H-6, H-5, H-4; H-3, H-2, α- and β-pyranose).

^{13}C-NMR (75 MHz, D2O) δ = 96.6 (C-1β), 94.9 (C-1α), 94.3 (C-1β), 92.3 (C-1α), 75.2, 74.1, 73.6, 73.0, 71.8, 71.5, 70.6, 69.6, 69.4, 69.1, 69.0, 68.8, 68.6, 68.3, 68.2, 67.8, 67.6 (C-2, C-3, C-4, C-5, C-6, α- and β-pyranose), 62.8, 62.9, 63.0 (C-7).

1-(*N*-Propargyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulose (111)

To a solution of D-*glycero*-D-*gluo* aldoheptose **48** (200 mg, 0.952 mmol) in a mixture of EtOH (4 mL), 1,4-dioxane (0.4 mL) and water (2 drops), propargylamine (61 µL, 0.952 mmol, 1.0 equiv.) and acetic acid (54 µL, 0.952 mmol, 1.0 equiv.) were added and the reaction mixture was stirred at 70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-propargylamino-modified ketose **111** (191 mg, 0.772 mmol) in a yield of 81%.



$C_{10}H_{17}NO_7$
Mw = 247.25 [g/mol]
Rf: 0.60 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: VAN, CAM
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 8:1:1%
$[\alpha]_D$ = + 50.9 (*c* 3.54, MeOH)
Yield: 81 %

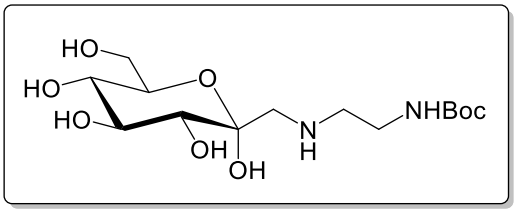
^{1}H-NMR (300 MHz, MeOH-d_4) δ = 3.84-3-63 (m, 4H, H-7, H-7', H-6, H-4), 3.46 (d, 1H, H-8), 3.32 (d, $J_{3,4}$ = 9.4 Hz, 1H, H-3), 3.32 (dd, $J_{5,4}$ = 9.9 Hz, $J_{5,6}$ = 8.7 Hz, 1H, H-5), 2.93 (d, $J_{1,1'}$ = 12.0 Hz, 1H, H-1), 2.85 (d, 1H, H-1'), 2.62 (t, 1H, H-10).

^{13}C-NMR (75 MHz, MeOH-d_4) δ = 98.1 (C-2), 82.3 (C-9), 75.7 (C-6), 74.2 (C-3), 74.0 (C-4), 73.2 (C-10), 71.6 (C-5), 62.8 (C-7), 54.9 (C-1), 38.9 (C-8).

HRMS (MALDI): *m/z* calcd for $[C_{10}H_{17}NO_6+H]^+$: 248.1134; found 248.1137 $[M+H]^+$.

1-(*N*-(*N*-*tert*-Butoxycarbonyl))ethylenediamino-1-deoxy-α-D-*gluco*-hept-2-ulose (123)

To a solution of D-*glycero*-D-*gluo* aldoheptose **48** (200 mg, 0.952 mmol) in a mixture of EtOH (5 mL), 1,4-dioxane (0.5 mL) and water (2 drops), *N*-(*tert*-butoxycarbonyl)ethylenediamine (152 mg, 0.952 mmol, 1.0 equiv.) and acetic acid (54 µL, 0.952 mmol, 1.0 equiv.) were added and the reaction mixture was stirred at 70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-(*tert*-butoxycarbonyl)ethylenediamino-modified ketose **123** (330 mg, 0.936 mmol) in a yield of 98%.



$C_{14}H_{28}N_2O_8$
Mw = 352.38 [g/mol]
Rf: 0.74 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: VAN, CAM
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 8:1:1%
$[\alpha]_D$ = + 24.9 (*c* 2.91, H_2O)
Yield: 98 %

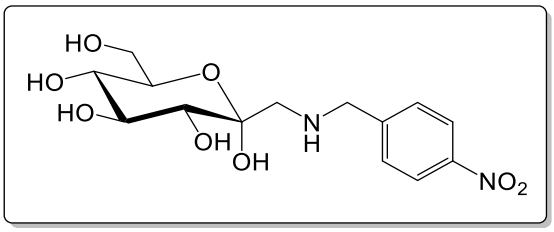
^{1}H-NMR (300 MHz, D_2O) δ = 3.94-3-66 (m, 4H, H-7, H-7', H-6, H-4), 3.50 (d, $J_{3,4}$ = 9.5 Hz, 1H, H-3), 3.47 (dd, $J_{5,4}$ = 9.3 Hz, $J_{5,6}$ = 9.4 Hz, 1H, H-5), 2.99 (d, $J_{1,1'}$ = 12.6 Hz, 1H, H-1), 2.86 (d, 1H, H-1'), 2.82 (t, 1H, H-8), 1.50 (Boc).

^{13}C-NMR (75 MHz, D_2O) δ = 158.6 (C-10), 97.9 (C-2), 80.1 (C-11), 75.7 (C-6), 74.4 (C-4), 74.0 (C-3), 71.7 (C-5), 62.8 (C-7), 55.5 (C-1), 50.4 (C-8), 40.8 (C-9), 28.8 (Boc).

HRMS (MALDI): *m/z* calcd for $[C_{14}H_{28}N_2O_8+H]^+$: 353.192; found 353.1928 $[M+H]^+$.

1-(*N*-4-Nitro-benzyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulose (113)

A solution of 4-nitro-benzylamine hydrochloride (269 mg, 1.43 mmol) in EtOH (5 mL) with 1,4-dioxane (0.5 mL) as co-solvent containing Et_3N (237 µL, 1.721 mmol, 1.2 equiv.) was applied to D-*glycero*-D-*gluo* aldoheptose **48** (300 mg, 1.43 mmol). The reaction mixture was stirred at 70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-(4-nitrobenzyl)amino-modified ketose **113** (312 mg, 0.907 mmol) in a yield of 64%.



$C_{14}H_{20}N_2O_8$
Mw = 344.32 [g/mol]
Rf: 0.78 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: UV, VAN, CAM
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 8:1:1%
$[\alpha]_D$ = + 26.2 (*c* 6.79, MeOH)
Yield: 64 %

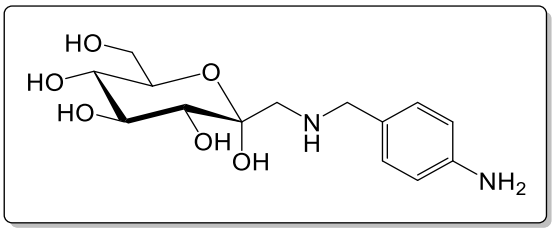
^{1}H-NMR (300 MHz, MeOH-d_4) δ = 7.96 (d, 2H, phenyl), 7.56 (d, 2H, phenyl), 4.09 (t, 2H, H-8), 3.59-3-34 (m, 4H, H-7, H-7', H-6, H-4), 3.13 (d, $J_{3,4}$ = 9.5 Hz, 1H, H-3), 3.06 (dd, $J_{5,4}$ = 9.4 Hz, 1H, H-5), 2.90 (bs, 2H, H-1, H-1').

^{13}C-NMR (75 MHz, MeOH-d_4) δ = 149.4, 141.5, 132.2, 124.8 (6C, phenyl), 96.7 (C-2), 75.1 (C-6), 74.5 (C-4), 74.1 (C-3), 71.3 (C-5), 62.3 (C-7), 54.1 (C-1), 52.1 (C-8).

ESIMS (*m/z*): calcd for $[C_{14}H_{20}N_2O_8+H]^+$: 345.129; found 345.129 $[M+H]^+$.

1-(*N*-4-Aminobenzyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulose (114)

To a solution of D-*glycero*-D-*gluo* aldoheptose **48** (300 mg, 1.43 mmol) in a mixture of EtOH (5 mL), 1,4-dioxane (0.5 mL) and water (2 drops), 4-aminobenylamine (161 µL, 1.43 mmol, 1.0 equiv.) and acetic acid (82 µL, 1.43 mmol, 1.0 equiv.) were added and the reaction mixture was stirred at 70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-(4-aminobenzyl)amino-modified ketose **114** (328 mg, 1.04 mmol) in a yield of 73%.



$C_{14}H_{20}N_2O_6$
Mw = 314.34 [g/mol]
Rf: 0.66 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: UV, VAN, CAM
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 8:1:1%
$[\alpha]_D$ = + 37.6 (*c* 1.82, MeOH)
Yield: 73 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 7.08 (d, 2H, phenyl), 6.69 (d, 2H, phenyl), 3.77 (dd, $J_{7,6}$ = 1.9 Hz, $J_{7,7'}$ = 10.9 Hz, 1H, H-7), 3.75-3.59 (m, 5-H, H-8, H-7', H-6, H-4), 3.29 (dd, $J_{5,4}$ = 8.4 Hz, $J_{5,6}$ = 9.3 Hz, 1H, H-5) 3.27 (d, $J_{3,4}$ = 9.3 Hz, 1H, H-3), 2.81 (d, $J_{1,1'}$ = 12.1 Hz, 1H, H-1), 2.76 (d, 1H, H1').

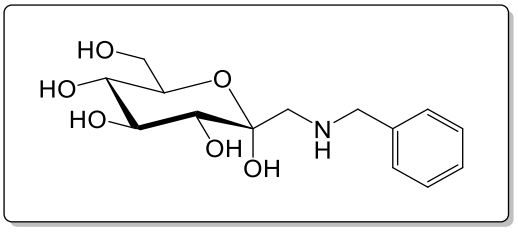
^{13}C-NMR (75 MHz, MeOH-d_4) δ = 147.8, 130.5, 129.9, 116.6 (6C, phenyl), 97.9 (C-2), 75.7, 74.0 (2C, C-6, C-4), 74.6 (C-3), 71.7 (C-5), 62.8 (C-7), 55.3 (C-1), 54.4 (C-8).

ESIMS (*m/z*): calcd for $[C_{14}H_{22}N_2O_6+H]^+$: 315.157; found 315.155 $[M+H]^+$.

1-(*N*-Benzyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulose (115)

To a solution of D-*glycero*-D-*gluo* aldoheptose **48** (150 mg, 0.714 mmol) in a mixture of EtOH (3 mL), 1,4-dioxane (0.3 mL) and water (2 drops), benylamine (78 µL, 0.714 mmol, 1.0 equiv.) and acetic acid (41 µL, 0.714 mmol, 1.0 equiv.) were added and the reaction mixture was stirred at

70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-benzylamino-modified ketose **115** (142 mg, 0.474 mmol) in a yield of 67%.



$C_{14}H_{21}NO_6$
Mw = 299.32 [g/mol]
Rf: 0.64 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 8:1:1%
$[\alpha]_D$ = + 46.5 (*c* 2.01, MeOH)
Yield: 67 %

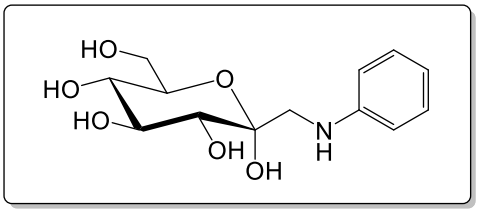
^{1}H-NMR (300 MHz, MeOH-d_4) δ = 7.37-7.19 (m, 5H, phenyl), 3.89-3.61 (m, 6H, H-8, H-7, H-7', H-6, H-4), 3.29 (d, $J_{3,4}$ = 9.34 Hz, 1H, H-3), 3.29 (dd, $J_{5,4}$ = 8.94 Hz, $J_{5,6}$ = 9.46 Hz, 1H, H-5), 2.84 (dd, $J_{1,1'}$ = 12.2 Hz, 1H, H-1), 2.78 (dd, 1H, H-1').

^{13}C-NMR (75 MHz, MeOH-d_4) δ = 140.6, 129.5, 129.4, 128.2 (6C, phenyl), 97.9 (C-2), 75.7 (C-6), 74.5 (C-3), 74.0 (C-4), 71.7 (C-5), 62.8 (C-7), 55.4 (C-1), 54.7 (C-8).

HRMS (MALDI): *m/z* calcd for $[C_{14}H_{21}NO_6+H]^+$: 300.1447; found 300.1445 $[M+H]^+$.

1-(*N*-Phenyl)amino-1-deoxy-α-D-*gluco*-hept-2-ulose (116)

To a solution of D-*glycero*-D-*gulo* aldoheptose **48** (100 mg, 0.476 mmol) in a mixture of EtOH (4 mL), 1,4-dioxane (0.4 mL) and water (2 drops), aniline (43 µL, 0.476 mmol, 1.0 equiv.) and acetic acid (27 µL, 0.476 mmol, 1.0 equiv.) were added and the reaction mixture was stirred at 70 °C for 2 days. Consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-phenylamino-modified ketose **116** (35 mg, 123 µmol) in a yield of 26%.



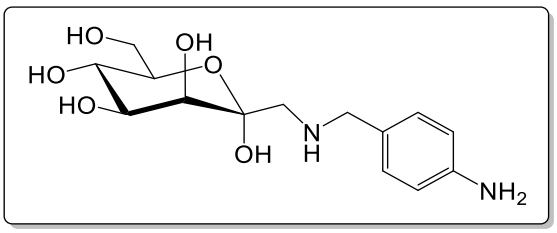
$C_{13}H_{19}NO_6$
Mw = 285.30 [g/mol]
Rf: 0.71 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 8:1:1%
Yield: 26 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ =7.10 (t, 2H, phenyl), 6.72 (d, 2H, phenyl), 6.62 (t, 1H, phenyl), 3.80 -3.75 (m, 3H, H-7, H-7', H-4), 3.71 (dd, $J_{6,6}$ = 9.2 Hz, $J_{6,7}$ = 9.3 Hz, 1H, H-6), 3.44 (d, 1H, H-1), 3.42 (d, 1H, $J_{3,4}$ = 9.36 Hz H-3), 3.41-3.33 (m, 1H, H-5), 3.19 (d, $J_{1,1'}$ = 12.4 Hz, 1H, H-1).

^{13}C-NMR (75 MHz, MeOH-d_4) δ =150.3, 129.9, 118.3, 114.4 (6C, phenyl), 98.7 (C-2), 76.0 (C-6), 74.0 (C-4), 73.5 (C-3), 71.5 (C-5), 62.6 (C-7), 50.7 (C-1).

1-(*N*-4-Aminobenzyl)amino-1-deoxy-α-D-*manno*-hept-2-ulose (118)

To a solution of D-*glycero*-D-*galacto*/D-*talo* aldoheptose **110** (300 mg, 1.43 mmol) in a mixture of EtOH (5 mL), 1,4-dioxane (0.5 mL) and water (2 drops), 4-aminobenylamine (161 µL, 1.43 mmol, 1.0 equiv.) and acetic acid (82 µL, 1.43 mmol, 1.0 equiv.) were added and the reaction mixture was stirred at 70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-(4-aminobenzyl)amino-modified ketose **118** (328 mg, 1.04 mmol) in a yield of 73%.



$C_{14}H_{20}N_2O_6$
Mw = 314.34 [g/mol]
Rf: 0.59 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 8:1:1%
$[\alpha]_D$ = + 5.75 (*c* 1.55, MeOH)
Yield: 73 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 7.08 (d, 2H, phenyl), 6.69 (d, 2H, phenyl), 3.85-3.75 (m, 3H, H-7, H-6, H-3), 3.74-3.65 (m, 4H, H-8, H-7', H-4), 3.61 (dd, $J_{5,4}$ =

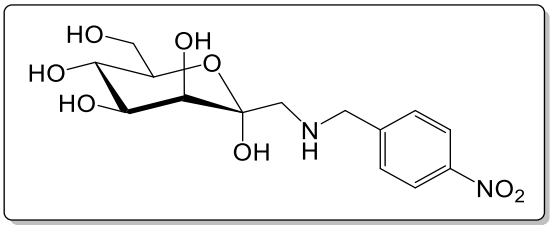
9.1 Hz, $J_{5,6}$ = 9.3 Hz, 1H, H-5) 2.89 (dd, $J_{1,1'}$ = 12.2 Hz, 1H, H-1), 2.79 (d, 1H, H1').

^{13}C-NMR (75 MHz, MeOH-d_4) δ = 148.0, 130.6, 129.3, 116.6 (6C, phenyl), 97.4 (C-2), 74.9 (C-3), 74.8 (C-4), 73.1 (C-6), 68.4 (C-5), 63.0 (C-7), 56.1 (C-1), 54.1 (C-8).

HRMS (MALDI): *m/z* calcd for $[C_{14}H_{20}N_2O_6+H]^+$: 315.1556; found 315.1550 $[M+H]^+$.

1-(*N*-4-Nitro-benzyl)amino-1-deoxy-α-D-*manno*-hept-2-ulose (119)

A solution of 4-nitro-benzylamine hydrochloride (269 mg, 1.43 mmol) in EtOH (5 mL) with 1,4-dioxane (0.5 mL) as co-solvent containing Et_3N (237 µL, 1.72 mmol, 1.2 equiv.) was applied to D-*glycero*-D-*galacto*/D-*talo* aldoheptose **110** (300 mg, 1.43 mmol). The reaction mixture was stirred at 70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-(4-nitrobenzyl)amino-modified ketose **119** (282 mg, 0.819 mmol) in a yield of 57%.



