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Synthesis of *C*-glycosyl type sugar-amino acid building blocks for "on surface" glycopeptides fabrication

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Meinen Eltern

“If something's important enough, you should try.
Even if the probable outcome is failure.”
- Elon Musk

“No experiment is ever a complete failure. It can always be used as a bad example.”
- Paul Dickson

Danksagung

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Last but not least gebührt mein größter Dank meinen Eltern, ohne deren Unterstützung mein Studium niemals möglich gewesen wäre.

Abstract

Carbohydrates fulfil a variety of functions in living organisms, either as monosaccharides, oligo- or polysaccharides, or glycoconjugates. By forming glycosides with different aglycons, the structural diversity by far exceeds nucleic acids and proteins. In natural products, various glycosidic bonds are found, with *O*- and *N*-glycosides being the most common. Over the years, numerous different methods for the synthesis of such compounds have been developed. Proteins are also mostly glycosylated, leading to glycoproteins. These play an important role in the three-dimensional folding of proteins, intracellular signalling, and function of the immune system. In recent years, *C*-glycosidic bonds have been found in nature and led to new classes of drugs as those bonds are metabolically stable. A long-known reaction for the formation of *C*-glycosyl-type sugars is the Amadori rearrangement. By the reaction of aldoses with amines, 1-amino-1-deoxy-ketoses are formed, which takes place in living organisms as a non-enzymatic glycation, related to the pathogenesis of diseases like diabetes or Alzheimer's disease, but can also be used in synthetic applications. As the surfaces of most cells are glycosylated, materials used for medical applications, like implants, have to mimic these to be biocompatible and resistant to the accumulation of biological foulants like proteins or bacteria. Therefore, in this work, two *C*-glycosidic sugar-amino acid glycoconjugates are synthesised, which will be used for the modification of thin film chitosan surfaces.

Kurzfassung

Kohlenhydrate erfüllen in lebenden Organismen eine Vielzahl von Funktionen, z.B. als Monosaccharide, Oligo- oder Polysaccharide oder Glycokonjugate. Durch die Bildung von Glycosiden mit unterschiedlichen Aglyconen übertrifft die strukturelle Vielfalt sogar die der Nukleinsäuren und Proteine. In Naturstoffen finden sich verschiedene glycosidische Bindungen, wobei *O*- und *N*-Glycoside am häufigsten vorkommen. Im Laufe der Jahre wurden zahlreiche verschiedene Methoden zur Synthese solcher Verbindungen entwickelt. Auch Proteine werden oft glycosyliert, was zu Glycoproteinen führt. Diese spielen eine wichtige Rolle bei der dreidimensionalen Faltung von Proteinen, der intrazellulären Signalübertragung und der Funktion des Immunsystems. In den letzten Jahren wurden auch *C*-glycosidische Bindungen in der Natur gefunden und führten zu neuen Wirkstoffklassen, da diese Bindungen metabolisch stabil sind. Eine seit langem bekannte Reaktion zur Bildung von *C*-Glycosyl ähnlichen Glycokonjugaten ist die Amadori-Umlagerung. Durch die Reaktion von Aldosen mit Aminen werden 1-Amino-1-desoxy-ketosen gebildet, was in lebenden Organismen als nicht-enzymatische Glykierung abläuft und mit der Pathogenese von Krankheiten wie Diabetes oder Alzheimer zusammenhängt, aber auch in der Synthese angewendet werden kann. Da die Oberflächen der meisten Zellen glycosyliert sind, müssen Materialien, die für medizinische Anwendungen verwendet werden, wie z.B. Implantate, diese nachahmen, um biokompatibel und resistent gegen die Ansammlung von biologischen Verunreinigungen wie Proteinen oder Bakterien zu sein. In dieser Arbeit werden zwei *C*-glycosidische Zucker-Aminosäure-Glycokonjugate synthetisiert, die zur Modifizierung von Dünnschicht-Chitosanoberflächen verwendet werden sollen.

Index

1 Introduction

1.1 Carbohydrates

The discovery of the wide variety of functions of carbohydrates, like the participation in cell-cell communication, cellular transportation, adhesion or in the immune system, has considerably increased research of this kind of molecules in recent years. Early on, carbohydrates were considered less interesting compared to other topics of biochemistry, also because the methods needed for the analysis of this complex class of compounds were not available. Nowadays, glycoproteins, glycolipids, lipopolysaccharides and many other molecules containing carbohydrates, so called glycoconjugates, which can be found in all cells in different forms, are interesting synthetic targets and potential drug candidates.[1,2]

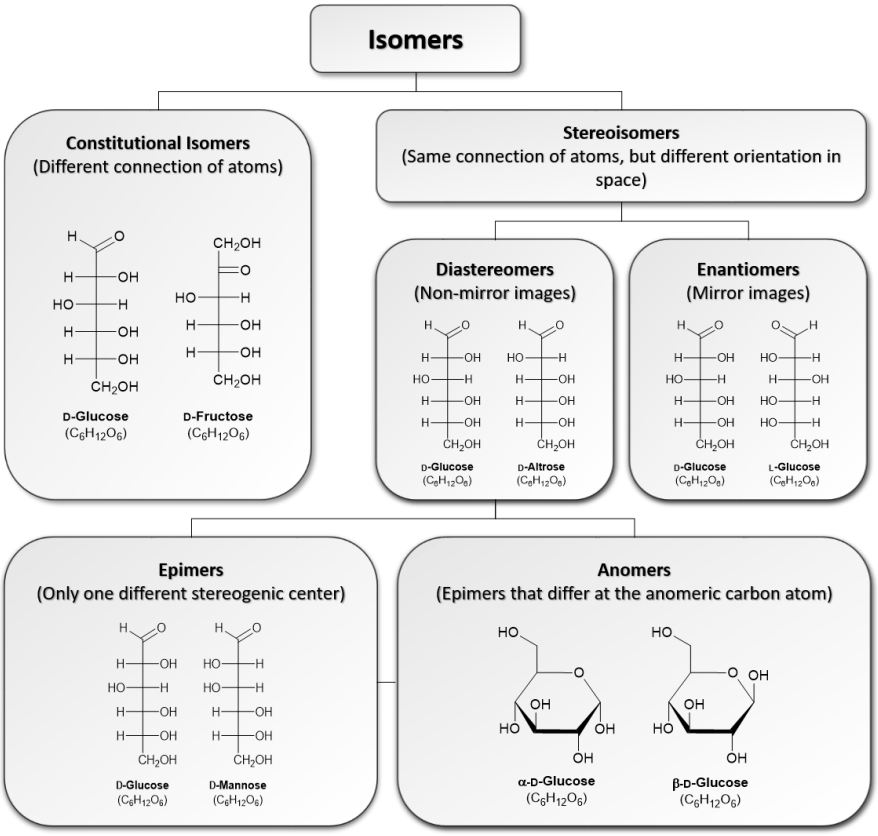


Figure 1: Examples of different types of isomers of carbohydrates. Adapted from literature.[1]

Historically, carbohydrates were defined with the empirical formula $C_n(H_2O)_n$, which explains their name. They are polyhydroxyaldehydes or -ketones, called aldoses or ketoses, respectively, creating a class of compounds with one of the highest densities of functional groups known. Nowadays, many derivatives with differing formulas and additional heteroatoms like nitrogen or sulfur can also be regarded as carbohydrates. The most common monosaccharides, commonly also called sugars, are pentoses, containing five carbon atoms and hexoses, containing six carbon atoms. Due to their high quantity of asymmetric carbon atoms, many different types of isomers exist (**Figure 1**). In fact, there are twelve different pentoses and 24 different hexoses, although mainly the D-configured carbohydrates exist in nature. In solution, they can form intramolecular cyclic hemiacetals or hemiketals, where five-membered rings are called furanoses and six-membered rings pyranoses. This cyclisation also creates a new stereo centre, called the anomeric centre.[3–6]

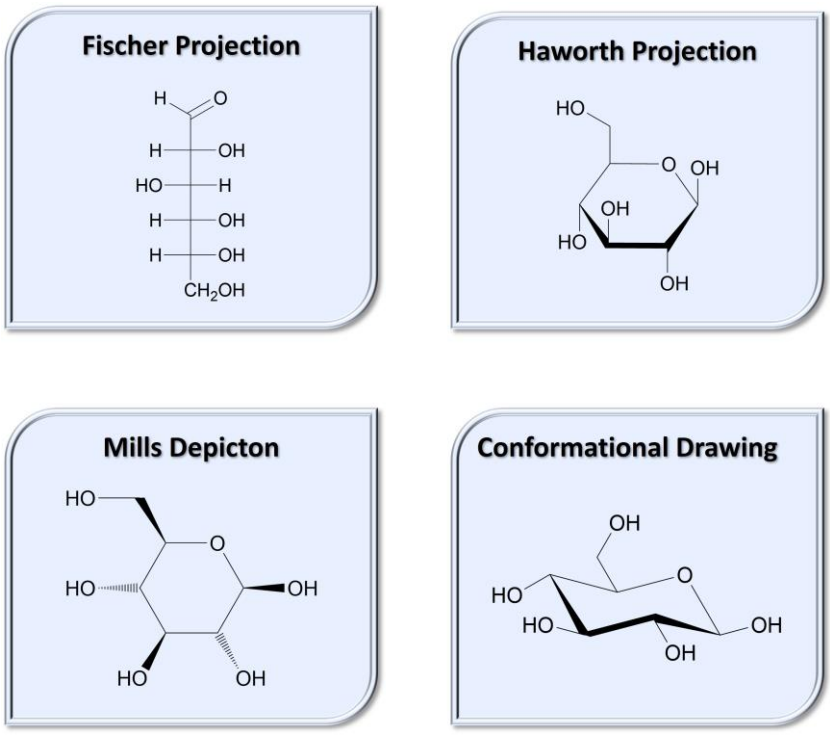


Figure 2: Four different projections of D-glucose.