$C_{14}H_{20}N_2O_8$
Mw = 344.32 [g/mol]
Rf: 0.68 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 8:1:1%
$[\alpha]_D$ = + 10.4 (*c* 1.99, MeOH)
Yield: 57 %

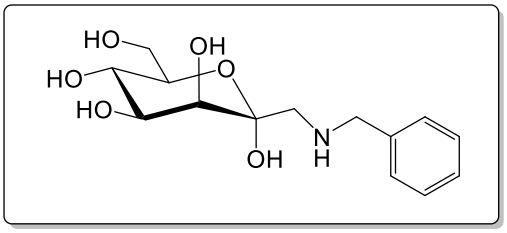
^{1}H-NMR (300 MHz, MeOH-d_4) δ = 8.22 (d, 2H, phenyl), 7.65 (d, 2H, phenyl), 4.05 (t, 2H, H-8), 3.89-3-67 (m, 5H, H-7, H-7', H-6, H-4, H-3), 3.63 (dd, $J_{5,4}$ = 8.99 Hz, $J_{5,6}$ = 9.12 Hz, 1H, H-5), 2.99 (d, $J_{1,1'}$ = 12.3 Hz, 1H, H-1), 2.86 (d, 1H, H-1').

^{13}C-NMR (75 MHz, MeOH-d_4) δ = 148.8 (C-9), 146.9 (C-12), 130.7 (C-10), 124.6 (C-11), 97.4 (C-2), 74.8, 74.87, 82.9 (3C, C-6, C-4, C-3), 68.3 (C-5), 62.8 (C-7), 55.8 (C-1), 53.2 (C-8).

HRMS (MALDI): *m/z* calcd for $[C_{14}H_{20}N_2O_8+H]^+$: 345.1298; found 345.1299 $[M+H]^+$.

1-(*N*-Benzyl)amino-1-deoxy-α-D-*manno*-hept-2-ulose (120)

To a solution of D-*glycero*-D-*galacto*/D-*talo* aldoheptose **110** (200 mg, 0.952 mmol) in a mixture of EtOH (4 mL), 1,4-dioxane (0.4 mL) and water (2 drops), benylamine (104 µL, 0.952 mmol, 1.0 equiv.) and acetic acid (54 µL, 0.952 mmol, 1.0 equiv.) were added and the reaction mixture was stirred at 70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-benzylamino-modified ketose **120** (159 mg, 0.531 mmol) in a yield of 56%.



$C_{14}H_{21}NO_6$
Mw = 299.32 [g/mol]
Rf: 0.69 ($CHCl_3$/MeOH/concd. NH4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH4OH 8:1:1%
$[\alpha]_D$ = + 10.6 (*c* 3.08, MeOH)
Yield: 56 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ =7.41-7.21 (m, 5H, phenyl), 3.88 -3.67 (m, 7H, H-8, H-7, H-7', H-6, H-4, H-3), 3.63 (dd, $J_{5,4}$ = 8.8 Hz, $J_{5,6}$ = 9.0 Hz, 1H, H-5), 2.93 (d, $J_{1,1'}$ = 12.2 Hz, 1H, H-1), 2.82 (d, 1H, H-1).

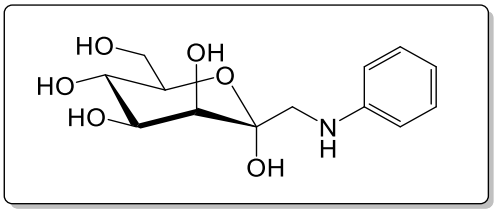
^{13}C-NMR (75 MHz, MeOH-d_4) δ =139.9, 129.6, 129.5, 128.4 (6C, phenyl), 97.4 (C-2), 74.9, 74.7, 73.1 (3C, C-6, C-4, C-3), 68.4 (C-5), 62.9 (C-7), 56.2 (C-1), 54.3 (C-8).

HRMS (MALDI): *m/z* calcd for $[C_{14}H_{21}NO_6+H]^+$: 300.1447; found 300.1448$[M+H]^+$.

1-(*N*-Phenyl)amino-1-deoxy-α-D-*manno*-hept-2-ulose (121)

To a solution of D-*glycero*-D-*galacto*/D-*talo* aldoheptose **110** (110 mg, 523 µmol) in a mixture of EtOH (1 mL), 1,4-dioxane (0.2 mL) and water (2 drops), aniline (47.8 µL, 523 µmol, 1.0 equiv.) and acetic acid (30.0 µL, 523 µmol, 1.0 equiv.) were added and the reaction mixture was stirred

at 70 °C for 2 days. Consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-phenylamino ketose **121** (35.0 mg, 123 µmol) in a yield of 24%.



$C_{13}H_{19}NO_6$
Mw = 285.30 [g/mol]
Rf: 0.76 ($CHCl_3$/MeOH/concd. NH4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH4OH 8:1:1%
$[\alpha]_D$ = + 21.5 (*c* 0.76, MeOH)
Yield: 24 %

^{1}H-NMR (500 MHz, CDCl3) δ =7.11 (dd, 2H, phenyl), 6.75 (d, 2H, phenyl), 6.65 (dd, 1H, phenyl), 3.90 (dd, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 9.4 Hz, 1H, H-4), 3.85 (d, 1H, H-3), 3.87 – 3.83 (m, 1H, H-7), 3.78 – 3.76 (m, 1H, H-6), 3.75 (dd, $J_{6,7'}$ = 5.3 Hz, $J_{7,7'}$ = 13.7 Hz, 1H, H-7'), 3.63 (dd, $J_{5,6}$ = 9.5 Hz, 1H, H-5), 3.43 (d, $J_{1,1'}$ = 12.7 Hz, 1H, H-1), 3.27 (d,1H, H-1').

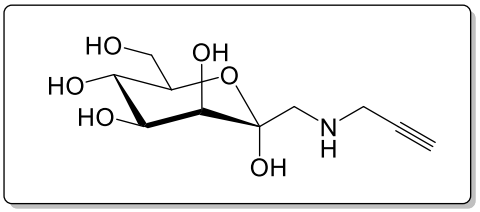
^{13}C-NMR (125 MHz, CDCl3) δ =150.2, 130.0, 118.7, 114.8 (6C, phenyl), 98.9 (C-2), 74.9 (C-6), 73.3 (C-3), 72.9 (C-4), 68.7 (C-5), 63.0 (C-7), 51.4 (C-1).

ESI MS calcd for $[C_{13}H_{19}NO_6+H]^+$: 286.1290; found *m/z* 286.129 $[M+H]^+$.

1-(*N*-Propargyl)amino-1-deoxy-α-D-manno-hept-2-ulose (122)

To a solution of D-glycero-D-galacto/D-talo aldoheptose **110** (467 mg, 2.22 µmol) in a mixture of EtOH (7 mL), 1,4-dioxane (1 mL) and water (2 drops), propargylamine (142 µL, 2.22 µmol, 1.0 equiv.) and acetic acid (127 µL, 2.22 µmol, 1.0 equiv.) were added and the reaction mixture was stirred at 70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-propargylamino-modified ketose **122** (420 mg, 1.70 µmol) in a yield of 77%.

$C_{10}H_{17}NO_7$



Mw = 247.25 [g/mol]
Rf: 0.58 ($CHCl_3$/MeOH/concd. NH4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH4OH 8:1:1%
$[\alpha]_D$ = + 13.2 (*c* 2.5, MeOH)
Yield: 77 %

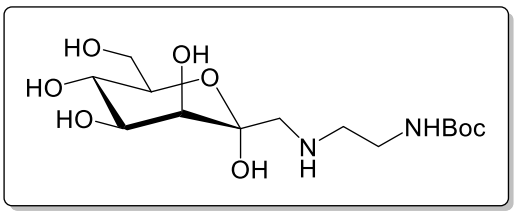
^{1}H-NMR (500 MHz, CDCl3) δ = 3.84 (dd, 1H, H-4), 3.82 (dd, $J_{7,6}$ = 2.2 Hz, 1H, H-7), 3.80 (d, $J_{3,4}$ = 3.3 Hz, 1H, H-3), 3.74 (dd, $J_{7,7'}$ = 11.5 Hz, $J_{7',6}$ = 5.5 Hz, 1H, H-7'), 3.72-3.69 (m, 1H, H-6), 3.62 (dd, $J_{4,5}$ = 9.4 Hz, $J_{5,6}$ = 9.5 Hz, 1H, H-5), 3.58 (d, 2H, H-8), 3.10 (d, $J_{1,1'}$ = 12.3 Hz, 1H, H-1), 2.96 (d, 1H, H-1'), 2.76 (t, 1H, H-10).

^{13}C-NMR (125 MHz, CDCl3) δ = 97.4 (C-2), 80.3 (C-9), 74.8 (2C, C-3, C-6), 74.5 (C-10), 72.9 (C-4), 68.2 (C-5), 62.8 (C-7), 55.4 (C-1), 38.6 (C-8).

ESI MS calcd for $[C_{10}H_{17}NO_6+H]^+$: 248.1134; found *m/z* 248.113 $[M+H]^+$.

1-(*N*-*tert*-Butoxycarbonyl))ethylenediamino-1-deoxy-α-D-*manno*-hept-2-ulose (123)

To a solution of D-*glycero*-D-*galacto*/D-*talo* aldoheptose **110** (281 mg, 1.34 mmol) in a mixture of EtOH (4 mL), 1,4-dioxane (0.4 mL) and water (2 drops), *N*-(*tert*-butoxycarbonyl)ethylenediamine (212 mg, 1.34 mmol, 1.0 equiv.) and acetic acid (76 µL, 1.34 mmol, 1.0 equiv.) were added and the reaction mixture was stirred at 70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 1/2/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 5/1 v/v containing 1% of concd. NH_4OH) gave 1-tert-butoxycarbonylethylenediamino-modified ketose **123** (438 mg, 1.24 mmol) in a yield of 93%.



$C_{14}H_{28}N_2O_8$
Mw = 352.38 [g/mol]
Rf: 0.79 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 5:1:1%
Yield: 93 %

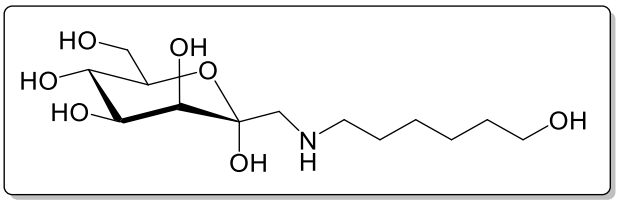
^{1}H-NMR (300 MHz, MeOH-d_4) δ =3.87-3.67 (m, 5H, H-7, H-7', H-6, H-4, H-3), 3.62 (dd, $J_{5,4}$ = 8.81 Hz, $J_{5,6}$ = 9.26 Hz, 1H, H-5), 3.25 (t, 2H, H-9), 3.07 (d, $J_{1,1'}$ = 12.3 Hz, 1H, H-1), 2.92 (s, 1H, H-1'), 2.87 (t, 2H, H-8), 1.55 (s, 9H, Boc).

^{13}C-NMR (75 MHz, MeOH-d_4) δ = 158.8 (C-10), 96.9 (C-2), 80.4 (C-11), 74.9, 74.8, 72.9 (3C, C-6, C-4, C-3), 68.2 (C-5), 62.8 (C-7), 56.1 (C-1), 50.2 (C-8), 39.8 (C-9), 28.7 (Boc).

ESIMS (*m/z*): calcd for $[C_{14}H_{28}N_2O_8+Na]^+$: 375.174; found 375.178 $[M+H]^+$.

1-(*N-(*6-Hydroxyhexyl)amino)-1-deoxy-α-D-*manno*-hept-2-ulose (124)

To a solution of D-*glycero*-D-*galacto*/D-*talo* aldoheptose **110** (300mg, 1.43 mmol) in a mixture of EtOH (5 mL), 1,4-dioxane (0.5 mL) and water (2 drops), 6-aminohexanol (167 mg, 1.43 mmol, 1.0 equiv.) and acetic acid (82µL, 1.43 mmol, 1.0 equiv.) were added and the reaction mixture was stirred at 70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 4/4/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-(6-hydroxyl)amino-modified ketose **124** (322 mg, 1.04 mmol) in a yield of 73%.



$C_{13}H_{27}NO_7$
Mw = 309.36 [g/mol]
Rf: 0.48 ($CHCl_3$/MeOH/concd. NH_4OH 4:4:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 8:1:1%
$[\alpha]_D$ = + 11.0 (*c* 1.79, MeOH)
Yield: 73 %

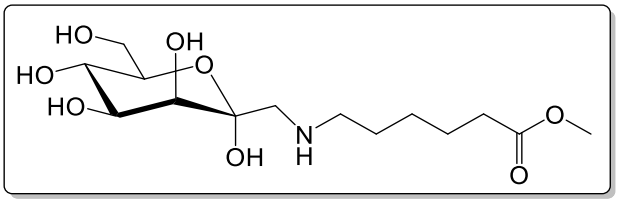
^{1}H-NMR (300 MHz, MeOH-d_4) δ =3.88-3.67 (m, 5H, H-7, H-7', H-6, H-4, H-3), 3.61 (dd, $J_{5,4}$ = 9.08 Hz, $J_{5,6}$ = 9.63 Hz, 1H, H-5), 3.55 (t, 2H, H-13), 2.90 (d, $J_{1,1'}$ = 12.2 Hz, 1H, H-1), 2.84 (d, 1H, H-1'), 2.66 (t, 2H, H-8), 1.63-1.48 (m, 4H, H-12, H-9), 1.46-1.29 (m, 4H, H-11, H-10).

^{13}C-NMR (75 MHz, MeOH-d_4) δ =97.4 (C-2), 74.8, 74.7 (2C, C-4, C-3), 73.1 (C-6), 68.4 (C-5), 62.9 (C-7), 62.8 (C-13), 56.9 (C-1), 50.6 (C-8), 33.5 (C-12), 30.1 (C-9), 28.0 (C-10), 26.8 (C-11).

HRMS (MALDI): *m/z* calcd for $[C_{13}H_{27}NO_7+H]^+$: 310.1866; found 310.1863 $[M+H]^+$.

1-(*N*-(5-(Methoxycarbonyl)pentyl)amino)-1-deoxy-α-D-*manno*-hept-2-ulose (125)

A solution of 6-aminohexanioc acid methyl ester hydrochloride (518 mg, 2.85 mmol, 2.0 equiv.) in EtOH (5 mL) with 1,4-dioxane (0.5 mL) as co-solvent containing Et_3N (396 µL, 2.85 mmol, 2.0 equiv.) was applied to D-*glycero*-D-*galacto*/D-*talo* aldoheptose **110** (300 mg, 1.43 mmol, 1.0 equiv.). The reaction mixture was stirred at 70 °C for 2 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 8/4/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 8/1 v/v containing 1% of concd. NH_4OH) gave 1-(5-Methoxycarbonyl-pentyl)amino-modified ketose **125** (336 mg, 0.996 mmol) in a yield of 70%.



$C_{14}H_{27}NO_8$
Mw = 337.37 [g/mol]
Rf: 0.52 ($CHCl_3$/MeOH/concd. NH_4OH 8:4:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 8:1:1%
$[\alpha]_D$ = + 10.7 (*c* 0.970, MeOH)
Yield: 70 %

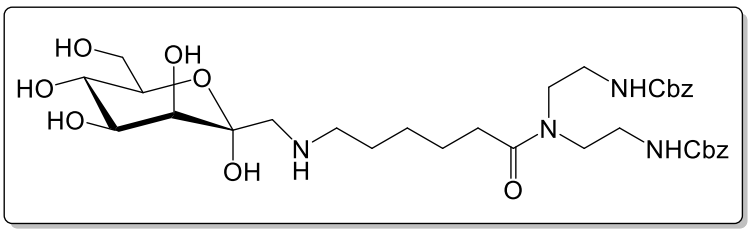
^{1}H-NMR (300 MHz, MeOH-d_4) δ = 3.87-3.57 (m, 9H, H-7, H-7', H-6, H-5, H-4, H-3, OMe), 3.04 (d, $J_{1,1'}$ = 12.3 Hz, 1H, H-1), 2.93 (d, 1H, H-1'), 2.78 (t, 2H, H-8), 2.35 (t, 2H, H-12), 1.71-1.52 (m, 4H, H-11, H-9), 1.45-1.31 (m, 2H, H-11).

^{13}C-NMR (75 MHz, MeOH-d_4) δ =175.8 (C-13), 96.9 (C-2), 74.9, (2C, C-4, C-3), 72.9 (C-6), 68.2 (C-5), 62.8 (C-7), 56.4 (C-1), 52.0 (OMe), 50.0 (C-8), 34.6 (C-12), 28.8 (C-9), 27.4 (C-10), 25.7 (C-11).

HRMS (MALDI): *m/z* calcd for $[C_{14}H_{27}NO_8+H]^+$: 338.1815; found 338.1811 $[M+H]^+$.

1-[N^1,N^7-(Di-benzyloxycarbonyl)-N^4-(aminopentenylcarbonyl)-dietyhlenetriamino]-1-deoxy-α-D-*manno*-hept-2-ulose (126)

To a solution of D-*glycero*-D-*galacto*/D-*talo* aldoheptose **110** (113mg, 0.538 mmol) in a mixture of EtOH (2 mL), 1,4-dioxane (0.5 mL) and water (2 drops), N^1,N^7-(di-benzyloxycarbonyl)-N^4-(aminopentenylcarbonyl)-dietyhlenetriamine **132** (261 mg, 0.538 mmol, 1.0 equiv.) and acetic acid (31µL, 0.538 mmol, 1.0 equiv.) were added and the reaction mixture was stirred at 70 °C for 4 days. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 3/1/1% v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 10/1 v/v containing 1% of concd. NH_4OH) gave compound **126** (184 mg, 0.272 mmol) in a yield of 51%.