To visualize carbohydrates, four different projections are common. Those are the Fisher projection, the Haworth projection, the Mills depiction and conformational drawings. In the two-dimensional Fisher projection, the highest oxidated carbon atom is drawn at the top, which is labelled as C-1. In Aldoses, this is the position of

the carbonyl group, while in ketoses, the carbonyl is mostly situated at C-2. The horizontal bonds are defined to point towards and the vertical bonds to point away from the viewer. The Haworth projection is suitable for rings and adds a three-dimensional perspective, although it does not show the actual conformation of the molecule. Substituents that are drawn to the right in the Fischer projection are drawn downwards in the Haworth projection and vice versa for the left substituents. A more classical way to show the stereo configuration is the Mills depiction, which is a two-dimensional drawing, showing the molecule in a plane from above.[7] The different projections are shown in **Figure 2**, using D-glucose as an example.

1.2 Glycosides

Carbohydrates can form so called glycosides. They consist of a glycon, which is the carbohydrate, and an aglycon, creating the glycosidic linkage.[6,8] Monosaccharides can form glycosidic bonds with other sugar moieties, resulting in oligo- or polysaccharides. Those oligosaccharides and glycoconjugates, like glycoproteins (Chapter **1.3**) and glycolipids, have a huge structural diversity, even exceeding proteins and nucleic acids and are playing an important role in living organisms as signalling molecules and in protein biosynthesis.[9]

Nearly all cell surfaces are glycosylated, which is relevant for the immune system in terms of pathogen recognition, innate immune responses and the development of autoimmune diseases and cancer. Bacterial cell walls also accumulate a large amount of polysaccharides, which can on the one hand promote the growth of colonies and prevent the attack and degradation by phagocytes, but on the other hand induces an adaptive immune response, which can be used in the development of vaccines.[10]

1.2.1 *O*-Glycosides

In nature, carbohydrates are often found as glycosides where the glycosidic linkage consists of an oxygen atom, forming acetals or ketals. This is called an *O*-glycosidic bond or an *O*-glycoside. For example, the monomers of many polysaccharides like cellulose, amylose, glycogen, chitin, hyaluronic acid or heparin are connected by *O*-glycosidic bonds (**Figure 3**). In the case of amylose and glycogen, this is done to

store glucose, an essential energy source for many living organisms, while reducing the osmotic pressure, which would be a consequence of the large number of monomers in solution.[11]

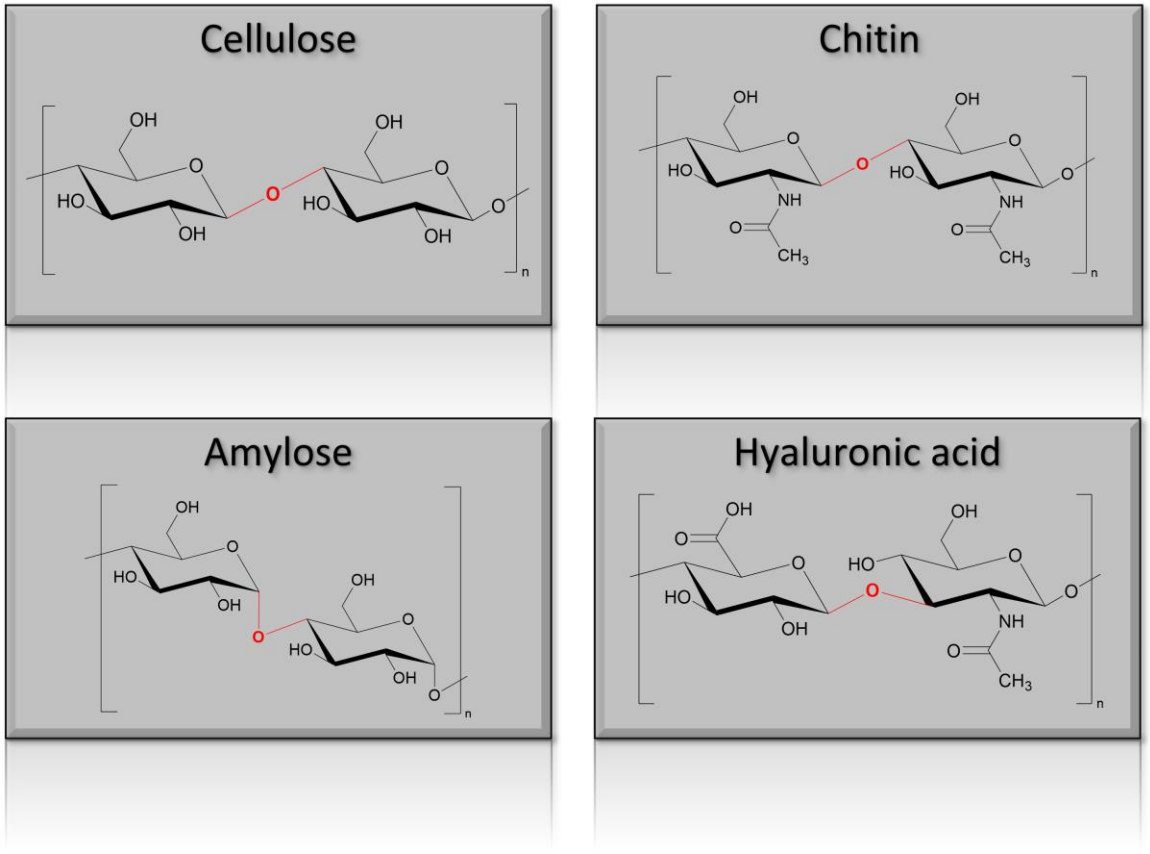


Figure 3: Examples of different polysaccharides with *O*-glycosidic bonds. The glycosidic bonds are drawn in red.

Over the years, many ways to synthesise such compounds have been established. Generally, the synthesis involves the reaction of a suitable protected sugar moiety with a leaving group at its anomeric centre, called glycosyl donor, with a glycosyl acceptor, containing a free hydroxyl group.[12] Two of the oldest and best known glycosylation methods are the Fischer glycosylation[13] and the Koenigs-Knorr[14] method.

Today, a much wider variety of different glycosylation reactions exist, involving glycosyl halides, thioglycosides, 1-*O*-acyl sugars, ortho esters, 1-*O*-carbonates, phosphate derivatives and many more.[15,16] Some strategies for the synthesis of such linkages are illustrated in **Figure 4**.

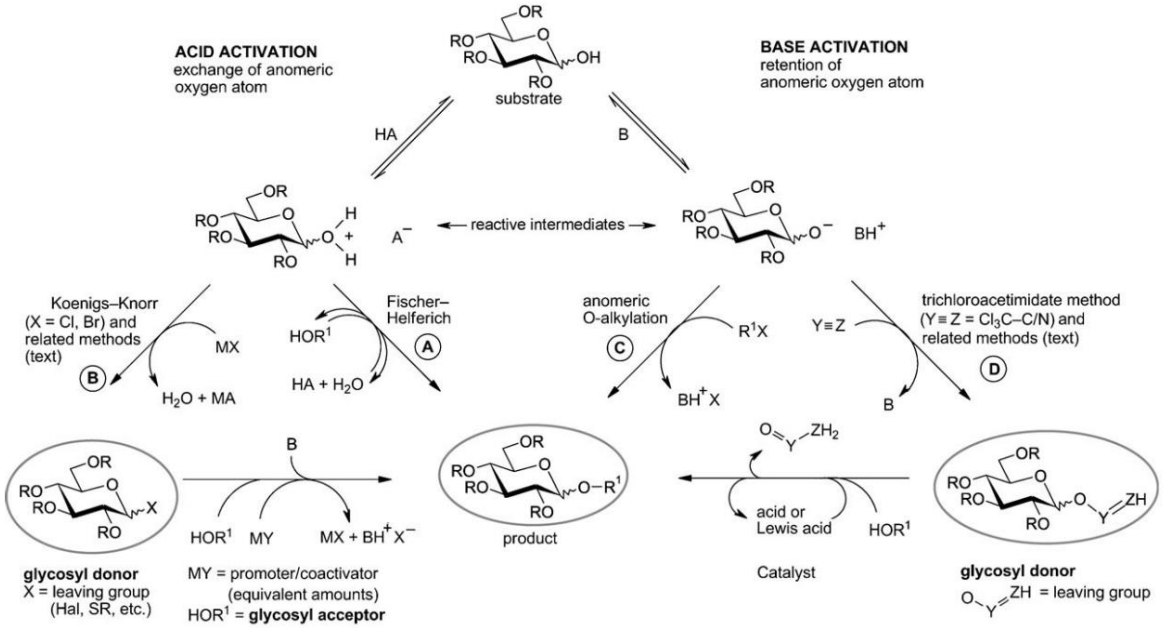


Figure 4: Different strategies and methods for the formation of *O*-glycosidic bonds. Reprinted from literature.[12]

This toolbox enables the synthesis of more complex natural products[17] such as lomaiviticin A[18], (-)-neonaucleoside A[19] or rebaudioside S[20] which all include at least one *O*-glycosidic bond (**Figure 5**).

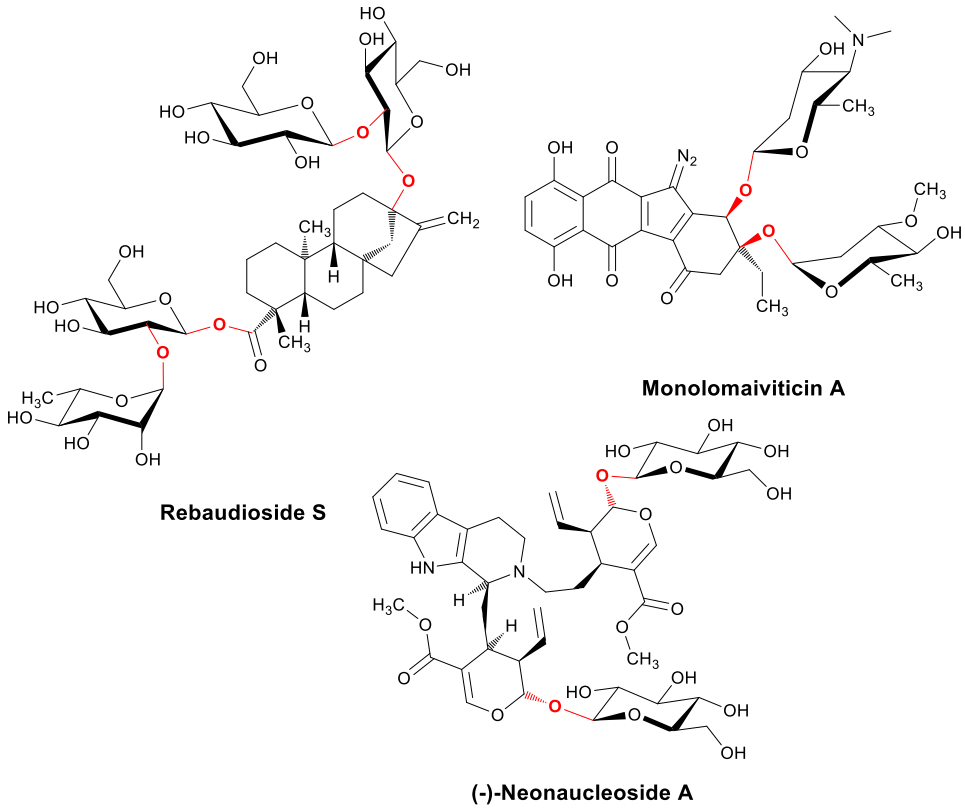


Figure 5: Natural products with *O*-glycosidic bonds. The glycosidic bonds are drawn in red.

1.2.2 *N*-Glycosides

In *N*-glycosides, the oxygen of the glycosidic linkage is replaced by a nitrogen atom, creating a C-*N*-C instead of a C-*O*-C linkage. Like *O*-glycosidic bonds, those resulting *N*-glycosidic bonds are abundant in nature and in living organisms. They can be found in glycoproteins, complex natural products like antibiotics and are a crucial part of DNA, RNA and cofactors for biological synthesis pathways.[21]

DNA and RNA carries genetic information and is therefore vital for living cells. They are linear polymers with chains of ribose or deoxyribose for RNA or DNA, respectively, which are linked by 3' → 5' phosphodiester bonds as their backbone. At each anomeric centre of the sugar moieties, one out of five common nucleobases is attached by a *N*-glycosidic bond. The sugar with a base attached is called a nucleoside, while with the phosphate group added, it is called a nucleotide. Nucleotide derivatives are essential biomolecules, like adenosine-5'-triphosphate, or short ATP, which is the most common direct source of energy for biological processes.[22] **Figure 6** shows examples of nucleotides and nucleosides with the *N*-glycosidic bonds highlighted in red.

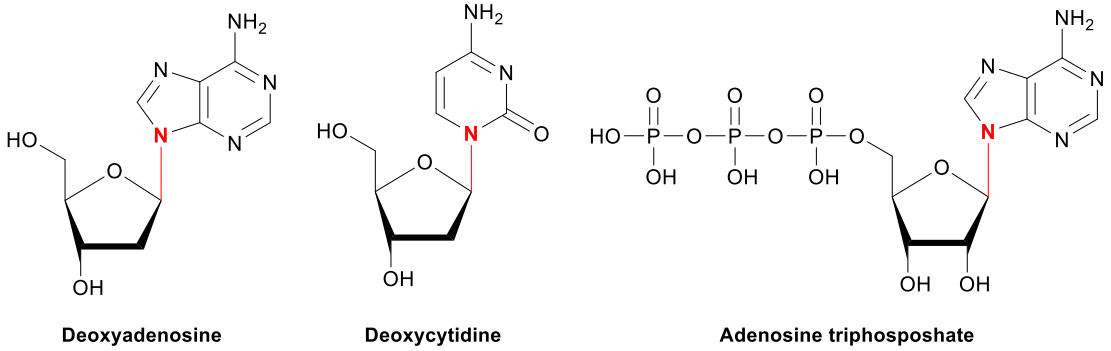
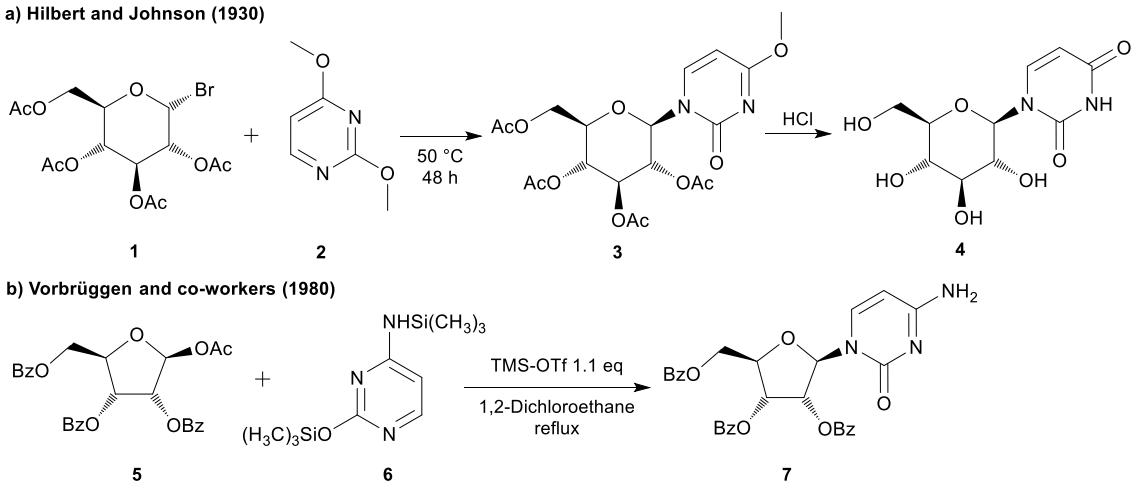


Figure 6: Examples for nucleotides and nucleosides with the *N*-glycosidic bonds highlighted in red.