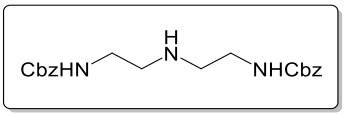
$C_{33}H_{48}N_4O_{11}$
Mw = 676.76 [g/mol]
Rf: 0.42
($CHCl_3$/MeOH/concd. NH_4OH 3:1:1%)
Detection: Van, Mly
Purification: SGC
$CHCl_3$/MeOH/concd. NH_4OH 10:1:1%
$[\alpha]_D$ = +5.68 (*c* 3.90, MeOH)
Yield: 51 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 7.31-7.13 (m, 10H, 2x phenyl), 4.97, 4.95 (s, 4H, 2x H-17), 3.81-3.61 (m, 5H, H-7, H-7', H-6, H-4, H-3), 3.57 (dd, $J_{4,5}$ = 8.39 Hz, $J_{5,6}$ = 9.39 Hz, 1H, H-5), 3.37-3.08 (m, 9H, 2x H-15, 2x H-14, H-1), 3.03 (d, $J_{1,1'}$ = 12.6 Hz, 1H, H-1), 2.86 (t, 2H, H-8), 2.23 (t, 2H, H-12), 1.64-1.34 (m, 4H, H-11, H-9), 1.29-1.14 (m, 2H, H-10).

^{13}C-NMR (75 MHz, MeOH-d_4) δ =175.9 (C-13), 158.8 (C-16), 138.4, 138.2, 129.5, 129.4, 129.1, 129.0, 128.8, 128.7 (12C, 2x phenyl), 95.9 (C-2), 75.1, 74.8, 72.5 (3C, C-6, C-4, C-3), 67.7 (C-5), 67.5, 67.4 (2C, 2X, C-17), 62.4 (C-7), 55.1 (C-1), 49.2 (C-8), 47.1 (C-14), 40.1, 39.7 (2C, 2x C-15), 33.3 (C-12), 27.0 (C-10), 26.4 (C-9), 26.6 (C-.11).

N^1-N^{10}-Dicarbobenzoxysperidine (128)

To a solution of diethylenetriamine (490 mg, 4.85mmol) in CH_2Cl_2 (15 mL), carbobenzoxyimidazole[218] (1.96, 9.69 mmol, 2 equiv.) and a catalytic amount of DMAP were added. The reaction mixture was stirred overnight at rt and the solvent was removed under reduced pressure. The product was recrystallized from EtOAc obtaining compound **128** (0.758 g, 2.04 mmol) as a white solid in 42% yield. The NMR data is in accordance with literature.[265]



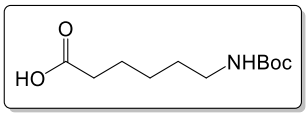
$C_{20}H_{25}N_3O_4$
Mw = 371.44 [g/mol]
Rf: 0.79 (EtOAc/MeOH/concd. NH_4OH 2:1:1%)
Detection: Van, Mly
Purification: Recrystallization from EtOAc
Yield: 42 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 7.47-7.27 (m, 10H, 2X phenyl), 5.13 (s, 4H, 2X H-4), 3.44 (t, 4H, 2X H-2), 3.12 (t, 4H, 2X H-1).

^{13}C-NMR (75 MHz, MeOH-d_4) δ =159.4 (2X C-3), 137.9, 129.5, 129.2, 129.0 (12C, phenyl), 67.8 (2X C-4), 49.5 (2X C-2), 38.8 (2X C-1).

***N*-(*tert*-Butoxycarbonyl)-amino hexcanoic acid[219] (130)**

To a solution of 6-amino hexanoic acid (3 g, 22.9 mmol) in a mixture of H_2O (20 mL) and acetone (20 mL), di-tert-butyl dicarbonate (5.44 g, 25.2 mmol, 1.1 equiv.) and Et_3N (6.34 ml, 45.7 mmol, 2 equiv.) were added and the reaction mixture was stirred overnight at rt. The organic solvent was removed under reduced pressure, the aqueous residue was acidified with 6% aqueous HCl and extracted with EtOAc. The organic phase was washed with brine, dried (Na_2SO_4) and filtered. After removal of the solvent under reduced pressure, compound **130** (3.95, 17.1 mmol) was obtained as a white solid (under 4°C) in 75% yield. The NMR data is in accordance with the literature.[219]



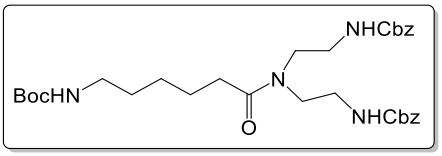
$C_{11}H_{21}NO_4$
Mw = 231.29 [g/mol]
Rf: 0.54 (EtOAc/MeOH/concd. NH_4OH 6:1:1%)
Detection: Van, Mly
Purification: crude
Yield: 75 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 3.06 (t, 2H, H-2), 2.32 (t, 2H, H-6), 1.46 (s, 9H, Boc), 1.69-1.60, 1.56-1.32 (m, 6H, H-3, H-4, H-5).

^{13}C-NMR (75 MHz, MeOH-d_4) δ =177.6 (C-1), 158.6 (C-7), 79.8 (C-8), 41.2 (C-6), 34.9 (C-2), 30.7, 27.4, 25.8 (3C,C-3,C-4,C-5), 28.8 (Boc).

N^1,N^7-(Di-benzyloxycarbonyl)-N^4-(butoxycarbonyl-aminopentenylcarbonyl)-dietyhlenetriamine (131)

To a solution of *N*-(*tert*-butoxycarbonyl)-amino hexcanoic acid **130** (312 mg, 1.35 mmol) in DMF (5 mL), HBTU (767 mg, 2.02 mmol, 1.5 equiv.), HoBt (276 mg, 2.02 mmol, 1.5 equiv.) and DIEA (704 µL, 4.04 mmol, 1.5 equiv.) were added and the reaction mixture was stirred for 20 minutes. After addition of N^1-N^{10}-dicarbobenzoxysperidine **128** (500 mg, 1.35mmol), the reaction mixture was stirred for further 8 hours. The solvents were removed under reduced pressure and the residue was purified bz column chromatography (Cy/EtOAc 20:1) to give compound **131** (322 mg, 0.551mmol) in 41%.[266]



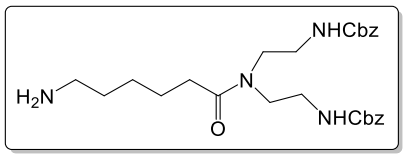
$C_{31}H_{44}N_4O_7$
Mw = 584.71 [g/mol]
Rf: 0.29 (C/EtOAc 1:4)
Detection: Van, Mly
Purification: SCG Cy/EtOAc 20:1
Yield: 41 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 7.45-7.26 (m, 10H, phenyl), 5.09 (d, 4H, H-1, H-8), 3.51-3.38 (m, 4H, H-4, H-5), 3.32-3.22 (m, 4H, H-3, H-6), 3.03 (t, 2H, H14), 2.33 (t, 2H, H-10), 1.45 (s,9H, Boc), 1.64-1.38, 1.38-1.21 (m ,6H, H-11, H-12, H-13)

^{13}C-NMR (75 MHz, MeOH-d_4) δ =176.4 (C-9), 158.9 (2C, C-2, C-7), 158.5 (C-15), 138.4, 138.3, 129.5-128.9 (12C, 2X phenyl), 79.8 (C-16), 67.6, 67.5 (2C, C-1, C-8), 49.2, 46.9 (2C, C-4, C-5), 41.2 (C-14), 40.2, 39.8 (2C, C-3, C-6), 33.8 (C-10), 28.8 (Boc), 30.7, 27.6, 26.2 (3C, C-11, C-12, C-13)

N^1,N^7-(Di-benzyloxycarbonyl)-N^4-(aminopentenylcarbonyl)-dietyhlenetriamine (132)

To a solution of compound **131** (500 mg, 0.855 mmol) in MeOH (5 mL), a solution of HCl (1 M in H_2O) was added dropwise until the pH of 1 was reached and the reaction mixture was stirred at rt for 3 hours. The reaction mixture was neutralized by addition of a satd aqueous sodium bicarbonate solution and extracted with CH_2Cl_2, dried (Na_2SO_4) and filtered. Removal of the solvent under reduced pressure and purification of the residue by column chromatography ($CHCl_3$/MeOH, 10/1 v/v containing 1% of concd. NH_4OH) gave compound **132** (303 mg, 0.625 mmol) in 73% yield.



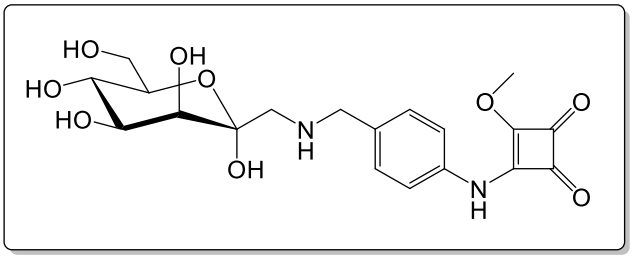
$C_{26}H_{36}N_4O_5$
Mw = 484.60 [g/mol]
Rf: 0.22 ($CHCl_3$/MeOH/concd. NH_4OH 5:1:1%)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 10:1:1%
Yield: 73 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 7.41-7.22 (m, 10H, 2x phenyl), 5.08, 5.06 (s, 4H, H-8, H-1), 3.47-3.37 (m, 4H, H-6, H-3), 3.29-3.19 (M, 4H, H-5, H-4), 2.73 (t, 2H, H-14), 2.33 (t, 2H, H-10), 1.64-1.41 (m, 4H, H-13, H-13), 1.39-1.24 (m, 2H, H-12).

^{13}C-NMR (75 MHz, MeOH-d_4) δ =176.1 (C-9), 158.9 (2C, C-7, C-2), 129.5, 129.4, 129.1, 129.0, 128.9, 128.8 (12 C, 2x phenyl), 67.5, 67.4 (2C, C-1, C-7), 49.2 (C-4), 47.1 (C-5), 41.4 (C-14), 40.2, 29.8 (2C, C-6, C-3), 33.5, 39.9, 27.2, 25.9 (4C, C-13, C-12,C-11, C-10).

3-methoxy-[1-(*N*-4-Aminobenzyl)amino-1-deoxy-α-D-*manno*-hept-2-ulose] 3-cyclobutene-1,2-dione (142)

To a solution of compound **118** (112mg, 0.356 mmol) in MeOH (5 mL), dimethyl squarate **137** (61 mg, 0.428 mmol, 1.2 equiv.) was added and the reaction mixture was stirred overnight at rt. Complete consumption of the starting material was indicated by TLC ($CHCl_3$/MeOH/NH_4OH, 8/4/1 v/v/v). The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 7/1 v/v containing 1% of concd. NH_4OH) gave compound **142** (33 mg, 0.078 mmol) in a yield of 22%.



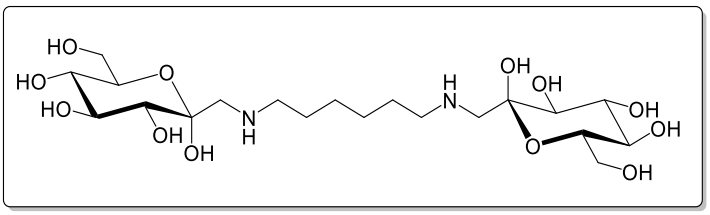
$C_{19}H_{24}N_2O_9$
Mw = 424.41[g/mol]
Rf: 0.42 ($CHCl_3$/MeOH/concd. NH_4OH 8:4:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 7:1:1%
Yield: 22 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 7.45-7.29 (m, 4H, phenyl), 4.46 (s, 3H, OMe), 3.95-3.59 (m, 5H, H-7, H-7', H-6, H-3, H-1), 3.41 (d, $J_{1,1'}$ = 14.3 Hz, 1H, H-1), 3.37-3.29 (m, 1H, H-5), 3.23 (dd, $J_{4,3}$ = 2.7 Hz, $J_{4,5}$ = 9.3 Hz, 1H, H-4).

^{13}C-NMR (75 MHz, MeOH-d_4) δ =189.9 (2C, C-14, C-15), 152.3 (C-16), 139.1, 130.3, 130.3, 121.2, 121.1 (phenyl), 130.9 (C-13), 99.4 (C-2), 75.7, 74.5, 74.3, 71.7 (4C, C-6, C-5, C-4, C-3), 62.8 (C-7), 61.5 (OMe), 53.9 (C-1).

Bis-*N*-(1-desoxy-α-D-*gluco*-hept-2-ulopyranosyl)-1,6-diaminohexane (143)

D-*glycero*-D-*gulo* aldoheptose **48** (300 mg, 1.43 mmol, 2 equiv.) was treated with 1,6-diaminohexane (83 mg, 0.714 mmol, 1 equiv.) in EtOH (8 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (82 µL, 1.43 mmol, 2 equiv.) at 70 C for 2 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 1/1 v/v containing 50% of conc. NH_4OH) gave dimeric product **143** (294 mg, 0.587 mmol) in a yield of 82%.



$C_{20}H_{40}N_2O_{12}$
Mw = 500.54 [g/mol]
Rf: 0.39 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 2:2:1
$[\alpha]_D$ = + 27.3 (*c* 1.06, H_2O)
Yield: 82 %

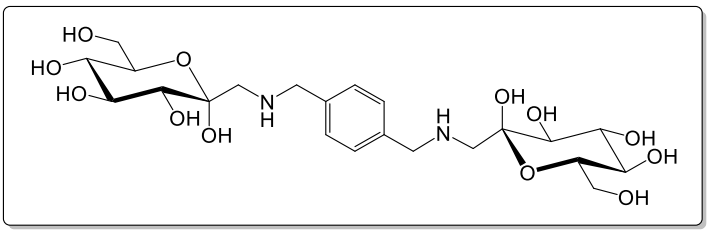
^{1}H-NMR (300 MHz, D_2O) δ = 3.90-3.63 (m, 8H, H-7, H-7', H-6, H-4), 3.44 (d, $J_{3,4}$ = 9.2 Hz, 2H, H-3), 3.38 (dd, $J_{5,4}$ = 9.4 Hz, $J_{5,6}$ = 10.4 Hz, 2H, H-5), 3.05 (d, $J_{1,1'}$ = 12.6 Hz, 2H, H-1), 2.96 (dd, 2H, H-1'), 2.80 (t, 4H, H-8), 1.58 (bs, 4H, H-9), 1.36 (bs, 4H, H-10).

^{13}C-NMR (75 MHz, D_2O) δ = 96.3 (C-2), 73.5, 72.4, 72.3 (3C, C-6, C-4, C-3), 69.5 (C-5), 60.7 (C-7), 53.6 (C-1), 48.9 (C-8), 26.9 (C-9), 25.8 (C-10).

HRMS (MALDI): *m/z* calcd for $C_{20}H_{40}N_2O_{12}$ $[M+H]^+$ 501.2660, found 501.2665.

Bis-*N*-(1-desoxy-α-D-*gluco*-hept-2-ulopyranosyl)-*p*-xylylenediamine (144)

D-*glycero*-D-*gulo* aldoheptose **48** (300 mg, 1.43 mmol, 2 equiv.) was treated with *p*-xylenyldiamine (97 mg, 0.714 mmol, 1 equiv.) in EtOH (5 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (82 µL, 1.43 mmol, 2 equiv.) at 70 °C for 2 days. The solvents were removed under reduced pressure and the crude product was passed through an ion exchange CG-120-II (Na+) Amberlite® resin column (H_2O; H_2O containing 1% of conc. NH_4OH) followed by purification on silica gel column chromatography ($CHCl_3$/MeOH, 1/1 v/v containing 25% of conc. NH_4OH) to give dimeric product **144** (281 mg, 0.540 mmol) in a yield of 76%.



$C_{22}H_{36}N_2O_{12}$
Mw = 520.53 [g/mol]
Rf: 0.37 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: IEC (H_2O; H_2O containing 1% of conc. NH_4OH); SGC ($CHCl_3$/MeOH/concd. NH_4OH 4:4:1)
$[\alpha]_D$ = + 34.3 (*c* 1.70, H_2O)
Yield: 76 %

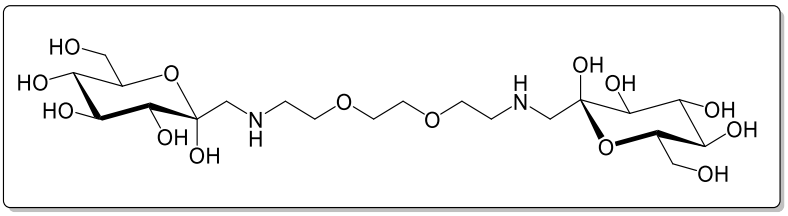
^{1}H-NMR (300 MHz, D_2O) δ = 7.43-7.36 (bs, 4H, phenyl), 3.92 (bs, 4H, H-8), 3.84-3.61 (m, 8H, H-7, H-7', H-6, H-4), 3.40 (d, $J_{3,4}$ = 9.4 Hz, 2H, H-3), 3.36 (dd, $J_{4,5}$ = 9.2 Hz, $J_{5,6}$ = 9.3 Hz, 2H, H-5), 2.97 (d, $J_{1,1'}$ = 12.5 Hz, 2H, H-1), 2.89 (d, 2H, H-1').

^{13}C-NMR (125 MHz, H_2O/D_2O 9/1 v/v) δ = 136.0, 129.3 (6C, phenyl), 96.5 (C-2), 73.6, 72.4, 72.3 (3C, C-6, C-4, C-3), 69.5 (C-5), 60.7 (C-7), 53.0 (C-1), 52.1 (C-8).

HRMS (MALDI): *m/z* calcd for $C_{22}H_{36}N_2O_{12}$ $[M+Na]^+$ 543.2166, found 543.2173.

Bis-*N*-(1-desoxy-α-D-*gluco*-hept-2-ulopyranosyl)-2-2'-(ethylenedioxy)-bis(ethylamine) (145)

D-*glycero*-D-*gulo* aldoheptose **48** (400 mg, 1.90 mmol, 2 equiv.) was treated with 2,2´-(ethylenedioxy)bis(ethylamine) (139 µL, 0.952 mmol, 1 equiv.) in EtOH (6 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (109 µL, 1.90 mmol, 2 equiv.) at 70 °C for 2 days. The solvents were removed under reduced pressure and the crude product was passed through an ion exchange CG-120-II (Na+) Amberlite® resin column (H_2O; H_2O containing 1% of conc. NH_4OH) followed by purification on silica gel column chromatography ($CHCl_3$/MeOH, 3/1 v/v containing 1% of conc. NH_4OH) to give 23% of dimeric compound **145** (118 mg, 0.222 mmol) and 56% of the monomeric product **146** (180 mg, 0.529 mmol).

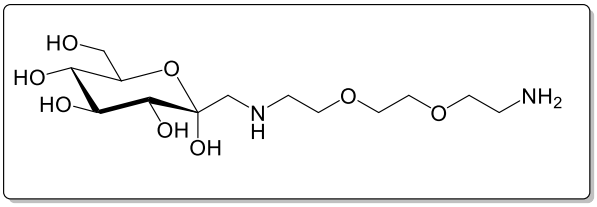


$C_{20}H_{40}N_2O_{14}$
Mw = 532.54 [g/mol]
Rf: 0.25 ($CHCl_3$/MeOH/concd. NH_4OH 1:3:1)
Detection: Van, Mly
Purification: IEC (H_2O; H_2O containing 1% of conc. NH_4OH); SGC ($CHCl_3$/MeOH/concd. NH_4OH 3:1:1%)
$[\alpha]_D$ = + 22.7 (*c* 2.23, H_2O)
Yield: 23 %

^{1}H-NMR (300 MHz, D_2O) δ = 3.84-3.64 (m, 12H, H-10, H-7, H-7', H-6, H-4,), 3.62 (t, 4H, H-9), 3.41 (d, $J_{3,4}$ = 9.4 Hz, 2H, H-3), 3.38 (dd, $J_{5,4}$ = 9.0 Hz, $J_{5,6}$ = 9.4 Hz, 2H, H-5), 2.89 (d, $J_{1,1'}$ = 12.8 Hz, 2H, H-1), 2.80 (t, 4H, H-8), 2.75 (dd, 2H, H-1').

^{13}C-NMR (75 MHz, D_2O) δ = 97.3 (C-2), 73.7, 72.2, 72.1 (3C, C-6, C-4, C-3), 69.6 (C-5), 69.5, 69.4 (2C, C-9, C-10), 60.8 (C-7), 53.9 (C-1), 48.2 (C-8).

HRMS (MALDI): *m/z* calcd for $C_{20}H_{40}N_2O_{14}D_2$ $[M+Na]^+$ 559.2690, found 559.2695.



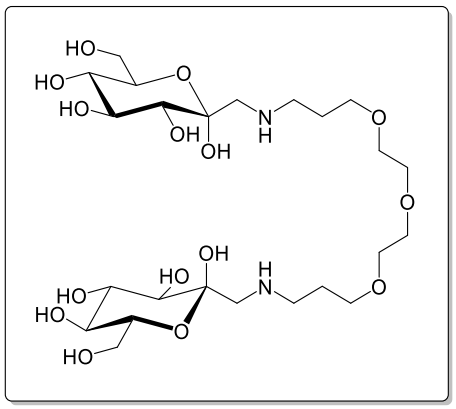
$C_{13}H_{28}N_2O_8$
Mw = 340.37 [g/mol]
Rf: 0.16 ($CHCl_3$/MeOH/concd. NH_4OH 1:3:1)
Detection: Van, Mly
Purification: IEC (H_2O; H_2O containing 1% of conc. NH_4OH); SGC ($CHCl_3$/MeOH/concd. NH_4OH 3:1:1%)
Yield: 56 %

^{1}H-NMR (300 MHz, D_2O) δ = 3.85-3.58 (m, 12H, H-12, H-11, H-10, H-9, H-7, H-7', H-6, H-4), 3.42 (d, $J_{3,4}$ = 9.4 Hz, 1H, H-3), 3.39 (dd, $J_{5,4}$ = 8.9 Hz, $J_{5,6}$ = 9.4 Hz, 1H, H-5), 3.15 (t, 2H, H-13), 2.94 (d, $J_{1,1'}$ = 12.7 Hz, 1H, H-1), 2.86 (t, 2H, H-8), 2.82 (dd, 1H, H-1').

^{13}C-NMR (75 MHz, D_2O) δ = 97.0 (C-2), 73.7, 72.3, 72.1 (3C, C-6, C-4, C-3), 69.6 (C-5), 69.5, 69.2, 69.1, 67.6 (4C, C-12, C-11, C-10, C-9), 60.7 (C-7), 53.8 (C-1), 48.2 (C-8), 39.3 (C-13).

Bis-*N*-(1-desoxy-α-D-*gluco*-hept-2-ulopyranosyl)-4,7,10-trioxa-1,13-tridecanediamine (147)

D-*glycero*-D-*gulo* aldoheptose **48** (300 mg, 1.43 mmol, 3 equiv.) was treated with 4,7,10-trioxa-1,13-tridecanediamine (104 µL, 0.476 mmol, 1 equiv.) in EtOH (4 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (55 µL, 0.952 mmol, 2 equiv.) at 70 °C for 2 days. The solvents were removed under reduced pressure and the crude product was passed through an ion exchange CG-120-II (Na+) Amberlite® resin column (H_2O; H_2O containing 1% of conc. NH_4OH) followed by purification on silica gel column chromatography ($CHCl_3$/MeOH, 1/1 v/v containing 25% of conc. NH_4OH) to give 58% of dimeric product **147** (168 mg, 0.278 mmol).



$C_{24}H_{48}N_2O_{15}$
Mw = 604.65 [g/mol]
Rf: 0.55 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: IEC (H_2O; H_2O containing 1% of conc. NH_4OH); SGC ($CHCl_3$/MeOH/concd. NH_4OH 4:4:1)
$[\alpha]_D$ = + 31.7 (*c* 2.83, H_2O)
Yield: 58 %

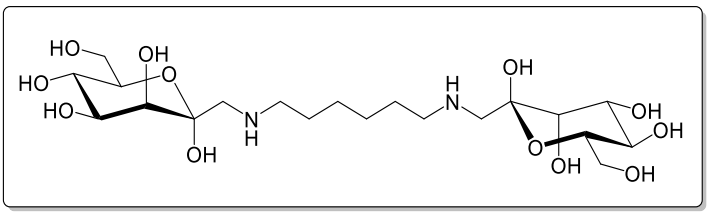
^{1}H-NMR (300 MHz, D_2O) δ = 3.85-3.62 (m,16H, H-12, H-11, H-7, H-7', H-6, H-4), 3.59 (t, 4H, H-10), 3.42 (d, $J_{3,4}$ = 9.3 Hz, 2H, H-3), 3.38 (dd, $J_{5,4}$ = 9.2 Hz, $J_{5,6}$ = 9.4 Hz, 2H, H-5), 2.98 (d, $J_{1,1'}$ = 12.6 Hz, 2H, H-1), 2.87 (d, 2H, H-1'), 2.81 (t, 4H, H-8), 1.82 (t, 4H, H-9).

^{13}C-NMR (125 MHz, H_2O/D_2O 9/1 v/v) δ = 95.4 (C-2), 73.4, 72.6, 72.5 (3C, C-6, C-4, C-3), 69.5, 69.4, 69.3 (3C, C-12, C-11, C-10), 68.4 (C-5), 60.6 (C-7), 53.3 (C-1), 46.6 (C-8), 25.9 (C-9).

HRMS (MALDI): *m/z* calcd for $C_{24}H_{48}N_2O_{15}$ $[M+H]^+$ 605.3133, found 605.3134.

Bis-*N*-(1-desoxy-α-D-*manno*-hept-2-ulopyranosyl)-1,6-diaminohexane (148)

D-*glycero*-D-*galacto*/D-*talo* aldoheptose **110** (204 mg, 0.971mmol, 2 equiv.) was treated with 1,6-diaminohexane (56 mg, 0.485 mmol, 1 equiv.) in EtOH (4 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (56 µL, 0.971 mmol, 2 equiv.) at 70 °C for 2 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 1/1 v/v containing 25% of conc. NH_4OH) gave dimeric product **148** (146 mg, 0.292mmol) in a yield of 60%.



$C_{20}H_{40}N_2O_{12}$
Mw = 500.54 [g/mol]
Rf: 0.39 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 4:4:1
$[\alpha]_D$ = + 11.0 (*c* 1.62, H_2O)
Yield: 60 %

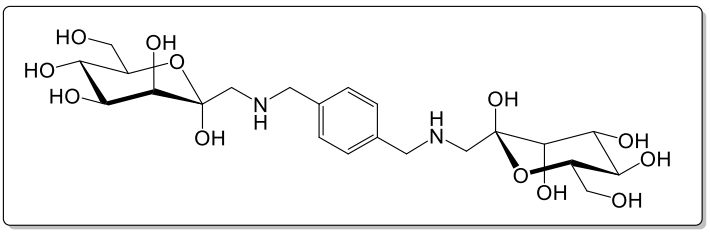
^{1}H-NMR (300 MHz, D_2O) δ = 3.98-3.86 (m, 10H, H-7, H-7', H-6, H-4, H-3), 3.66 (dd, $J_{5,4}$ = 9.3 Hz, $J_{5,6}$ = 9.5 Hz, 2H, H-5), 3.42 (d, $J_{1,1'}$ = 12.9 Hz, 2H, H-1), 3.22 (d, 2H, H-1'), 3.11 (t, 4H, H-8), 1.74 (bs, 4H, H-9), 1.42 (bs, 4H, H-10).

^{13}C-NMR (75 MHz, D_2O) δ = 95.1 (C-2), 73.5, 72.1, 70.6 (3C, C-6, C-4, C-3), 66.1 (C-5), 60.9 (C-7), 53.2 (C-1), 48.1 (C-8), 25.2 (C-9), 24.8 (C-10).

HRMS (MALDI): *m/z* calcd for $C_{20}H_{40}N_2O_{12}$ $[M+H]^+$ 501.2660, found 501.2664.

Bis-*N*-(1-desoxy-α-D-*manno*-hept-2-ulopyranosyl)-*p*-xylylenediamine (149)

D-*glycero*-D-*galaco/*D-*talo* aldoheptose **110** (300 mg, 1.43 mmol, 2 equiv.) was treated with *p*-xylenyldiamine (97 mg, 0.714 mmol, 1 equiv.) in EtOH (5 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (82 µL, 1.43 mmol, 2 equiv.) at 70 °C for 2 days. The solvents were removed under reduced pressure and the crude product was passed through an ion exchange CG-120-II (Na+) Amberlite® resin column (H_2O; H_2O containing 1% of conc. NH_4OH) followed by purification on silica gel column chromatography ($CHCl_3$/MeOH, 1/1 v/v containing 25% of conc. NH_4OH) to give dimeric product **149** (226 mg, 0.434 mmol) in a yield of 61%.



$C_{22}H_{36}N_2O_{12}$
Mw = 520.53 [g/mol]
Rf: 0.60 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: IEC (H_2O; H_2O containing 1% of conc. NH_4OH); SGC ($CHCl_3$/MeOH/concd. NH_4OH 4:4:1)
$[\alpha]_D$ = + 14.1 (*c* 3.4, H_2O)
Yield: 61 %

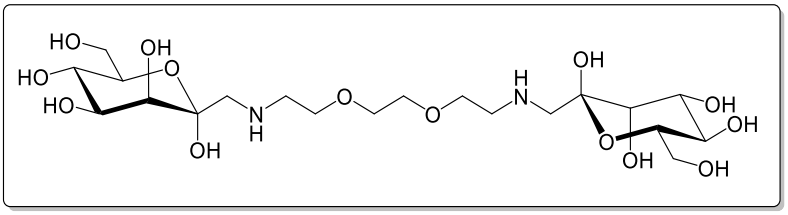
^{1}H-NMR (300 MHz, D_2O) δ = 7.46-7.39 (bs, 4H, phenyl), 4.00 (bs, 4H, H-8), 3.93-3.80 (m, 6H, H-7, H-4, H-3), 3.79-3.66 (m, 4H, H-7', H-6), 3.59 (dd, $J_{4,5}$ = 9.5 Hz, $J_{5,6}$ = 9.6 Hz, 2H, H-5), 3.01 (dd, $J_{1,1'}$ = 13.4 Hz, 2H, H-1), 2.97 (d, 2H, H-1').

^{13}C-NMR (125 MHz, H_2O/D_2O 9/1 v/v) δ = 135.8, 129.3 (6C, phenyl), 96.7 (C-2), 73.1 (C-6), 71.6, 70.9 (2C, C-4, C-3), 66.6 (C-5), 61.1 (C-7), 53.1 (C-1), 51.9 (C-8).

HRMS (MALDI): *m/z* calcd for $C_{22}H_{36}N_2O_{12}$ $[M+H]^+$ 521.2347, found 521.2346.

Bis-*N*-(1-desoxy-α-D-glucohept-2-ulopyranosyl)-2-2'-(ethylenedioxy)-bis(ethylamine) (150)

D-*glycero*-D-*galaco*/D-*talo* aldoheptose **110** (400 mg, 1.90 mmol, 2 equiv.) was treated with 2,2´-(ethylenedioxy)bis(ethylamine) (139 µL, 0.952 mmol, 1 equiv.) in EtOH (6 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (109 µL, 1.90 mmol, 2 equiv.) at 70 °C for 4 days. The solvents were removed under reduced pressure and the crude product was passed through an ion exchange CG-120-II (Na+) Amberlite® resin column (H_2O; H_2O containing 1% of conc. NH_4OH) followed by purification on silica gel column chromatography ($CHCl_3$/MeOH, 3/1 v/v containing 1% of conc. NH_4OH) to give 29% of dimeric product **150** (148 mg, 0.278 mmol) and 55% of monomeric product **151** (178 mg, 0.523 mmol).

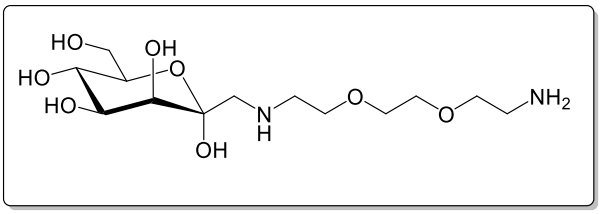


$C_{20}H_{40}N_2O_{14}$
Mw = 532.54 [g/mol]
Rf: 0.31 ($CHCl_3$/MeOH/concd. NH_4OH 1:3:1)
Detection: Van, Mly
Purification: IEC (H_2O; H_2O containing 1% of conc. NH_4OH); SGC ($CHCl_3$/MeOH/concd. NH_4OH 3:1:1%)
$[\alpha]_D$ = + 11.5 (*c* 1.73, H_2O)
Yield: 29 %

^{1}H-NMR (300 MHz, D_2O) δ = 3.97-3.87 (m, 4H, H-4, H-3), 3.86-3.67 (m, 14H, H-10, H-9, H-7, H-7', H-6), 3.64 (dd, $J_{5,4}$ = 9.4 Hz, $J_{5,6}$ = 9.5 Hz, 2H, H-5), 3.39 (d, $J_{1,1'}$ = 12.9 Hz, 2H, H-1), 3.33-3.20 (m, 4H, H-8), 3.24 (dd, 2H, H-1').

^{13}C-NMR (125 MHz, D_2O) δ = 97.0 (C-2), 73. 2, 71.5, 70.9 (3C, C-6, C-4, C-3), 69.5 (C-10), 68.4 (C-5), 66.7 (C-9), 61.1 (C-7), 53.7 (C-1), 47.9 (C-8).

HRMS (MALDI): *m/z* calcd for $C_{20}H_{40}N_2O_{14}$ $[M+Na]^+$ 555.2377, found 555.2379.



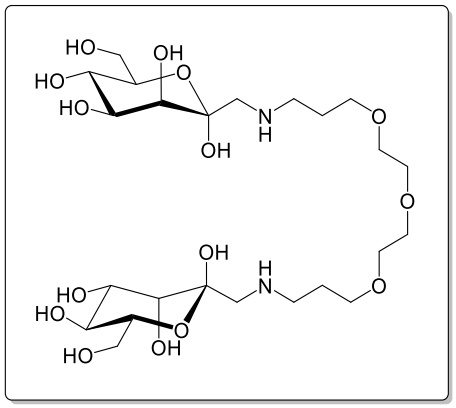
$C_{13}H_{28}N_2O_8$
Mw = 340.37[g/mol]
Rf: 0.17 ($CHCl_3$/MeOH/concd. NH_4OH 1:3:1)
Detection: Van, Mly
Purification: IEC (H_2O; H_2O containing 1% of conc. NH_4OH); SGC ($CHCl_3$/MeOH/concd. NH_4OH 3:1:1%)
Yield: 55 %

^{1}H-NMR (300 MHz, D_2O) δ = = 3.99-3.56 (m, 14H, H-12, H-11, H-10, H-9, H-7, H-7', H-6, H-5, H-4, H-3), 3.46 (d, $J_{1,1'}$ = 13.0 Hz, 1H, H-1), 3.27 (d, 1H, H-1'), 3.38-3.15 (4H, H-13, H-8).

^{13}C-NMR (125 MHz, D_2O) δ = 94.9 (C-2), 73.5, 72.3, 70.6 (3C, C-6, C-4, C-3), 66.1 (C-5), 69.7, 66.4, 65.1 (4C, C-12, C-11, C-10, C-9), 60.8 (C-7), 53.3 (C-1), 47.4 (C-8), 39.1 (C-13).