Other important nucleoside derivatives are nucleoside antibiotics. Despite their antibacterial properties, they can also be active as antiviral, antifungal, or antitumor substances, just to name a few of their biological functions. This has led to a toolbox of reactions for the formation of *N*-glycosidic nucleotide bonds. One of the first methods used in synthesis of more complex nucleosides was the Vorbrüggen reaction, which is a modification of the Hilbert-Johnson synthesis. The original Hilbert-Johnson synthesis[23] uses *O*-alkylated pyrimidines **2** as glycosyl acceptors

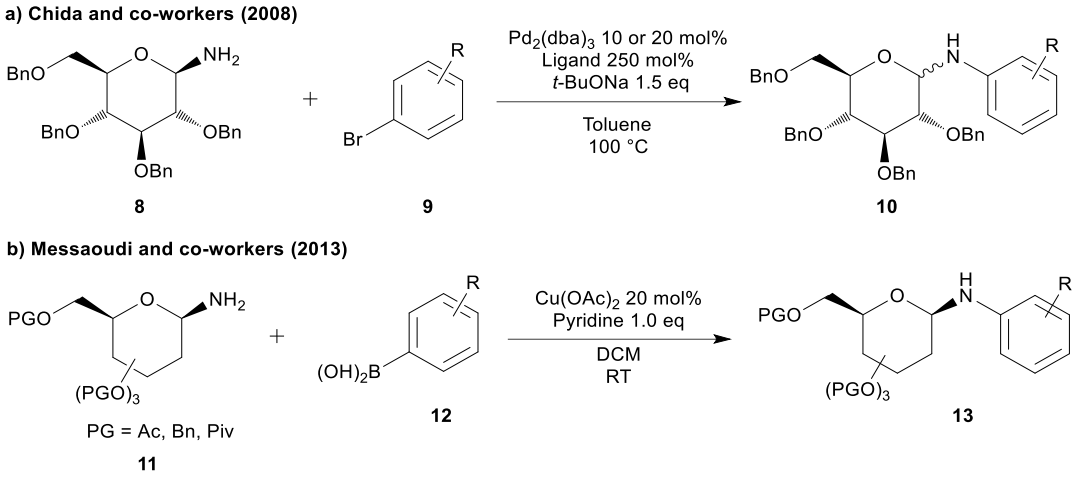
and glycosyl halides **1** as glycosyl donors (**Scheme 1a**) while in the Vorbrüggen modification, per-*O*-acetylated sugars **5** are used as donors and silylated pyrimidines **6** or acylated purines as acceptors with stannic chloride or TMS-OTf as a promoter, which greatly increases the yield (**Scheme 1b**).[24] As more methods for *O*-glycosylations have been established, nucleoside synthesis has also benefitted from a variety of new donors and promoters, which makes *N*-glycosylation often the most efficient step in the nucleoside synthesis.[25]



Scheme 1: Methods for the synthesis of *N*-glycosides by (a) Hilbert and Johnson and (b) Vorbrüggen and co-workers.

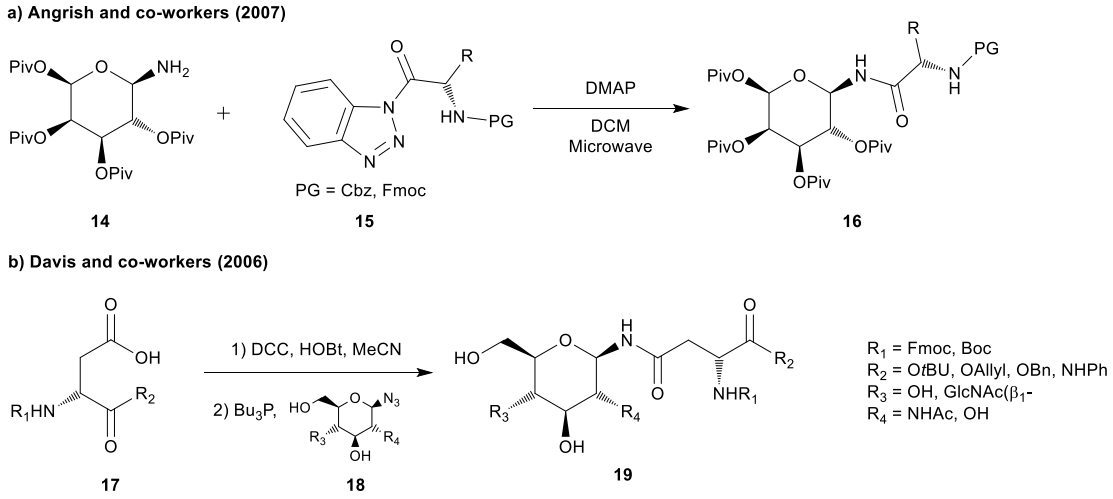
Apart from the synthesis of nucleosides, methods for a variety of aglycons have been developed. Sangwan, Khanam and Mandakl recently reviewed modern methods for the *N*-functionalization of sugars, where a few examples will be explained in the following.[26]

Chida and co-workers[27] used a Buchwald-Hartwig reaction to couple aryl and heteroaryl bromides **9** to per-*O*-benzylated D-glucopyranosylamine **8** at elevated temperatures, which has been used for different total syntheses (**Scheme 2a**). As this method only works for this specific substrate, Messaoudi and co-workers[28] used aryl boronic acids **12** and copper(II) acetate as a catalyst at room temperature, which expanded the scope to various carbohydrates **11** and yielded *N*-aryl glycosides **13** solely as β-anomers (**Scheme 2b**).



Scheme 2: *N*-Glycosylation methods by (a) Chida and co-workers and (b) Messaoudi and co-workers.

For the synthesis of *N*-glycosyl amides **16** Angrish and co-workers[29] used a microwave assisted method, where tetra-*O*-pivaloyl-β-D-galactopyranosylamine (**14**) is reacted with *N*-(Cbz-α-aminoacyl)benzotriazoles **15** at 100 W irradiation and 60 °C for 75 minutes (**Scheme 3a**). Davis and co-workers[30] have developed a three-component one-pot Staudinger reaction, which yields *N*-linked glyco-amino acids **19** starting from unprotected sugar azides **18** and a suitable protected amino acid **17** (**Scheme 3b**). They have proven that this methodology works for a linear polypeptide synthesis approach, where the glyco-amino acid is used as a building block, as well as a convergent approach, where the sugar moiety is attached to an existing polypeptide chain. The importance of *N*-glycosides in glycopeptides will be further discussed in chapter **1.3**.



Scheme 3: *N*-Glycosylation methods by (a) Angrish and co-workers and (b) Davis and co-workers.

1.2.3 *C*-Glycosides

In *C*-Glycosides, the glycosidic linkage is established by a carbon-carbon bond instead of a heteroatomic bond. Although their research has received less attention than heteroatomic glycosides for a long time, nowadays many bioactive *C*-glycosidic compounds have been identified as natural products of plants and microorganisms.[31–33]