Bis-*N*-(1-desoxy-α-D-*manno*-hept-2-ulopyranosyl)-4,7,10-trioxa-1,13-tridecanediamine (152)

D-*glycero*-D-*galaco*/D-*talo* aldoheptose **110** (448 mg, 2.13 mmol, 3 equiv.) was treated with 4,7,10-trioxa-1,13-tridecanediamine (156 µL, 0.711 mmol, 1 equiv.) in EtOH (6 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (82 µL, 1.43 mmol, 2 equiv.) at 70 °C for 4 days. The solvents were removed under reduced pressure and the crude product was passed through an ion exchange CG-120-II (Na+) Amberlite® resin column (H_2O; H_2O containing 1% of conc. NH_4OH) followed by purification on silica gel column chromatography ($CHCl_3$/MeOH, 1/1 v/v containing 25% of conc. NH_4OH) to give 54% of dimeric product **152** (234 mg, 0.387mmol).



$C_{24}H_{48}N_2O_{15}$
Mw = 604.65 [g/mol]
Rf: 0.54 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: IEC (H_2O; H_2O containing 1% of conc. NH_4OH); SGC ($CHCl_3$/MeOH/concd. NH_4OH 4:4:1)
$[\alpha]_D$ = + 12.9 (*c* 2.24, H_2O)
Yield: 54 %

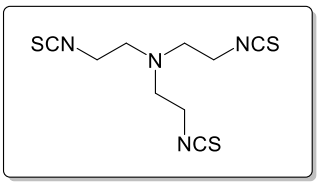
^{1}H-NMR (300 MHz, D_2O) δ = 3.94-3.83 (m, 6H, H-7, H-4, H-3), 3.78-3.71 (m, 4H, H-7', H-6), 3.69-3.65 (s, 12H, H-12, H-11, H-10), 3.62 (dd, 2H, H-5), 3.13 (d, $J_{1,1'}$ = 12. 5 Hz, 2H, H-1), 3.08 (d, 1H, H1'), 2.98 (t, 4H, H-8), 1.90 (m, 4H, H-9).

^{13}C-NMR (125 MHz, H_2O/D_2O 9/1 v/v) δ = 96.5 (C-2), 73.1 (C-6), 71,8, 70.9 (2C, C-4, C-3), 69.5, 69.3, 68.8 (3C, C-12, C-11, C10), 66.6 (C-5), 61.1 (C-7), 53.9 (C-1), 46.5 (C-8), 26.9 (C-9).

HRMS (MALDI): *m/z* calcd for $C_{24}H_{48}N_2O_{15}$ $[M+H]^+$ 605.3133, found 605.3135.

Tris(2-isocyanatoethyl)amine (162)[230b]

To a solution of tris(2-aminoethyl)amine **158** (297 µL, 2.00 mmol) in THF (8 mL), DCC (1.65 g, 8 mmol, 4 equiv.) and CS_2 (3.2 mL, large excess) dissolved in THF (8 mL) was added dropwise at -10 °C. The reaction mixture was warmed up to rt and stirred overnight. Diethyl ether (5 mL) was added and the precipitate solid of N,N'-dicyclohexyl-thiourea was filtered off and washed with diethyl ether (5 mL). The filtrate was evaporated and the resulting solid was washed with cyclohexane followed by purification on silica gel column chromatography ($CHCl_3$) to give 56% of compound **162** (306 mg, 1.12 mmol). The NMR data is in accordance with literature.[230b]



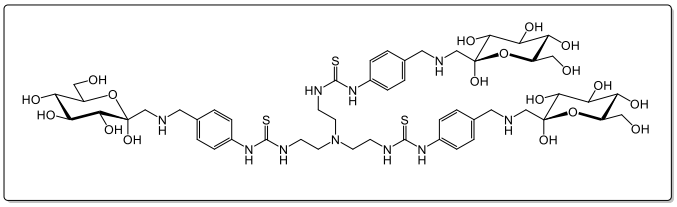
$C_9H_{12}N_4S_3$
Mw = 272.40 [g/mol]
Rf: 0.83 ($CHCl_3$)
Detection: Van, Mly
Purification: SGC ($CHCl_3$)
Yield: 56 %

^{1}H-NMR (300 MHz, $CDCl_3$) δ = 3.57 (t, 6H, H-2), 2.92 (t, 6H, H-1).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 132.1 (C-3), 54.2 (C-2), 44.2 (C-1).

Tris-*N*-(1-desoxy-α-D-*gluco*-hept-2-ulopyranosyl)-(2-isocyanatoethyl)amine (**163**)[232]

To a solution of compound **114** (294 mg, 0.936 mmol, 3 equiv.) in DMF (10 mL), tris(2-isocyanatoethyl)amine **162**[230b] (85 mg, 0.312 mmol) and DIPEA (160 µ, 0.936 mmol, 3 equiv.) were added and the reaction mixture was stirred for 3 days at rt. Water was added to allow dialysis (100 mwco, 72 hours, 7x 25 mL), and compound **163** was isolated in 67% yield.



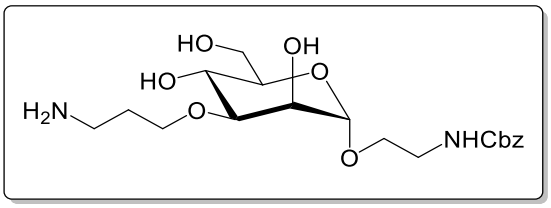
$C_{51}H_{78}N_{10}O_{18}S_3$
Mw = 1216.42 [g/mol]
TLC: $CHCl_3$/MeOH/concd. NH_4OH 1:2:1
Detection: Van, Mly
Purification: dialysis
Yield: 67 %

^{1}H-NMR (500 MHz, DMSO-d_6) δ = 7.42-7.13, 7.07-6.91, 6.58-6,47 (m, 12H, 3x phenyl), 4.12-3.26 (m, 30H, 3x H-14, 3x H-8, 3x H-7, 3x H-7', 3x H-6, 3x H-5, 3x H-4, 3x H-3), 3.25-2.58 (m, 12H, 3x H-15, 3x H-1, 3x H-1').

^{13}C-NMR (125 MHz, DMSO-d_6) δ = 180.6, 180.3, 180.1 (3C, 3x C-13), 147.8, 128.8, 128.7, 128.6, 123.6, 113.8 (18C, 3x phenyl), 91.3, 91.1, 91.0 (3C, 3x C-2), 74.8, 74.7, 74.3, 71.3, 71.2, 67.0, 66.9, 66.8 (15C, 3x C-6, 3x C-5, 3x C-4, 3x C-3), 63.4. 63.3 (3C, 3x C-7), 54.5, 54.4, 54.3 (3C, 3x C-1), 52.6, 52.5, 52.4 (3C, 3x C-15), 49.7, 49.6, 49.5 (3C, 3x C-8), 40.1, 39.9, 39.8 (3C, 3x C-14).

2-(Benzyloxycarbonyl)amnioethyl 3-*O*-(3-aminopropyl)-α-D-mannopyranoside (166)[233]

To a solution of 2-(benyloxycarbonyl)-3-*O*-(3-phthalimidopropyl)- α-D-mannopyranoside (**165**)[233-234] (175 mg, 0.321 mmol) in MeOH (10 mL) was treated with hydrazine monohydrate (56 µL, 1.16 mmol, 3.6 equiv.) and stirred for 5 hours at 50 °C. The reaction mixture was filtered through a bed of celite, the solvent was removed under reduced pressure and the residue was taken up in H_2O, the pH was adjusted to 5 with 6% aqueous HCl and extracted thoroughly with EtOAc. Removal of the solvent under reduced pressure and purification by column chromatography ($CHCl_3$/MeOH, 6/1 v/v containing 1% of conc. NH_4OH) gave compound **166** (106 mg, 0.256 mmol) in 80% yield.



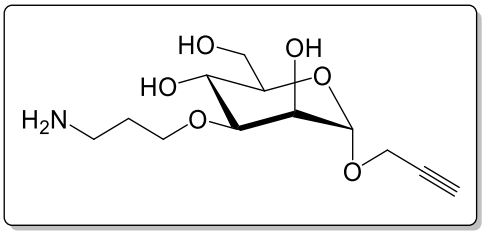
$C_{19}H_{30}N_2O_8$
Mw = 414.46 [g/mol]
Rf: 0.33 ($CHCl_3$/MeOH/concd. NH_4OH 4:4:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 6:1:1%
Yield: 80 %

^{1}H-NMR (300 MHz, D_2O) δ = 7.47-7.33 (5H, phenyl), 5.09 (bs, 2H, PhCH_2O), 4.85 (dd, $J_{1,2}$ = 1.65 Hz, 1H, H-1), 4.05 (bs, 1H, H-2), 3.80 (dd, $J_{6,6'}$ = 12.3 Hz, $J_{6,5}$ = 2.3 Hz, 1H, H-6), 3.78-3.45 (m, 8H, OCH_2CH$_2$CH$_2$NH$_2$, CH_2CH$_2$NH, H-6', H-5, H-4, H-3), 3.42-3.27 (bs, 2H, CH$_2$CH_2NH), 2.78-2.67 (bs, 2H, OCH$_2$CH$_2$CH_2NH$_2$), 1.74 (t, 2H, OCH$_2$CH_2CH$_2$NH$_2$).

^{13}C-NMR (125 MHz, D_2O) δ = 158.4 (C=O), 136.6, 128.8, 128.4, 127.6 (6C, phenyl), 99.6 (C-1), 78.7 (C-3), 72.8 (C-5), 67.4 (CH_2CH_2NH), 66.8 ($PhCH_2O$), 66.6 (C-2), 66.3 ($OCH_2CH_2CH_2NH_2$), 65.6 (C-4), 60.8 (C-6), 40.1 (CH_2CH_2NH), 37.8 ($OCH_2CH_2CH_2NH_2$), 30.9 ($OCH_2CH_2CH_2NH_2$).

Propynyl-3-*O*-(3-aminopropyl) α-D-mannopyranoside (169)[233]

To a solution of 2-propynyl-3-*O*-(3-phthalimidopropyl) α-D-mannopyranoside (**168**)[233, 235] (200 mg, 0.493 mmol) in MeOH (10 mL) was treated with hydrazine monohydrate (89 µL, 1.83 mmol, 3.7 equiv.) and stirred for 5 hours at 50° C. The reaction mixture was filtered through a bed of celite, the solvent was removed under reduced pressure and the residue was taken up in H_2O, the pH was adjusted to 5 with 6% aqueous HCl and extracted thoroughly with EtOAc. Removal of the solvent under reduced pressure and purification by column chromatography ($CHCl_3$/MeOH, 6/1 v/v containing 1% of conc. NH_4OH) gave compound **169** (121 mg, 0.440 mmol) in 89% yield.



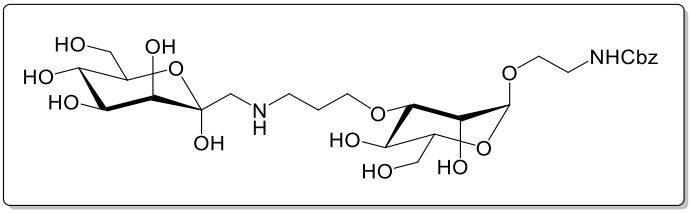
$C_{12}H_{21}NO_6$
Mw = 275.30 [g/mol]
Rf: 0.16 ($CHCl_3$/MeOH/concd. NH_4OH 4:4:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 6:1:1%
Yield: 86 %

^{1}H-NMR (300 MHz, D_2O) δ = 5.06 (d, 1H, H-1), 4.32 (dd, 2H, $OCH_2C{\equiv}CH$), 4.15 (dd, $J_{2,3}$ = 3.3 Hz, $J_{2,1}$ = 1.9 Hz 1H, H-2), 3.88 (dd, $J_{6,6'}$ = 12.1 Hz, $J_{6,5}$ = 1.9 Hz, 1H, H-6), 3.84-3.61 (m, 6H, $OCH_2CH_2CH_2NH_2$, $OCH_2C{\equiv}CH$, H-6', H-5, H-4), 3.58 (dd, $J_{3,4}$ = 8.3 Hz, $J_{3,2}$ = 3.2 Hz, 1H, H-3), 2.93 (t, 2H, $OCH_2CH_2CH_2NH_2$), 1.91-1.81 (m, 2H, $OCH_2CH_2CH_2NH_2$).

^{13}C-NMR (125 MHz, D_2O) δ = 98.7 (C-1), 78.6 (C-3), 78.4 ($C{\equiv}CH$), 73.2 (C-5), 67.5 ($OCH_2CH_2CH_2NH_2$), 66.5 (C-2), 65.5 (C-4), 60.8 (C-6), 54.6 ($OCH_2C{\equiv}CH$), 37.9 ($OCH_2CH_2CH_2NH_2$), 29.2 ($OCH_2CH_2CH_2NH_2$).

1-(*N*-Benzyloxycarbonylaminoethyl-α-D-mannopyranoside-3-*O*-yl]propyl)amino-1-deoxy-α-D-*manno*-hept-2-ulose (170)

D-*glycero*-D-*galaco/*D-*talo* aldoheptose **110** (222 mg, 1.06 mmol, 1 equiv.) was treated with 2-(benzyloxycarbonyl)amnioethyl 3-*O*-(3-aminopropyl)-α-D-mannopyranoside **166** (436 mg, 1.05 mmol, 1 equiv.) in EtOH (5 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (60 µL, 1.06 mmol, 1 equiv.) at 70 °C for 5 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 1/1 v/v containing 1% of conc. NH_4OH) gave product **171** (250 mg, 0.412 mmol) in 39% yield.



$C_{26}H_{42}N_2O_{14}$
Mw = 606.62 [g/mol]
Rf: 0.59 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 1:1:1%
$[\alpha]_D$ = + 18.6 (c 1.95, H_2O)
Yield: 39 %

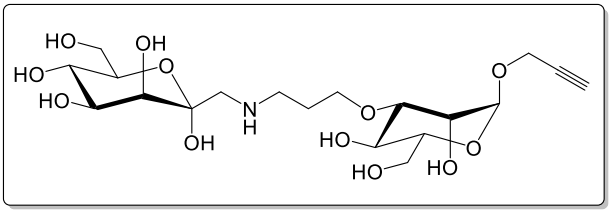
^{1}H-NMR (300 MHz, D_2O) at pH= 7 H/D exchange; pH=8: δ = 7.39-7.27 (s, 5H, phenyl), 5.00 (dd, 2H, H-20), 4.87 (bs, 1H, H-11, pH=7), 4.01 (s, 1H, H-12), 3.95-3.45 (m, 15H, H-17, H-16, H-16', H-15, H-14, H-13, H-10, H-7, H-7', H-6, H-5, H-4, H-3), 3.28 (q, 2H, H-18), 3.13 (d, 1H, $J_{1,1'}$ = 12.8 Hz, 1H, H-1), 3.04 (d,1H, H-1'), 2.99 (t, 2H, H-8), 2.00-1.74 (m, 2H, H-9).

^{13}C-NMR (125 MHz, H_2O/D_2O 9/1 v/v)) δ = 158.4 (C=O), 136.5, 128.8, 128.4, 127.6 (6C, phenyl), 99.6 (C-11), 95.6 (C-2), 78.9 (C-13), 73.3, 72.1, (2C, C-4, C-3), 72.7 (C-15), 70.7 (C-6), 67.5, 66.8, 66.4 (3C, C-20, C-17, C-10), 66.5 (C-12), 66.3 (C-14), 65.5 (C-5), 60.9, 60.7 (2C, C-16, C-7), 53.7 (C-1), 47.1 (C-8), 40.1 (C-18), 25.9 (C-9).

HRMS (MALDI): *m/z* calcd for $C_{26}H_{42}N_2O_{14}$ $[M+H]^+$ 607.2714, found 607.2717.

1-(*N*-[Propynyl-β-D-mannopyranodise-3-*O*-yl]propyl)amino-1-deoxy-α-D-*manno*-hept-2-ulose (171)

D-*glycero*-D-*galaco/*D-*talo* aldoheptose **110** (178 mg, 0.847 mmol, 1.2 equiv.) was treated with 2-propynyl, 3-*O*-(3-aminopropyl)-α-D-mannopyranoside **169** (190 mg, 0.690 mmol, 1equiv.) in EtOH (4 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (48 µL, 0.847 mmol, 1.2 equiv.) at 70 °C for 5 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 2/1 v/v containing 1% of conc. NH_4OH) gave product **171** (81 mg, 0.173 mmol) in 25% yield.



$C_{19}H_{33}NO_{12}$
Mw = 467.47 [g/mol]
Rf: 0.81 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 2:1:1%
$[\alpha]_D$ = = + 37.9 (c 0.912, H_2O)
Yield: 25 %

^{1}H-NMR (300 MHz, D_2O) δ = 5.04 (bs, 1H, H-11), 4.31 (dd, 2H, H-17), 4.13 (bs, 1H, H-12), 3.94-3.59 (m, 12H, H-16, H-16', H-15, H-14; H-10, H-7, H-7', H-6, H-5, H-4, H-3), 3.56 (dd, $J_{13,12}$ = 3.5 Hz, $J_{13,14}$= 9.2 Hz, 1H, H-13), 3.17-2.88 (m, 4H, H-1, H-1', H-8), 1.99-1.86 (m, 2H, H-9).

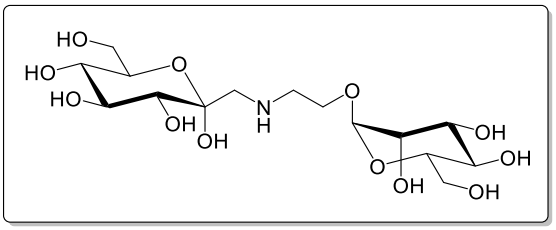
^{13}C-NMR (125 MHz, H_2O/D_2O 9/1 v/v)) δ = 98.6 (C-11), 95.0 (C-2), 78.7 (C-13), 78.6 (C-18), 76. 1 (C-19), 73.4, 73.0, 72.1, 70.6, 67.3, 66.4, 66.1, 65.5 (8C, C-15, C-14, C-12, C-10, C-6, C-5, C-4, C-3), 60.8, 60.7 (2C, C-16, C-7), 54.5 (C-17), 53.6 (C-1), 47.2 (C-8), 25.2 (C-9).

HRMS (MALDI): *m/z* calcd for $C_{19}H_{33}NO_{12}$ $[M+H]^+$ 468.2081, found 468.2085.

***N*-[(α-D-Mannopyranosyl)ethyl]amino-1-deoxy-α-D-*gluco*-hept-2-ulose (173)**

D-*glycero*-D-*gulo* aldoheptose **48** (105 mg, 0.500 mmol, 1.1 equiv.) was treated with 2-aminoethyl-α-D-mannopyranoside **172**[188b] (100 mg, 0.448 mmol, 1 equiv.) in EtOH (4 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (26 µL, 0.448 mmol, 1 equiv.) at 70 °C for 4 days. The solvents were removed under reduced pressure and subsequent column

chromatography ($CHCl_3$/MeOH, 1/1 v/v containing 25% of conc. NH_4OH) gave product **173** (84 mg, 0.202 mmol) in 45% yield.



$C_{15}H_{29}NO_{12}$
Mw = 415.39 [g/mol]
Rf: 0.43 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 4:4:1
$[\alpha]_D$ = + 46.8 (c 1.15, H_2O)
Yield: 45 %

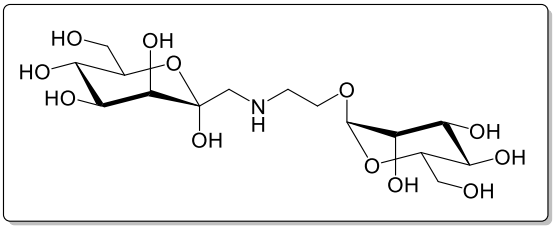
^{1}H-NMR (300 MHz, D_2O) δ = = 4.88 (d, $J_{10,11}$ = 1.8 Hz 1H, H-10), 4.02-3.57 (m, 12H, H-15, H-15', H-14, H-13, H-12, H-11; H-9, H-7, H-7', H-6, H-4), 3.46 (d, $J_{3,4}$ = 9.6 Hz, 1H, H-3), 3.41 (dd, $J_{5,4}$ = 9.5 Hz, $J_{5,6}$ = 9.6 Hz, 1H, H-5), 3.21 (d, 1H, H-1), 3.25-3.16 (m, 2H, H-8), 3.17 (d, $J_{1,1'}$ = 12.0 Hz, 1H, H-1').

^{13}C NMR (75 MHz, D_2O) δ = 100.0 (C-10), 95.5 (C-2), 73.4, 73.0 (2C, C-6, C-4), 72.7 (C-14), 72.6 (C-3), 70.5, 69.9 (2C, C-13, C-11), 69.4 (C-5), 66.7 (C-12), 63.3 (C-9), 60.9, 60.6 (2C, C-15, C-7), 53.1 (C-1), 47.6 (C-8).

HRMS (MALDI): *m/z* calcd for $C_{15}H_{29}NO_{12}$ $[M+H]^+$ 416.1768, found 416.1768.

_N_-[(α-D-Mannopyranosyl)ethyl]amino-1-deoxy-α-D-*manno*-hept-2-ulose (174)

D-*glycero*-D-*galaco*/D-*talo* aldoheptose **110** (94 mg, 0.448 mmol, 1 equiv.) was treated with 2-aminoethyl-α-D-mannopyranoside **172**[188b] (100 mg, 0.448 mmol, 1 equiv.) in EtOH (4 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (26 µL, 0.448 mmol, 1 equiv.) at 70 °C for 4 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 1/1 v/v containing 25% of conc. NH_4OH) gave product **174** (120 mg, 0.289 mmol) in 65% yield.



$C_{15}H_{29}NO_{12}$
Mw = 415.39 [g/mol]
Rf: 0.33 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 4:4:1
$[\alpha]_D$ = + 33.9 (c 1.36, H_2O)
Yield: 65 %

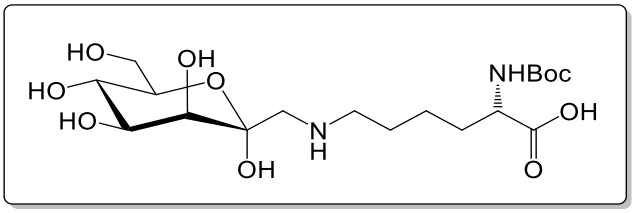
^{1}H-NMR (300 MHz, D_2O) δ = = 4.89 (bs, 1H, H-10), 4.05-3.69 (m, 11H, H-15, H-15', H-13, H-11, H-9, H-7, H-7', H-6, H-4, H-3), 3.68-3.54 (m, 3H, H-14, H-12; H-5), 3.46 (d, $J_{1,1'}$ = 12.9 Hz, 1H, H-1), 3.37 (t, 2H, H-8), 3.27 (d, 1H, H-1').

^{13}C NMR (75 MHz, D_2O) δ = 99.9 (C-10), 96.5 (C-2), 73.2, 71.9 (2C, C-4, C-3), 72.9, 70.9 (2C, C-13, C-12), 70.5 (C-6), 69.9 (C-11), 66.7, 66.5 (2C, C-14, C-5), 64.7 (C-9), 61.0, 60.9 (2C, C-15, C-7), 53.7 (C-1), 47.7 (C-8).

HRMS (MALDI): *m/z* calcd for $C_{15}H_{29}NO_{12}$ $[M+H]^+$ 417.1846, found 417.1807.

1-*N*-[(5S)-(*tert*-Butoxycarbonylamino)-5-(hydroxycarbonyl)pentyl]-amino-1-deoxy-α-D-*manno*-hept-2-ulose (176)

D-*glycero*-D-*galaco*/D-*talo* aldoheptose **110** (200 mg, 0.952 mmol, 1. equiv.) was treated with N_α-(*tert*-butoxycarbonyl)-L-lysine **175** (234 mg, 0.950 mmol, 1 equiv.) in EtOH (4 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (55 µL, 0.952 mmol, 1 equiv.) at 70 °C for 4 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 6/1 v/v containing 1% of conc. NH_4OH) gave product **176** (263 mg, 0.600 mmol) in 63% yield.



$C_{18}H_{34}N_2O_{10}$
Mw = 438.47 [g/mol]
Rf: 0.32 ($CHCl_3$/MeOH/concd. NH_4OH 4:4:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 6:1:1%
$[\alpha]_D$ = + 21.8 (*c* 2.87, MeOH)
Yield: 63 %

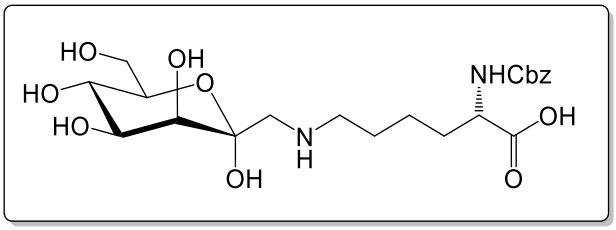
^{1}H-NMR (300 MHz, MeOH-d_4) δ = 3.87 (t, 1H, H-12), 3.82-3.73 (m, 3H, H-7, H-6, H-3), 3.72-3.59 (m, 2H, H-7', H-4), 3.57 (dd, $J_{5,4}$ = 8.5 Hz, $J_{5,6}$ = 9.5 Hz, 1H, H-5), 3.25 (d, 1H, H-1), 3.07 (d, $J_{1,1'}$ = 12.7 Hz, 1H, H-1'), 2.93 (t, 2H, H-8), 1.79-1.51 (m, 4H, H-9, H-11), 1.41-1.27 (bs, 11H, H-10, Boc).

^{13}C-NMR (75 MHz, MeOH-d_4) δ = 179.5 (C-13), 157.6 (C-14), 96.1 (C-2), 80.1 (C-15), 75.1 (C-4), 74.6 (C-3), 72.5 (C-6), 67.8 (C-5), 62.5 (C-7), 56.7 (C-12), 54.9 (C-1), 49.2 (C-8), 33.6 (C-11), 28.8 (Boc), 26.3 (C-9), 23.6 (C-10).

HRMS (MALDI): *m/z* calcd for $C_{18}H_{34}N_2O_{10}$ $[M+H]^+$ 439.2292, found 439.2293.

1-*N*-[(5S)-(Benzyloxycarbonylamino)-5-(methoxycarbonyl)pentyl]-amino-1-deoxy-α-D-*manno*-hept-2-ulose (178)

D-*glycero*-D-*galaco/*D-*talo* aldoheptose **110** (200 mg, 0.952 mmol, 1. equiv.) was treated with N_α-(benzyloxycarbonyl)-L-lysine **177** (267 mg, 0.952 mmol, 1 equiv.) in EtOH (4 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (55 µL, 0.952 mmol, 1 equiv.) at 70 °C for 4 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 6/1 v/v containing 1% of conc. NH_4OH) gave product **178** (292 mg, 0.618 mmol) in 65% yield.



$C_{21}H_{32}N_2O_{10}$
Mw = 472.49 [g/mol]
Rf: 0.23 ($CHCl_3$/MeOH/concd. NH_4OH 4:4:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 6:1:1%
$[\alpha]_D$ = + 12.2 (*c* 0.545, MeOH)
Yield: 65 %

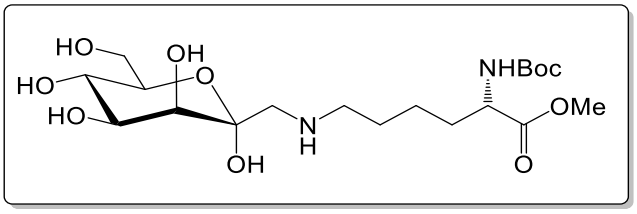
^{1}H-NMR (300 MHz, MeOH-d_4) δ = 7.38-7.23 (m, 5H, phenyl), 5.05 (bs, 2H, H-15), 4.07-3.96 (m, 1H, H-12), 3.86 (d, $J_{3,4}$ = 3.2 Hz, 1H, H-3), 3.86-3.73 (m, 3H, H-7, H-7', H-6), 3.72-3.66 (m, 1H, H-4), 3.65 (dd, $J_{5,4}$ = 8.5 Hz, $J_{5,6}$ = 9.4 Hz, 1H, H-5), 3.29 (d, 1H, H-1), 3.09 (d, $J_{1,1'}$ = 12.2 Hz, 1H, H-1'), 2.97 (t, 2H, H-8), 1.91-1.59 (m, 4H, H-9, H-11), 1.42 (q, 2H, H-10).

^{13}C-NMR (75 MHz, MeOH-d_4) δ = 179.1 (C-13), 158.1 (C-14), 138.4, 129.5, 128.9, 128.8 (6C, phenyl), 96.1 (C-2), 75.1 (C-4), 74.8 (C-3), 72.6 (C-6), 67.8 (C-5), 67.4 (C-15), 62.5 (C-7), 57.2 (C-12), 55.1 (C-1), 49.2 (C-8), 33.5 (C-11), 26.4 (C-9), 23.5 (C-10).

HRMS (MALDI): *m/z* calcd for $C_{21}H_{32}N_2O_{10}$ $[M+H]^+$ 473.2135, found 473.2137.

1-*N*-[(5S)-(*tert*-Butoxycarbonylamino)-5-(methoxycarbonyl)pentyl]-amino-1-deoxy-α-D-*manno*-hept-2-ulose (180)

D-*glycero*-D-*galaco/*D-*talo* aldoheptose **110** (300 mg, 1.43 mmol, 1. equiv.) was treated with methyl N_α-(*tert*-butoxycarbonyl)-L-lysinate **179**[236] (370 mg, 1.42 mmol, 1 equiv.) in EtOH (5 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (82 µL, 1.43 mmol, 1 equiv.) at 70 °C for 3 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 6/1 v/v containing 1% of conc. NH_4OH) gave product **180** (438 mg, 0.968 mmol) in 68% yield.



$C_{19}H_{36}N_2O_{10}$
Mw = 452.50 [g/mol]
Rf: 0.67 ($CHCl_3$/MeOH/concd. NH_4OH 4:4:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 6:1:1%
$[\alpha]_D$ = + 0.63 (*c* 2.06, MeOH)
Yield: 68 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 4.15-4.05 (m, 1H, H-12), 3.89 (d, $J_{3,4}$ = 3.3 Hz, 1H, H-3), 3.87-3.77 (m, 3H, H-7, H-7', H-6), 3.76-3.67 (m, 4H, H-4, OCH_3), 3.66 (dd, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 9.3 Hz, 1H, H-5), 3.37 (d, $J_{1,1'}$ = 12.3 Hz, 1H,

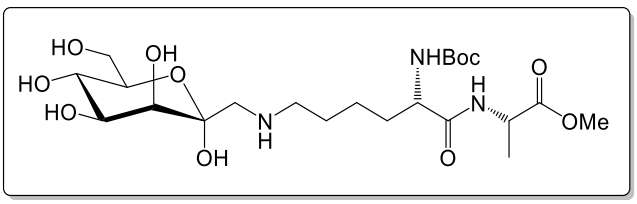
H-1), 3.15 (d, 1H, H-1'), 3.06 (t, 2H, H-8), 1.91-1.59 (m, 4H, H-9, H-11), 1.53-1.33 (bs, 11H, H-10, Boc).

^{13}C-NMR (75 MHz, MeOH-d_4) δ = 174.7 (C-13), 158.1 (C-14), 95.9 (C-2), 80.7 (C-15), 75.1 (C-4), 74.8 (C-3), 72.4 (C-6), 67.6 (C-5), 62.3 (C-7), 55.1 (C-1), 54.8 (C-12), 52.7 (OCH_3), 49.2 (C-8), 32.0 (C-11), 28.7 (Boc), 26.2 (C-9), 23.9 (C-10).

HRMS (MALDI): *m/z* calcd for $C_{19}H_{36}N_2O_{10}$ $[M+H]^+$ 453.2448, found 453.2448.

Methyl *N*-(*tert*-butyloxycarbonyl)-L-lysinyl-*N*^{6}-(1-deoxy-α-D-*manno*-hept-2-ulose)-L-alaninate (185)

D-*glycero*-D-*galaco*/D-*talo* aldoheptose **110** (150 mg, 0.714 mmol, 1. equiv.) was treated with methyl N_α-(*tert*-butoxycarbonyl)-L-lysinyl-L-anlaninate **184**[236, 267] (237 mg, 0.715 mmol, 1 equiv.) in EtOH (3 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (41 µL, 0.714 mmol, 1 equiv.) at 70 °C for 3 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 6/1 v/v containing 1% of concd. NH_4OH) gave product **185** (231 mg, 0.441 mmol) in 62% yield.



$C_{22}H_{48}N_3O_{11}$
Mw = 523.58 [g/mol]
Rf: 0.73 ($CHCl_3$/MeOH/concd. NH_4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 6:1:1%
$[\alpha]_D$ = - 12.3 (*c* 0.86, MeOH)
Yield: 62 %

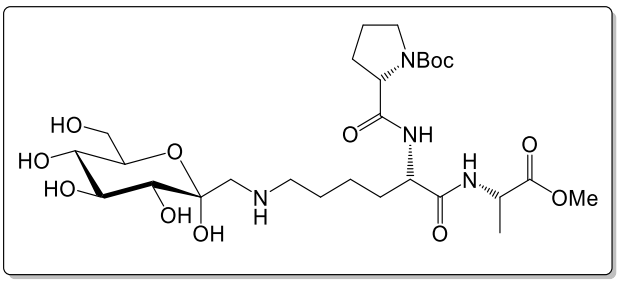
^{1}H-NMR (300 MHz, MeOH-d_4) δ = 4.42 (q, 1H, H-16), 4.10-4.00 (m, 1H, H-12), 3.87-3.80 (m, 3H, H-7, H-6, H-3), 3.79-3.68 (m, 5H, H-7', H-4, OCH_3), 3.64 (dd, $J_{4,5}$ = 8.9 Hz, $J_{5,6}$ = 9.5 Hz, 1H, H-5), 3.20 (d, $J_{1,1'}$ = 12.5 Hz 1H, H-1), 3.02 (d, 1H, H-1'), 2.92 (t, 2H, H-8), 1.84-1.56 (m, 4H, H-9, H-11), 1.52-1.35 (m, 14H, H-18, H-10, Boc).

^{13}C-NMR (75 MHz, MeOH-d_4) δ = 174.7, 174.6 (2C, C-17, C-13), 157.8 (C-14), 96.4 (C-2), 80.7 (C-15), 75.0, 74.9 (2C, C-4, C-3), 72.7 (C-6), 67.9 (C-5), 62.6 (C-7), 55.8 (C-1), 55.4 (C-12), 52.8 (OCH_3), 49.4 (C-8), 49.2 (C-16), 32.9 (C-11), 28.7 (Boc), 27.5 (C-9), 23.9 (C-10), 17.4 (C-18).

HRMS (MALDI): *m/z* calcd for $C_{22}H_{41}N_3O_{11}$ $[M+H]^+$ 524.2819, found 524.2819.

Methyl *N*-(*tert*-butyloxycarbonyl)-L-prolinyl-N^6-(1-deoxy-α-$_D$-*gluco*-hept-2-ulose)-L-lysinyl-L-alaninate (198)

D-*glycero*-D-*gulo* aldoheptose **48** (340 mg, 1.62 mmol, 1. equiv.) was treated with methyl *N*-(*tert*-butoxycarbonyl)-L-prolyl-L-lysyl-L-alaninate **188**[236, 267] (693 mg, 1.62 mmol, 1 equiv.) in EtOH (5 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (93 µL, 1.62 mmol, 1 equiv.) at 70 °C for 4 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 3/1 v/v containing 1% of conc. NH_4OH) gave product **198** (392 mg, 0.632 mmol) in 39% yield.