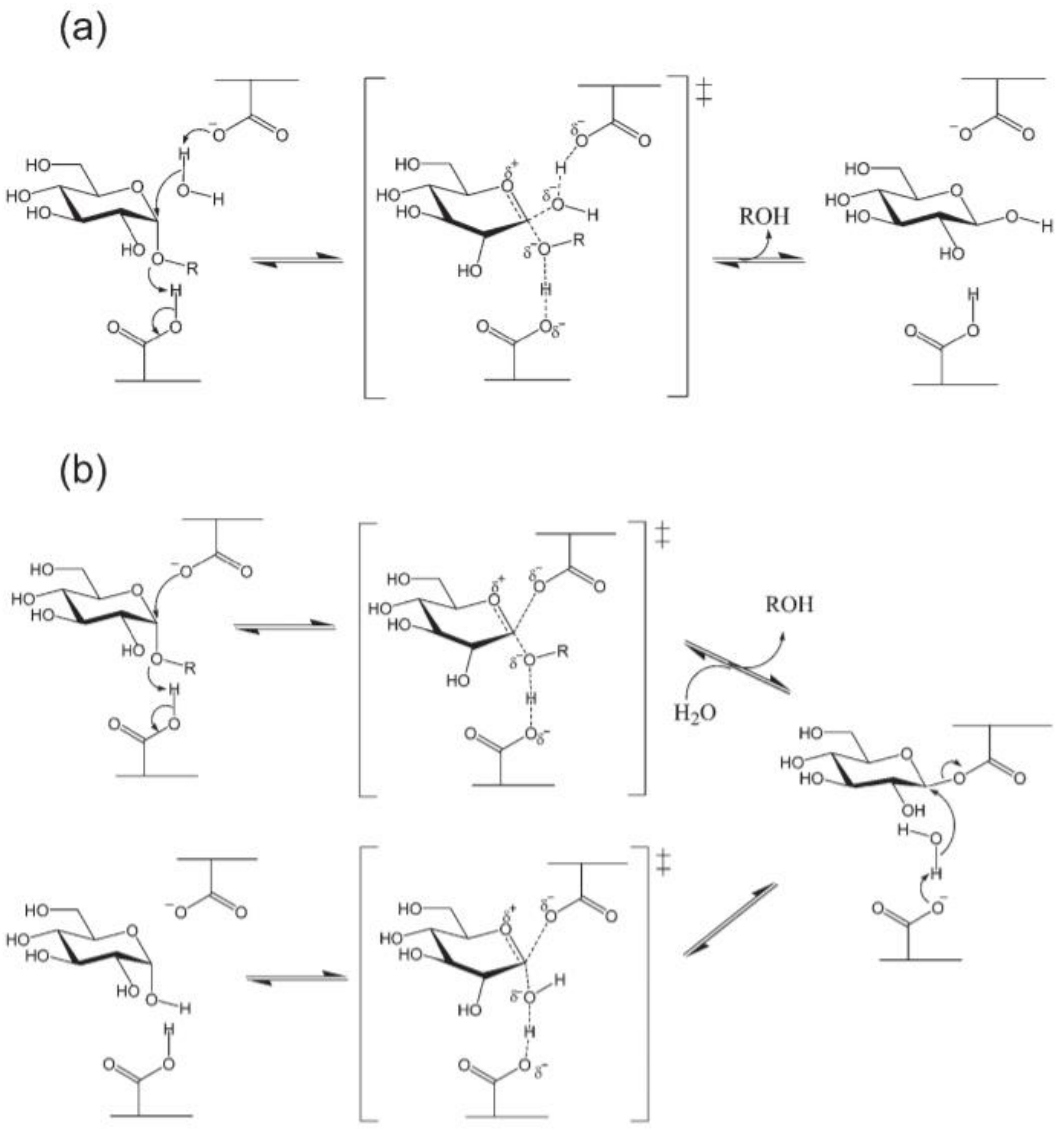


Figure 7: Mechanisms of (a) inverting and (b) retaining glycosidases. Reprinted from literature.[34]

An interesting property of *C*-glycosides is their stability against enzymatic cleavage in contrast to heteroatomic glycosides. Enzymes which can cleave glycosidic bonds are called glycoside hydrolases or short glycosidases. For *O*-glycosidases, there are

two general types with different mechanisms, the retaining and the inverting glycosidases, which are illustrated in **Figure 7**.[34] Due to their wide abundance in nature and their connection to various diseases, they are an interesting target for the development of inhibitors, as, for example, demonstrated by Thonhofer[35], Weber[36] and Wolfsgruber[37]. For *C*-glycosides, there are no glycosidases known by now, making this kind of bonds practically stable in biological systems.[38]

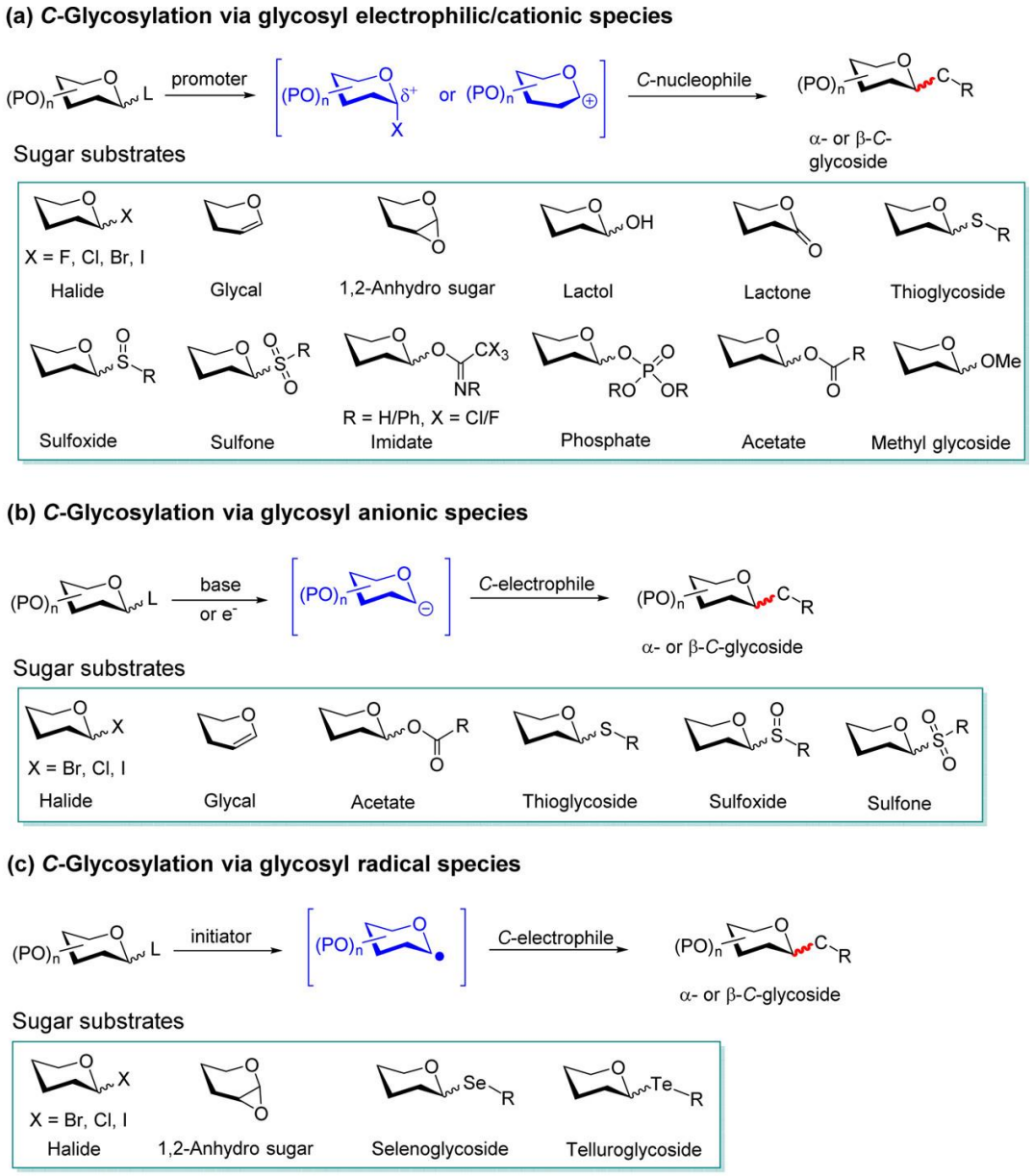


Figure 8: Concepts for the synthesis of *C*-glycosides. Part 1. Reprinted from literature.[39]

This metabolic stability makes *C*-glycosides promising candidates for therapeutics, for example against cancer[40], bacterial infections[41] or diabetes[42]. Therefore, various

synthetic methods with different scopes have been developed. There are numerous comprehensive reviews, for example from Postema (1992),[43] which summarises the literature from 1983 to 1991 and from Du, Linhardt and Vlahov (1998),[44] summarising publications from 1994 to 1998. More recently, Yang and Yu (2017)[39] reviewed literature from 2000 to 2016, from which a few examples will be presented below.

There are generally six different concepts for the formation of *C*-glycosidic bonds, namely *C*-glycosylation via electrophilic/cationic glycosyl donors, via anionic glycosyl donors, via radical glycosyl donors, via transition metal complexes, rearrangements, or sugar ring formations (**Figure 8**, **Figure 9**).[39]