$C_{27}H_{48}N_4O_{12}$
Mw = 620.70 [g/mol]
Rf: 0.84 ($CHCl_3$/MeOH/concd. NH4OH 1:2:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH4OH 8:1:1%
$[\alpha]_D$ = - 29.1 (c 3.65, MeOH)
Yield: 39 %

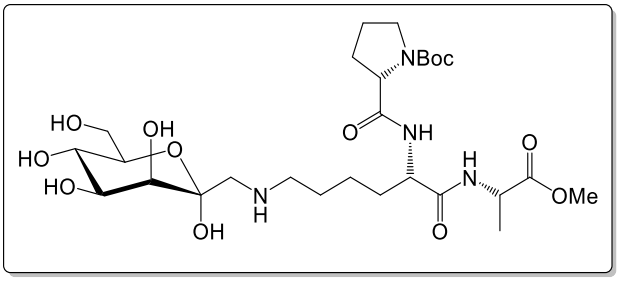
^{1}H-NMR (300 MHz, MeOH-d_4) δ = 4.46-4.32 (m, 2H, H-18, H-14), 4.28-4.19 (m, 1H, H-12), 3.84-3.60 (m, 4H, H-7, H-7', H-6, H-4), 3.72 (3H, OCH_3), 3.56-3.38 (2H, H-21), 3.31 (dd, $J_{4,5}$ = 9.6 Hz, $J_{5,6}$ = 9.6 Hz, 1H, H-5), 3.29 (d, $J_{3,4}$ = 9.6 Hz, 1H, H-3), 2.86 (d, $J_{1,1'}$ = 12.3 Hz 1H, H-1), 2.78 (d, 1H, H-1'), 2.74-2.56 (m, 2H, H-8), 2.34-2.12 (m, 1H, H-11), 2.04-1.65 (m, 5H, H-19, H-11, H-9), 1.64-1.36 (m, 16H, H-20, H-16, H-10, Boc).

^{13}C-NMR (75 MHz, MeOH-d_4) particular peaks in the peptide part were found in doublets due to rotameric appearance:
δ = 175.4, 175.2, 174.5, 174.0, 173.9 (5C, 2x C-17, C-15, 2x C-13), 156.5, 156.0 (2C, 2x C-22), 97.9 (C-2), 81.4, 81.3 (2C, 2x C-23), 75.7 (C-4), 74.5 (C-3), 74.0 (C-6), 71. 7 (C-5), 62.8 (C-7), 61.4, 61.3 (2C, 2x C-12), 56.1 (C-1), 54.4 (C-18), 52.8 (OMe), 50.8 (C-8), 49.4 (C-14), 47.9 (C-21), 33.4, 33.0 (2X C-11), 32.5, 31.4 (2x C-9), 30.3, 30.2 (2x C-20), 28.8 (Boc), 25.5, 24.7 (2x C-19), 24.5, 24.3 (2x C-10), 17.4 (C-16).

HRMS (MALDI): *m/z* calcd for $C_{27}H_{48}N_4O_{12}$ $[M+H]^+$ 621.3347, found 621.3348.

Methyl *N*-(*tert*-butyloxycarbonyl)-L-prolinyl-*N*^{6}-(1-deoxy-α-D-*manno*-hept-2-ulose)-L-lysinyl-L-alaninate (190)

D-*glycero*-D-*galaco*/D-*talo* aldoheptose **110** (165 mg, 0.785 mmol, 1. equiv.) was treated with methyl *N*-(*tert*-butoxycarbonyl)-L-prolyl-L-lysyl-L-alaninate **188**[236, 267] (335 mg, 0.782 mmol, 1 equiv.) in EtOH (4 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (45 µL, 0.785 mmol, 1 equiv.) at 70 °C for 4 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 3/1 v/v containing 1% of conc. NH_4OH) gave product **190** (239 mg, 0.385 mmol) in 49% yield.



$C_{27}H_{48}N_4O_{12}$
Mw = 620.70 [g/mol]
0.81: 0.81 ($CHCl_3$/MeOH/concd. NH_4OH 8:4:1)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 3:1:1%
$[\alpha]_D$ = - 44.7 (c 2.62, MeOH)
Yield: 49 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 4.44-4.30 (m, 2H, H-18, H-14), 4.25-4.18 (m, 1H, H-12), 3.87-3.79 (m, 3H, H-7, H-6, H-3), 3.79-3.67 (m, 2H, H-7', H-4), 3.70 (s, 3H, OCH_3), 3.63 (dd, $J_{4,5}$ = 8.7 Hz, $J_{5,6}$ = 9.0 Hz, 1H, H-5), 3.53-3.34 (2H, H-21), 3.31 (d, 1H, H-1), 3.09 (d, $J_{1,1'}$ = 12.5 Hz, 1H, H-1'),

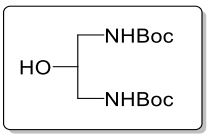
2.99 (t, 2H, H-8), 2.31-2.11 (m, 1H, H-11), 2.01-1.63 (m, 7H, H-20, H-19, H-11, H-9), 1.56-1.33 (m, 14H, H-16, H-10, Boc).

^{13}C-NMR (75 MHz, MeOH-d_4) particular peaks in the peptide part were found in doublets due to rotameric appearance:
δ = 175.5, 175.3, 174.5, 173.8, 173.6 (5C, 2X C-17, C-15, 2x C-13), 156.4, 155.9 (2C, 2x C-22), 96.0 (C-2), 81.4, 81.3 (2C, 2x C-23), 75.1 (C-4), 74.6 (C-3), 72.4 (C-6), 67.7 (C-5), 62.4 (C-7), 61.4, 61.3 (2C, 2x C-12), 55.0 (C-1), 54.2, 54.1 (2C. 2x C-18), 52.8 (OMe), 49.4 (C-14), 49.1 (C-8), 47.9 (C-21), 32.8, 31.6 (2x C-11), 32.5 (C-9), 28.8 (Boc), 26.4, 26.3 (2x C-19), 25.5, 24.7 (2x C-20), 23.8, 23.7 (2x C-10), 17.3 (C-16).

HRMS (MALDI): *m/z* calcd for $C_{27}H_{48}N_4O_{12}$ $[M+H]^+$ 621.3347, found 621.3349.

3-(*tert*-Butoxycarbonylamnio-2-hydroxxy-propyl)-carbamic acid *tert* butyl ester (192)[241]

To a solution of 1,3-diamino-2-propanol (2.0 g, 22.2 mmol) in a mixture of CH_2Cl_2 (20 mL) and MeOH (20 mL), di-*tert*-butyl dicarbonate (9.69 g, 44.4 mmol, 2 equiv.) was added slowly. The reaction mixture was stirred at rt for 30 minutes. The solvents were removed under reduced pressure and the residue was taken up in CH_2Cl_2, washed consequently with 6% aqueous HCl and satd aqueous sodium bibarbonate, dried (Na_2SO_4) and filtered. Removal of the solvent under reduced pressure and purification of the residue by column chromatography (Cy/EtOAc 4:1) gave compound **192** (6.38 g, 21.9 mmol) in 99% as white powder.



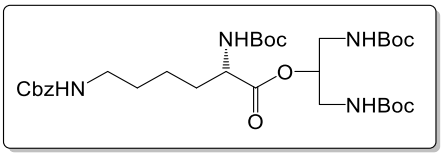
$C_{13}H_{26}N_2O_5$
Mw = 290.36 [g/mol]
Rf: 0.72 (C/EtOAc 1:2)
Detection: Mly
Purification: SGC Cy/EtOAc 4:1
Yield: 99 %

^{1}H-NMR (300 MHz, $CDCl_3$) δ = 5.25-5.06 (bs, 2x NH), 3.82-3.67 (m, 1H, H-1). 3.31-3.06 (m, 4H, H-2), 1.43 (s, Boc).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 157.5 (2x C-3), 79.9 (2x C-4), 71.2 (C-1), 43.8 (2x C-2), 28.6 (Boc).

1,3-Diamino-2-*O*-(N_{ε}-*tert*-butoxycarbonyl-L-lysinyl-1,2-di-*N*-*tert*-butoxycarbonyl-propane (193)[242]

To a solution of N_{α}-(benzyloxycarbonyl)-L-lysine **(191)** (812 mg, 2.14 mmol) in CH_2Cl_2 (20 mL), 3(-*tert*-butoxycarbonylamino-2-hydroxy-propyl)-carbamic acid tert-butyl ester **192** (620 mg, 2.14 mmol) and EDCl (409 mg, 2.63 mmol, 1.2 equiv.) were added and the reaction mixture was stirred overnight at rt. The solvent was removed under reduced pressure and subsequent column chromatography ($CHCl_3$) gave product **193** (1.24 g, 1.91 mmol) in 89% yield.



$C_{32}H_{52}N_4O_{10}$
Mw = 652.79 [g/mol]
Rf: 0.82 ($CHCl_3$/MeOH 5:1)
Detection: Mly
Purification: SGC $CHCl_3$
Yield: 89 %

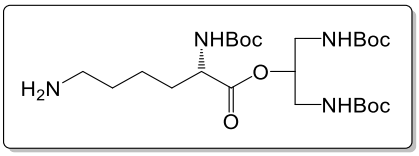
^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.31 (bs, 5H, phenyl), 5.43-5.14 (4H, NH), 5.1 (s, 2H, H-8), 4.83 (bs, 1H, H-10), 4.09 (dd, 2H, H-2), 3.27 (s, 4H, H-11), 3.16 (dd, 2H, H-6), 1.85-1.56 (m, 2H, H-3), 1.55-1.14 (m, 22H, H-5, H-4, Boc).

^{13}C-NMR (75 MHz, $CDCl_3$) δ = 172.2 (C-1), 156.8, 156.5, 156.3, 155.9 (4C, C-14, 2X C-12, C-7), 136.7, 128.5, 128.1, 128.0 (6C, phenyl), 80.1, 79.6 (3C, C-15, 2x C-13), 72.9 (C-10), 66.6 (C-8), 53.9 (C-2), 40.4, 40.3, 40.2 (3C, C-6, 2x C-11), 31.5 (C-3), 31.4 (C-5), 29.4 (Boc), 22.4 (C-4).

HRMS (MALDI): *m/z* calcd for $C_{32}H_{52}N_4O_{10}$ $[M+Na]^+$ 675.3581, found 675.3583.

1-{*N*-[1,3-Diamino-di-*N*-*tert*-butoxycarbonyl-prop-3-yl](5S)-(*tert*-butoxycarbonylamino)-5-(hydroxycarbonyl)pentyl}amino-1-deoxy-α-D-*manno*-hept-2-ulose (194)

To a solution of compound **193** (1.24 g, 1.91 mmol) in MeOH (20 mL), a catalytic amount of $Pd(OH)_2$/C was added and the reaction mixture was stirred under hydrogen at ambient pressure for 2 hours. After filtration and removal of the solvent under reduced pressure, the residue is purified by column chromatography ($CHCl_3$/MeOH, 5/1 v/v containing 1% of concd. NH_4OH) obtaining product **194** (839 mg, 1.62 mmol) in 85% yield.



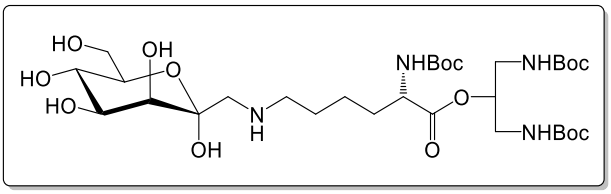
$C_{24}H_{46}N_4O_8$
Mw = 518.65[g/mol]
Rf: 0.73 ($CHCl_3$/MeOH/concd. NH_4OH 3:1:1%)
Detection: Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 5:1:1%
Yield: 85 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 4.95 (bs, 1H, H-7), 4.10 (dd, 1H, H-2), 3.32-3.07 (m, 2H, H-6), 2.71 (t, 4H, H-8), 1.94-1.78 (m, 1H, H-3), 1.76-1.63 (m, 3H, H-5, H-3), 1.61-1.34 (m, 29H, H-4, Boc).

^{13}C-NMR (75 MHz, MeOH-d_4) δ = 173.5 (C-1), 158.3, 158.1, 158.0 (3C, C-11, 2x C-9), 80.6, 80.2 (3C, C-12, 2x C-10), 73.9 (C-7), 55.1 (C-2), 41.8 (C-6), 40.5 (2C, 2x C-8), 31.7 (C-3), 28.8 (2x Boc), 27.9 (C-5), 23.8 (C-4).

***N*$_\varepsilon$-(1-Deoxy-α-D-*manno*-hept-2-ulosepyranosyl)-*N*$_\alpha$-*tert*-butoxycarbonyl-*O*-(1,3-diamino-di-*N*,*N*-*tert*-butoxycarbonyl-prop-3-yl)-L-lysine (195)**

D-*glycero*-D-*galaco*/D-*talo* aldoheptose **110** (135 mg, 0.642 mmol, 1. equiv.) was treated with amine **194** (334 mg, 0.644 mmol, 1 equiv.) in EtOH (3 mL) and 1,4-dioxane as co-solvent in the presence of acetic acid (37 µL, 0.644 mmol, 1 equiv.) at 70 °C for 5 days. The solvents were removed under reduced pressure and subsequent column chromatography ($CHCl_3$/MeOH, 5/1 v/v containing 1% of concd. NH_4OH) gave product **195** (232 mg, 0.327 mmol) in 51% yield.



$C_{31}H_{58}N_4O_{14}$
Mw = 710.82 [g/mol]
Rf: 0.70 ($CHCl_3$/MeOH/concd. NH_4OH 3:1:1%)
Detection: Van, Mly
Purification: SGC $CHCl_3$/MeOH/concd. NH_4OH 5:1:1%
$[\alpha]_D$ = - 2.24 (*c* 3.96, MeOH)
Yield: 51 %

^{1}H-NMR (300 MHz, MeOH-d_4) δ = 5.00-4.93 (bs, 1H, H-14), 4.16-4.06 (m, 1H, H-12), 3.95-3.63 (m, 6H, H-7, H-7', H-6, H-5, H-4, H-3), 3.46-3.19 (m, 3H, H-15, H-1), 3.19 (d, $J_{1,1'}$ = 12.5 Hz, 1H, H-1'), 3.09 (t, 2H, H-8), 2.00-1.64 (m, 4H, H-9, H-11), 1.59-1.36 (bs, 29H, H-10, Boc).

^{13}C NMR (75 MHz, MeOH-d_4) δ = 173.5 (C-13), 158.4, 158.3, 158.2 (3C, C-18, 2x C-16), 95.9 (C-2), 80.7, 80.4 (3C, C-19, 2x C-17), 75.1, 74.7, 74.1, 72.4 (4C, C-14, C-6, C-4, C-3), 67.6 (C-5), 62.3 (C-7), 55.1 (C-1), 55.0 (C-12), 49.2 (C-8), 41.9 (C-15), 31.8 (C-11), 28.8 (Boc), 26.1 (C-9), 23.9 (C-10).

HRMS (MALDI): *m/z* calcd for $C_{31}H_{58}N_4O_{14}$ $[M+H]^+$ 711.4028, found 711.4023.

2.3. Bioassays

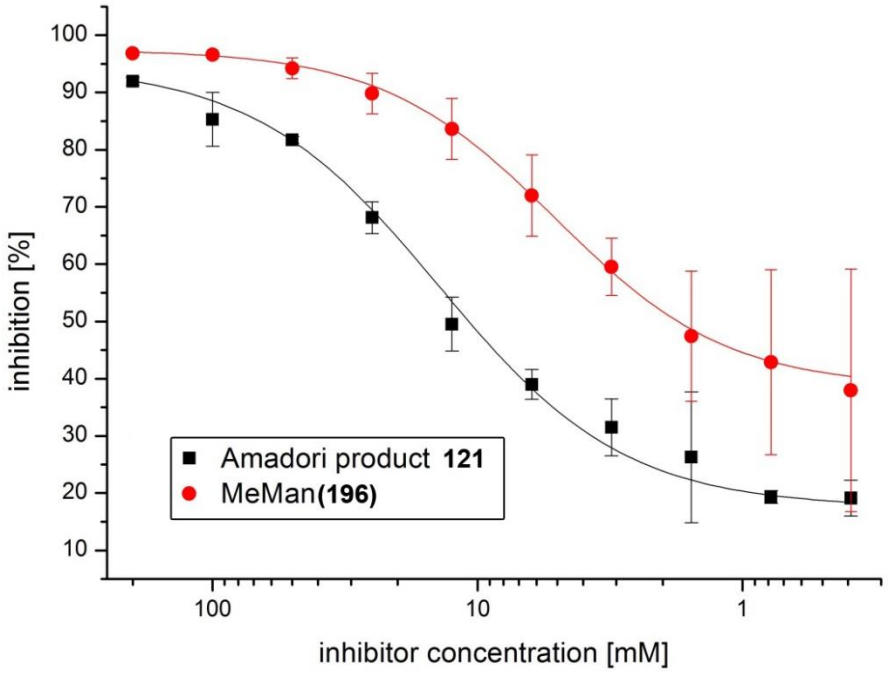
2.3.1. Inhibition-adhesion studies

Media and buffer solutions: Carbonate buffer solution (pH 9.6): sodium carbonate (1.59 g) and sodium hydrogen carbonate (2.52 g) were dissolved in distilled deionized water (1.00 L). PBS buffer solution (pH 7.2): sodium chloride (8.00 g), potassium chloride (200 mg), sodium hydrogen phosphate dihydrate (1.44 g) and potassium dihydrogen phosphate (200 mg) were dissolved in distilled deionized water (1.00 L). PBST buffer solution (pH 7.2): PBS buffer + Tween® 20 (0.05% v/v). LB medium: tryptone (10.0 g), sodium chloride (10.0 g) and yeast extract (5.00 g) were dissolved in distilled deionized water (1.00 L); after autoclavation chloramphenicol (50.0 mg) and ampicillin (100 mg) were added. The buffer pH values were adjusted with aqueous 0.1 M HCl or 0.1 M NaOH solution.

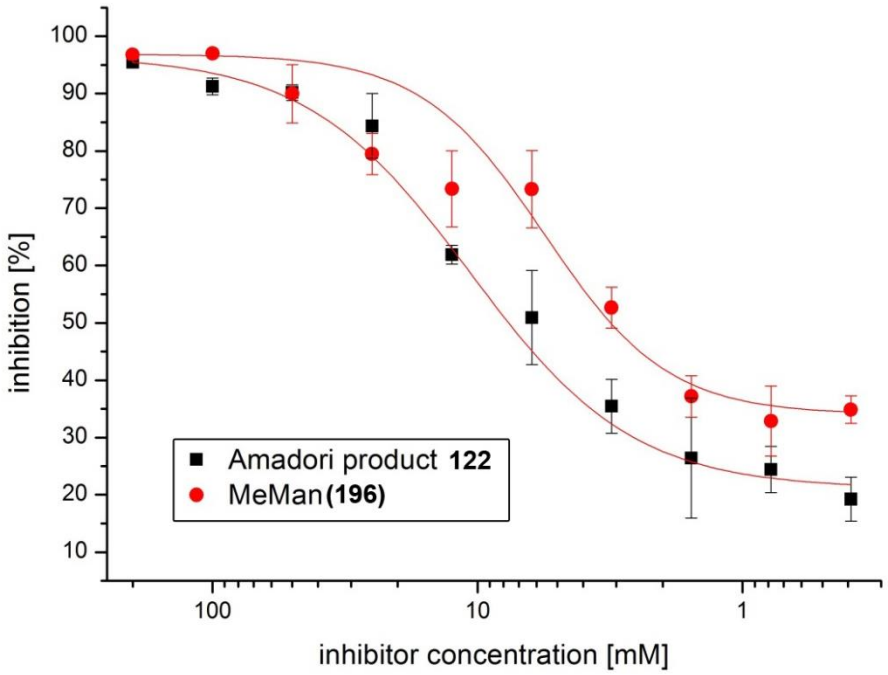
Cultivation of bacteria: *E. coli* bacteria (strain pPKL1162)[268] were cultured from a frozen stock in LB medium and incubated overnight at 37 °C. After centrifugation and washing twice with PBS buffer (2.00 mL), the bacteria pellet was suspended in PBS buffer and the suspension was adjusted to OD_{600} = 0.4 (2 mg/mL) with PBS.

GFP assay: The published assay[246] was adapted and modified as follows: Black 96-well microtiter plates (Nunc, MaxiSorp) plates were treated with a solution of mannan from *Saccharomyces cerevisiae* (1.2 mg/mL in carbonate buffer, 120 µL/well) and desiccated overnight at 37 °C. After washing for three times with PBST (150 µL/well), the wells were blocked with PVA (1% in PBS, 120 µL/well) for 4 h at 4 °C. Subsequently, the plates were washed twice with PBST (150 µL/well) and once with PBS (150 µL/well). Solutions of Amadori compounds **121** and **122** as well as MeMan (**196**) were prepared (200 mM in PBS) and serial dilutions of each solution added to the mannan-coated plates (50 µl/well). Then the bacterial suspension (OD_{600} = 0.4, 50 µL/well) was added and the plates were incubated for 1 h at 37 °C and 100 rpm. After washing twice with PBS (150 µL), the wells were filled with PBS (100 µL/well) and the fluorescence intensity (485 nm/535 nm) was determined.

Each compound was tested at least in triplicate and in parallel with the standard inhibitor MeMan (**196**) on the same plate.



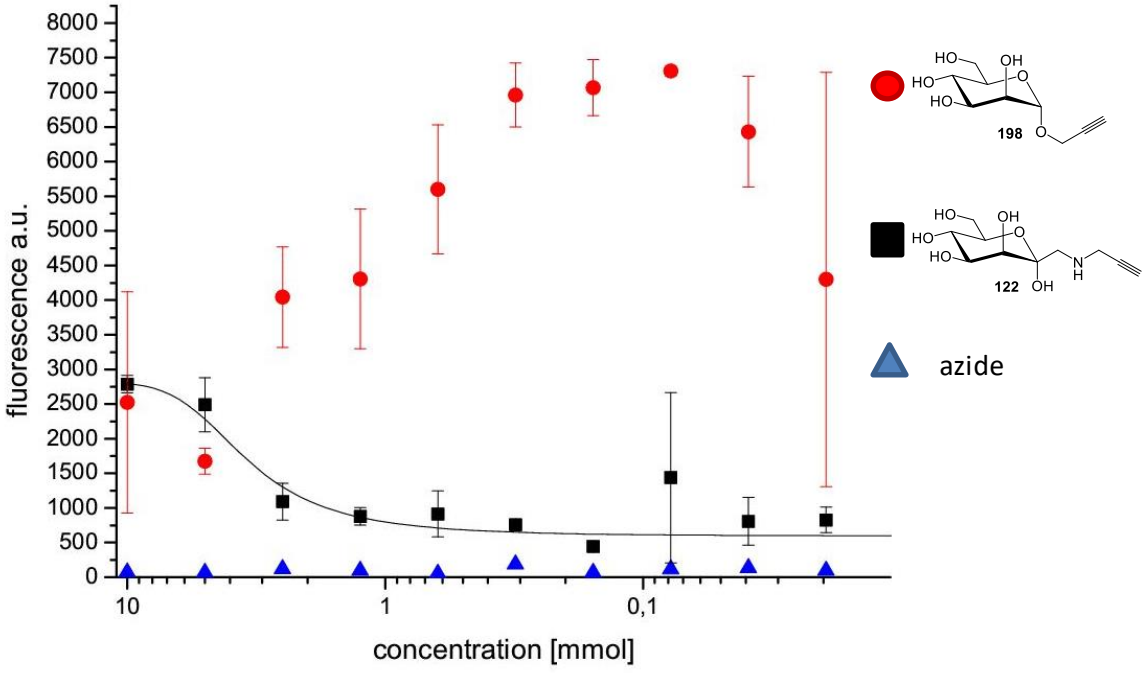
Scheme 60: Inhibition curves obtained with Amadori product **121** from inhibition of type 1 fimbriae-mediated bacterial adhesion to mannan. MeMan (**196**) was tested on the same microtiter plate. The sigmoidal concentration-response curves were fitted by non-linear regression. Error bars are standard deviations from multiple (at least three) testing results on one plate.



Scheme 61: Inhibition curves obtained with Amadori product **122** from inhibition of type 1 fimbriae-mediated bacterial adhesion to mannan. MeMan (**196**) was tested on the same microtiter plate. The sigmoidal concentration-response curves were fitted by non-linear regression. Error bars are standard deviations from multiple (at least three) testing results on one plate.

5.3.2 Adhesion study

A black 96-well microtiter plates, which is functionalized with an active ester, was immobilized with *O*-(2-aminoethyl)-*O'*-(2-azidoethyl)pentaethylene (100 µL/well). Solutions of compound **122** (10mM in MeOH) as well as propargyl α-D-mannopyranoside **198** (10mM in MeOH) were prepared and added to the microtiter plate (50 µL/well), following by addition of CuI (10mM in MeOH) as well as DIPEA (13mM in MeOH). After serial dilution the plate was shaken for 8 hours at room temperature and 300 rpm. After washing the plate three times with PBS (150 µL/well), the bacterial suspension (OD_{600} = 0.4, 50 µL/well) was added and the plates were incubated for 1 h at 37 °C and 100 rpm. After washing three times with PBS (150 µL), the wells were filled with PBS (100 µL/well) and the fluorescence intensity (485 nm/535 nm) was determined.



Scheme 62: Bacterial adhesion of GFP-tagged *E.coli* bacteria to glycoarray of potential inhibitor on a polystyrene microtiter plate.

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6. APPENDIX

6.3. List Figures

6.4. List of Scheme

6.5. List of Table

7. CURRICULUM VITAE

PERSONAL INFORMATION

First name/Surname	Cornelia Hojnik, MSc, BSc
Adress	Hubertusweg 7, 9150 Bleiburg
Nationality	Austria
Date of birth	16 December 1987
E-mail	c.hojnik@tugraz.at
Mobile	0664 4153888

EDUCATION

2012-2015	Doctorate degree in chemistry at Graz University of Technology
Jänner 2014 bis April 2014	Internship at Christina Albertina University of Kiel
2009-2012	Master degree in chemistry at University of Graz And Graz University of Technology
2006-2009	Bachelor degree in chemistry at University of Graz and Graz University of Technology
1998-2006	Grammer School in St. Paul, Carinthia, Austria
1994-1998	Elementary school in Beliburg, Carinthia, Austria

WORK EXPERIENCE

Since October 2016	Employment: Omega Bittner Main responsibilities: R&D, project management

June 2012 to September 2015	Employment: Graz University of Technology – Doctorate Main responsibilities: The Amadori rearrangement as a bioconjugation method
April 2011 to June 2012	Employment: Graz University of Technology – Master student Main responsibilities: Synthesis of substituted Pyrrolo[3,2-*c*]-and [2,3-*b*]pyridine
August 2010	Employment: Mohorjeva-Hermagoras Main responsibilities: Organizing the dirstibution Of school books in Carinthia
October 2009 – January 2010	Employment: University of Graz – Teaching assistant Main responsibilities: Monitoring students in laboratory
August – September 2009	Employment: Graz University of Technology – Scientific employee Main responsibilities: Development of novel electrode materials for lithium-ion batteries
May –July 2009	Employment: University of Graz – Bachelor student Main responsibilities: Asymmetirc C=C bond bioreduction of activated enolethers using enoate reductases

August 2008	Employment: Mohorjeva-Hermagoras Main responsibilities: Organizing the dirstibution of school books in Carinthia
2004 – 2010	Employment: MAHLE Filtersysteme Austria GmbH Main responsibilities: Working in the manufacture

PERSONAL SKILLS AND COMPETENCES

Languages

Mother tongue	German
Slovene	C1 in understanding – B2 in writing and spoken Interations
Englisch	C1 in understanding and writing – B2 in spoken Interations
French	A2 in understanding, writing and spoken interactions
Latin	C1 in writing

Social skills and competences

- Good communication skills gained through my experiences as a teaching assistant
- Flexible, self-reliant as well as the ability to work in a team, working experiences in industry and different working groups at university

Organization skills and competences

Experienced in project and team management gained during my experience as teaching assistant, through seminars and group working activities

8. PUBLICATIONS

Publications in international peer reviewed Journals

1. *"The Amadori Rearrangement for Carbohydrate Conjugation: Scope and Limitations"*

   Hojnik, C.; Mueller, A.; Gloe, T.-E.; Lindhorst, T. K.; Wrodnigg, T. M. *Eur. J. Org. Chem*. 2016, 4328-4337.

2. *" Are D manno-configured Amadori products inhibitors of the bacterial lectin FimH?"*

   Gloe, T.-E.; Stamer, I.; Hojnik, C.; Wrodnigg, T. M.; Lindhorst, T. K. *Beilstein J. Org. Chem*. 2015, 11, 1096-1104.

Lectures

1. *"The Amadori rearrangement as conjugation method: Scope and Limitations"*

   Hojnik, C.; Gloe, T.-E.; Landemarre, L.; Lebl, R.; Lindhorst, T. K.; Schalli, M.; Stütz, A. E. ; Thonhofer, Weber, P.; M.; Wrodnigg, T. M.; Zoidl, M.

   20th Austrian Carbohydrate Workshop, 11-12th February 2016, Vienna, Austria

2. *"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, 12th February 2015, Graz, Austria

3. "*Synthesis of carbacyclic inhibitors for β-galactosidases"*

   Schalli, M.; Fischer, R.; Gonzalez-Santana, A.; Hojnik, C.; Lebl, R.; Thonhofer, M.; Stütz, A. E.; Weber, P.; Withers, S. G.; Wrondigg, T. M.; Zoidl, M.

   20th Austrian Carbohydrate Workshop, 11-12th February 2016, Vienna, Austria

4. "*Synthesis and biological evaluation of C-5a extended derivatives of 4-epi-isofagomine."*

   Thonhofer, M.; Fischer, R.; Gonzalez-Santana, A.; Hojnik, C.; Lebl, R.; Saf, R.; Schalli, M.; Stütz, A.; Torvisco Gomez, A.; Weber, P.; Withers, S. G.; Wrodnigg, T.; Zoidl, M.

20th Austrian Carbohydrate Workshop, 11-12th February 2016, Vienna, Austria

4. "*Chemical synthesis of C-5a modified derivatives of the powerful β-glucosidase inhibitor Isofagomine"*

Weber, P.; Fischer, R.; Gonzalez-Santana, A.; Hojnik, C.; Lebl, R.; Saf, R.; Schalli, M.; Stütz, A.; Torvisco Gomez, A.; Withers, S. G.; Wrodnigg, T.; Zoidl, M.

20th Austrian Carbohydrate Workshop, 11-12th February 2016, Vienna, Austria

5. "*The Staudinger/aza-Wittig - Grignard reaction cascade: Stereochemical considerations and biological evaluation"*

Zoidl, M.; Gonzalez-Santana, A.; Hojnik, C.; Lebl, R.; Schalli, M.; Stütz, A. E.; Torvisco Gomez, A.; Thonhofer, M.; Weber, P.; Withers, S. G.; Wrodnigg, T. M.

20th Austrian Carbohydrate Workshop, 11-12th February 2016, Vienna, Austria

6. "*The Staudinger/aza-Wittig nucleophile reaction cascade: Scope and limitations."*

Lebl, R.; .Hojnik, C.; Lebl, R.; Schalli, M.; Stütz, A. E.; Thonhofer, M.; Weber, P.; Wrodnigg, T. M.; Zoidl. M.

20th Austrian Carbohydrate Workshop, 11-12th February 2016, Vienna, Austria

8. *"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, 12th February 2015, Graz, Austria

9. *" Snythesis od D-glacto-validamine derivatives."*

Schalli, M.; Hojnik, C.; Thonhofer, M.; Stütz, A. E.; Wrondigg, T. M.; Zoidl, M.

19th Austrian Carbohydrate Workshop, 12th February 2015, Graz, Austria

10. *"Diversity Oriented Synthesis of Iminoalditol Building Blocks for Glycoprobes."*

Zoidl, M.; Gonzalez Santana, A. .; Hojnik, C.; Saf, R.; Schalli, M.; Stütz, A.; Thonhofer, M.; Withers, S.; Wrodnigg, T.:

Mini Symposium on Glycomics, Glycochemistry and Glycobiology. 5th September 2014, Vienna, Austria

11. *"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.; Torvisco Gomez, A.; Withers, S. G.; Wrodnigg, T.; Zoidl, M.

Mini Symposium on Glycomics, Glycochemistry and Glycobiology. 5th September 2014, Vienna, Austria

12. *"Alternative Binder Systems for Lithium Ion Batteries"*

Stangl, C.; Bayer, C.; Fruhwirth, O.; Fuchsbichler, B.; God, C.; Hohl, R.; Hojnik, C.; Kaltenböck, L.; Kren, H.; Sternad, M.; Koller, S.

216th ECS Meeting, 5th October 2009, Vienna, Austira

Posters

1. *"The Amadori Rearrangement: A Versatile Reaction for the Synthesis of Neoglycoconjugates."*

Hojnik, C.; Gloe, T.; Lindhorst, T.; Schalli, M.; Stütz, A. E.; Thonhofer, M.; Wrodnigg, T. M.; Zoidl, M.

23rd International Symposium on Glycoconjugate, 15-20th September 2015, Croatia.

2. *"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, 01.-05th of June 2014, Canada.

3. *"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

4. *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 Glycoconjugate, 15-20th September 2015, Croatia.

5. *"Glycolipid Mimetics: Lipophilic 4-epi-Isofagomine Derivatives as Chemical Chaperons for GM1-Gangliosidosis and Morquio B."*

   Thonhofer M.; Fischer R.; Gonzales-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 Glycoconjugate, 15-20th September 2015, Croatia.

5. *"Glycolipid Mimetics: Fluorine containing Isoiminosugars as Chemical Chaperons for GM1-Gangliosidosis and Fabry´s Disease."*

   Stütz A. E.; Fischer R.; Gonzales-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 Glycoconjugate, 15-20th September 2015, Croatia.

5. *"Glycolipid Mimetics: Lipophilic Carbasugars as inhibitors and Chemical Chaperons for GM1-Gangliosidosis."*

   Schalli M.; Fischer R.; Gonzales-Santana A.; Hojnik C.; Paschke E.; Stütz A. E.; Thonhofer M.; Withers S.G.; Wrodnigg T. M.; Zoidl M.

   23rd International Symposium on Glycoconjugate, 15-20th September 2015, Croatia.

6. *"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 Glycoconjugate, 15-20th September 2015, Croatia.

7. "*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, 22.-26th of June 2014, Bratislava.

8. *"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, 01.-05th of June 2014, Canada.

9. *"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, 01.-05th of June 2014, Canada.

10. *"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, 01.-05th of June 2014, Canada.

12. *"Synthesis and Biological Evaluation of Potential Therapeutic Compounds for GM1 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, 01.-05th of June 2014, Canada.

13. *"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

14. *"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

15. *„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

17. *"6-Azido-6-deoxy-D-fructose as substrate for the Staudinger-aza-Wittig-cyanide multicomponent reaction."*

Lebl, R.; Hojnik, C.; Schalli, M.; Thonhofer, M.; Stütz, A. E., Wrodnigg, T. M.

15th Austrian Chemistry Days, 23.-26th of September 2013, Graz, Austria

18. *"Synthesis of 7-deazaguanine analogues including a novel biotransformation step"*

Wilding, B.; Hojnik, C.; Winkler, M.; Klempier, N.

Challenges in Organic Chemistry and Chemical Biology (ISACS7), 12th of June, Edinburgh, UK