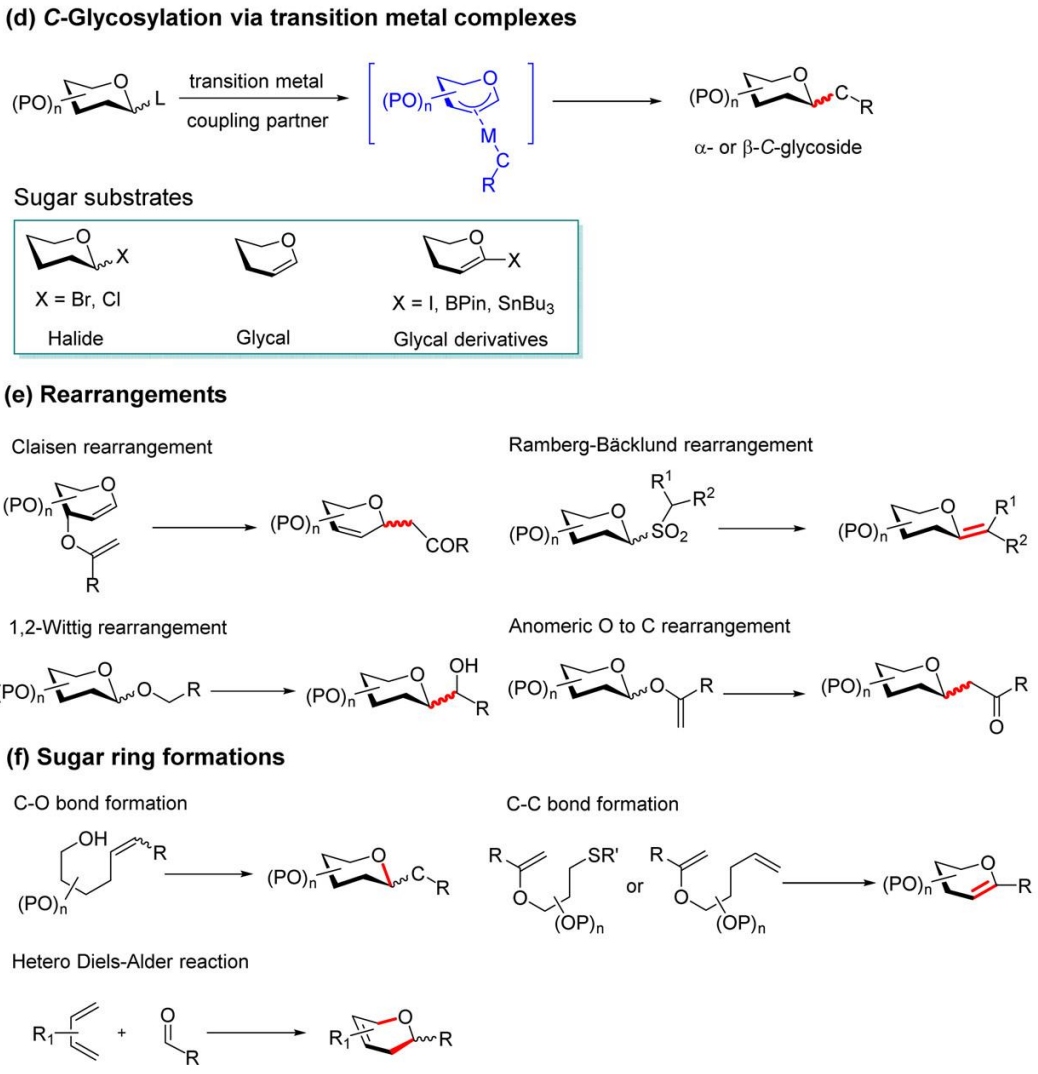
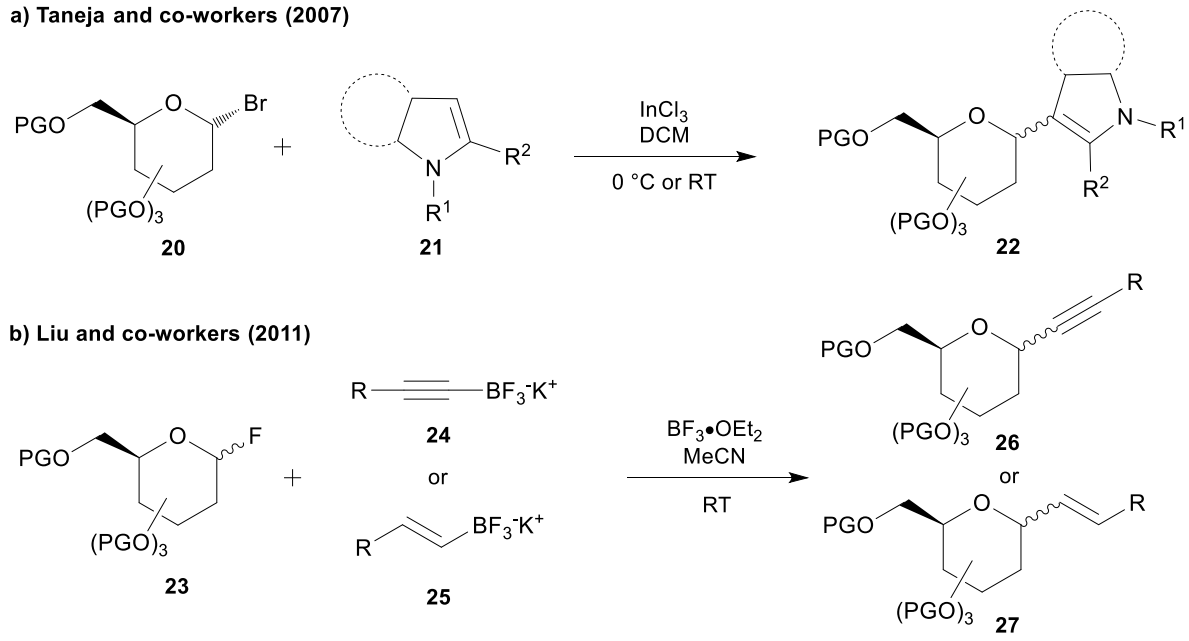


Figure 9: Concepts for the synthesis of *C*-glycosides. Part 2. Reprinted from literature.[39]

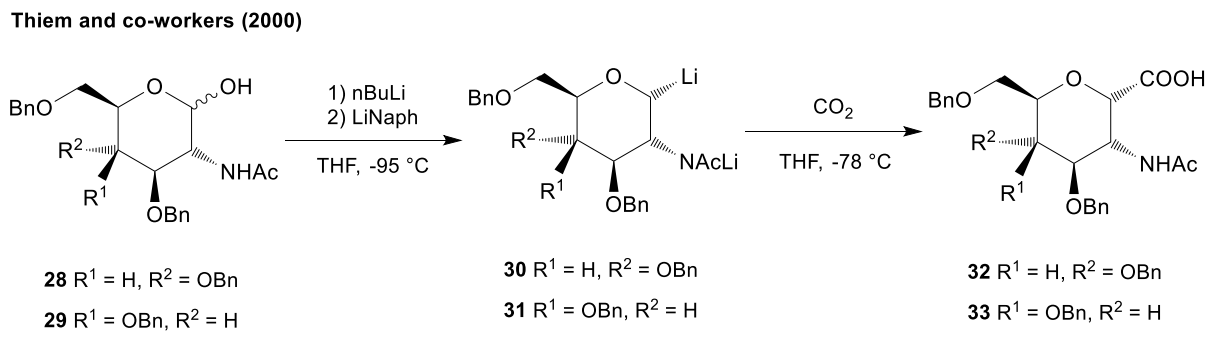
C-Glycosidic indoles and pyrroles **22** can be obtained by the reaction of glycosyl bromides **20** with electron-rich indole or pyrrole derivatives **21** and indium(III)chloride as a catalyst, as shown by Taneja and co-workers (**Scheme 4a**).[45] The yields of this reaction to compounds **22** were generally above 60 % and a > 1:9 1,2-*trans*-configuration could be observed. Starting from glycosyl fluorides **23**, the reaction with potassium organotrifluoroborates **24** or **25** and $BF_3 \bullet OEt_2$ as a promoter in acetonitrile gives the desired *C*-glycosides **26** or **27** in high yields. Liu and co-workers[46] have demonstrated this reaction with a variety of different carbohydrates as well as organotrifluoroborates (**Scheme 4b**).



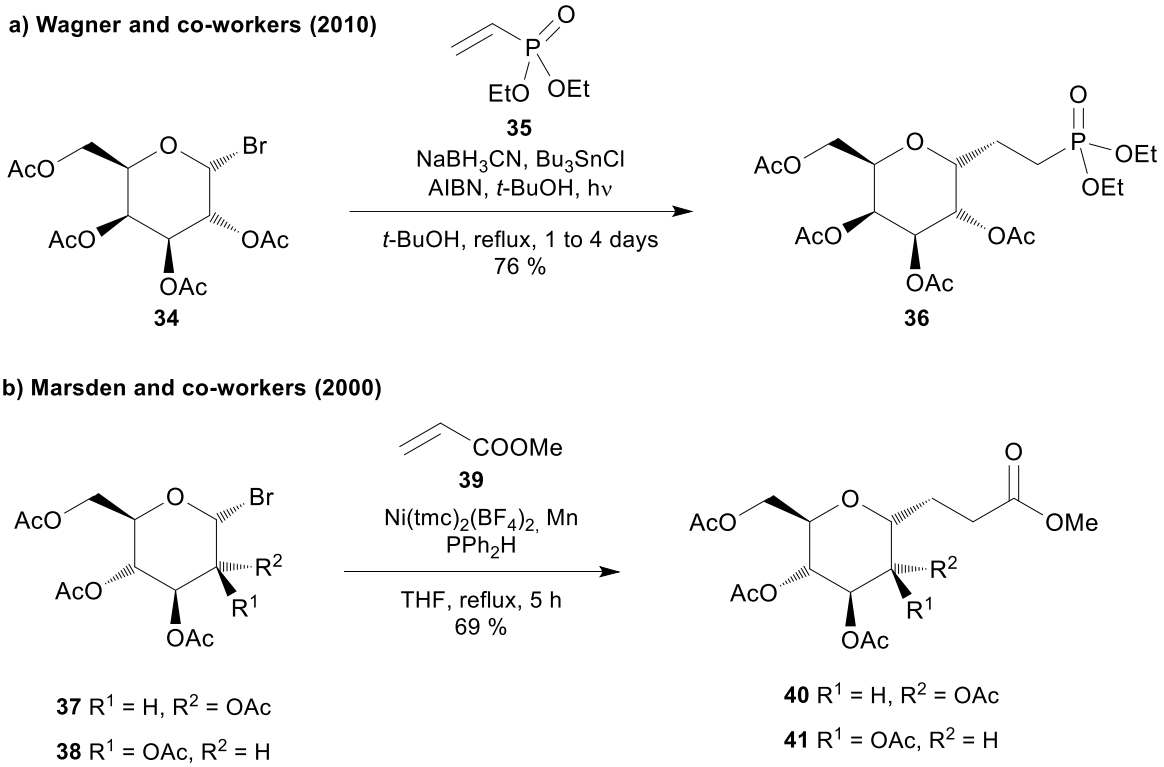
Scheme 4: *C*-Glycosylation methods used by (a) Taneja and co-workers and (b) Liu and co-workers.

Thiem and co-workers[47] used a dianion approach developed by Kessler and co-workers,[48] which involves anionic sugar species **30** or **31** that react with carbon dioxide as an electrophile to give 1-*C*-α-carboxylic acids **32** or **33** (**Scheme 5**).

Using a radical approach, Wagner and co-workers[49] used per-*O*-acetylated galactosyl bromide **34** and diethyl vinylphosphonate **35** with AIBN as a radical starter to synthesise galactosyl phosphonate **36** (**Scheme 6a**). Marsden and co-workers[50] used a similar approach to obtain glycosyl esters **40** and **41**, using an acrylic acid ester **39**, a nickel catalyst and manganese (**Scheme 6b**).

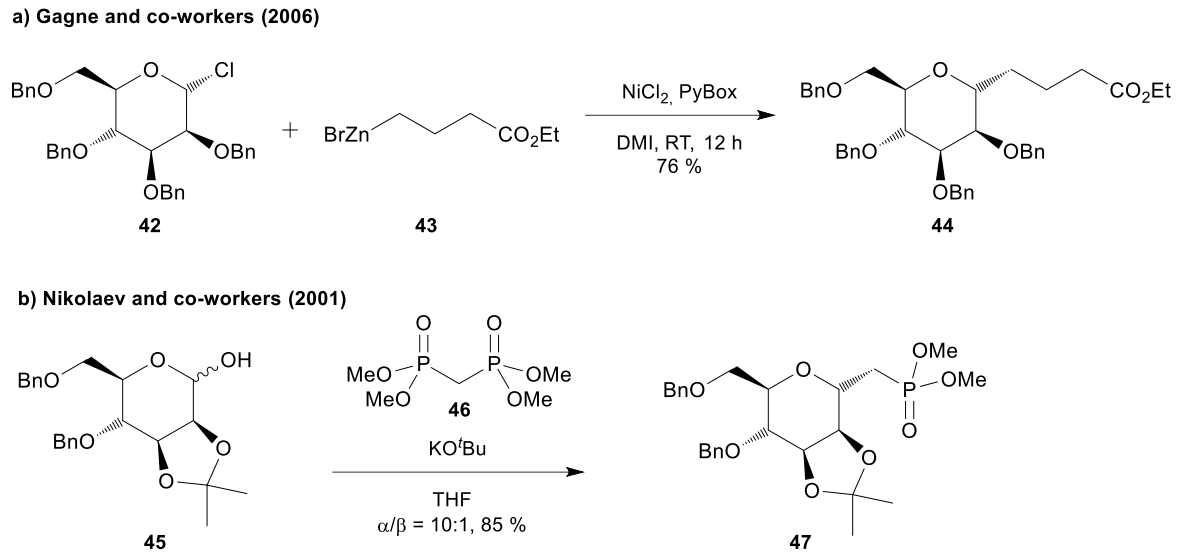


Scheme 5: *C*-Glycosylation method used by Thiem and co-workers.



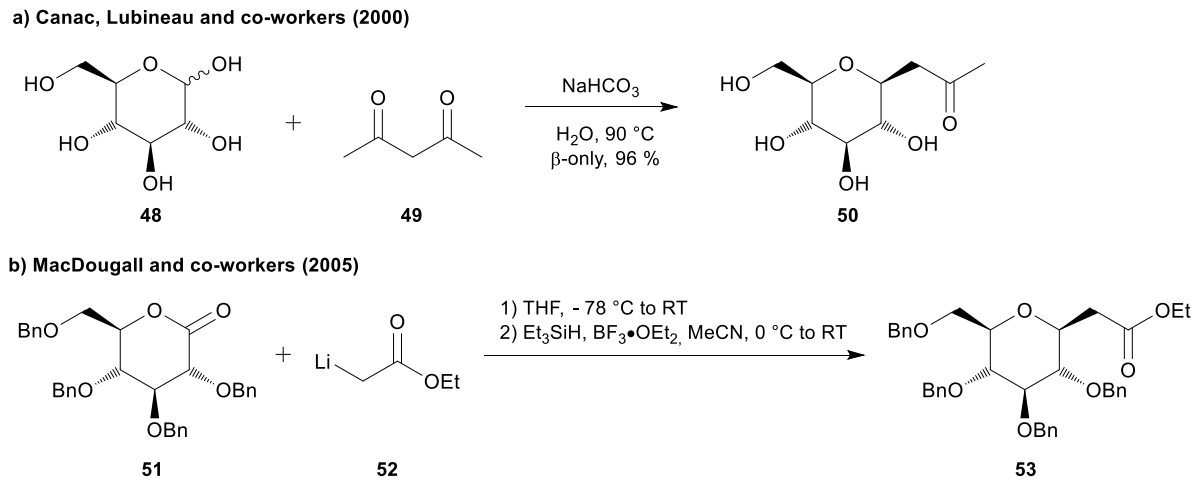
Scheme 6: *C*-Glycosylation methods used by (a) Wagner and co-workers and (b) Marsden and co-workers.

The Negishi cross-coupling of mannosyl chloride **42** with alkyl zinc reagents **43** using nickel(II) chloride as a catalyst and PyBox as a ligand led to *C*-glycosides **44** with high α-stereoselectivity, while for glucosyl bromides no selectivity was achieved, as shown by Gagné and co-workers (**Scheme 7a**).[51] Nikolaev and co-workers[52] employed a Horner-Wadsworth-Emmons reaction with phosphonate **46** on sugar **45** followed by a spontaneous intramolecular 1,4-Michael addition to *C*-glycoside **47** (**Scheme 7b**).



Scheme 7: *C*-glycosylation methods used by (a) Gagne and co-workers and (b) Nikolaev and co-workers.

Canac, Lubineau and co-workers[53] developed a method for the synthesis of β-*C*-glycosidic ketones **50** using unprotected sugars like D-glucose (**48**) under aqueous conditions (**Scheme 8a**). They have utilised a Knoevenagel-condensation with pentane-2,4-dione (**49**) which further undergoes an intramolecular Michael-addition and a retro-Claisen condensation to give product **50**. MacDougall and co-workers[54] used a method developed by Kishi and co-workers[55] starting with protected gluconolactone **51**. Reaction with lithiated ethyl acetate **52** and subsequent reduction with triethylsilane and boron trifluoride etherate gives *C*-glycoside **53** (**Scheme 8b**).



Scheme 8: *C*-Glycosylation methods used by (a) Canac, Lubineau and co-workers and (b) MacDougall and co-workers.

1.3 Glycoproteins

Proteins synthesised by eukaryotes as well as some bacteria and archaea are often modified co- or post-translational. A common modification is the glycosylation of asparagine sidechains, creating *N*-glycosidic linkages. While *O*-glycosidic bonds with amino acids like serine or threonine are also possible, they are less frequent.[56] Albert Neuberger has verified the existence of glycoproteins in egg-white in 1938, although they were supposed well before that time.[57] **Figure 10** shows a schematic representation of a glycosylated protein.[58]

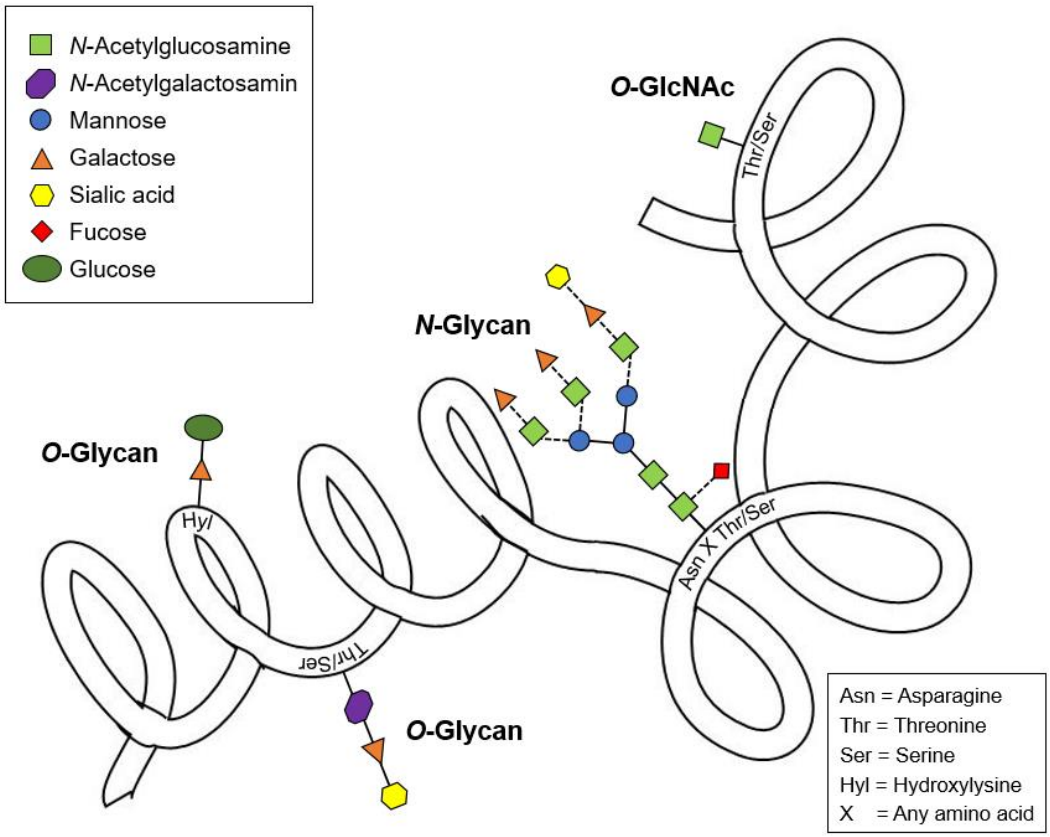


Figure 10: Schematic drawing of a glycosylated protein. Adapted from literature.[58]

Apweiler and co-workers analysed the SWISS-PROT database, a protein sequence database, in 1999 for the abundance of glycosylated proteins. The amino acid sequence NXS/T, where X is any amino acid except proline, possesses possible *N*-glycosylation sites. The authors have concluded, that more than half of the proteins found in nature could be glycosylated, although at the time of the analysis, only 10.6 % of the proteins in the database were proven to be glycoproteins.[59]

1.3.1 Glycosylation process

About 3-4 % of the human genome encodes for the enzymes and tools necessary for the glycosylation machinery. Although many glycosylation pathways are known and characterised, new ones are still found. The known human glycoproteins can consist of ten different carbohydrates: D-glucose, D-galactose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, D-glucuronic acid, D-mannose, *N*-acetylneuraminic acid, D-xylose, D-ribose and L-fucose, with the latter sugar representing one of the few L-configured monosaccharides in nature. In many cases, *N*-glycosylation is initiated in the endoplasmic reticulum (ER) co-translational by an oligosaccharyl-transferase complex by attaching a prebuild oligosaccharide. The first monosaccharide linked to the asparagine sidechain is mostly GlcNAc. Further processing involves trimming of sugar residues by glycosidases followed by assembly of the specific epitopes conducted by glycosyltransferases as well as branching to bi-, tri- or tetra-antennary core structures (**Figure 11**).[60]

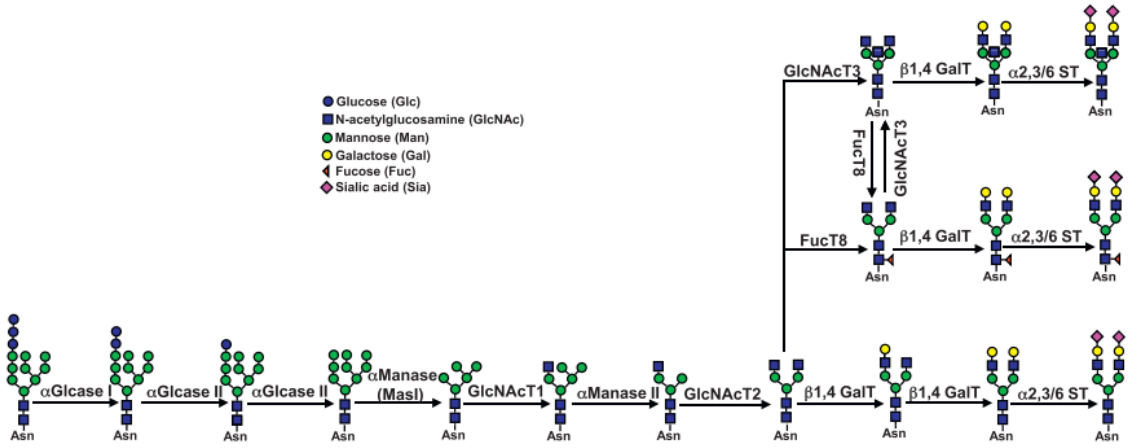


Figure 11: Synthesis pathway of *N*-glycosylated proteins in mammals. These are just some examples of glycan structures with many more possible. Reprinted from literature.[61]

The core structure directly linked to the protein is well preserved in eukaryotes. In archaea, the diversity of different structures is the highest, while in bacteria, it is already limited and only present in a few species (**Figure 12**). The huge diversity of eukaryotic glycans is a result of late modifications in the ER and the Golgi apparatus.[62]

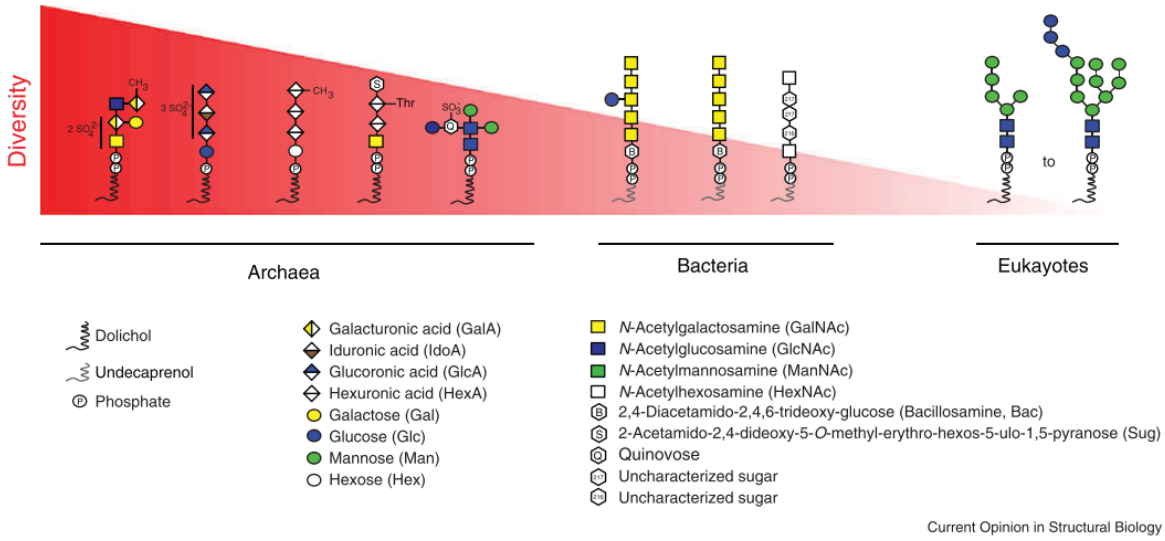


Figure 12: Differences in the diversity of glycan structures between archaea, bacteria and eukaryotes. Reprinted from literature.[62]

1.3.2 Biological functions[63–65]

The biological roles of glycoproteins are diverse and still subject to research, also helping to reveal several human glycosylation disorders in recent years (**Figure 13**). Generally, three different categories can be distinguished: (1) structural and modulatory properties, (2) intrinsic recognition processes and (3) extrinsic recognition processes.

Regarding the structural and modulatory properties, glycosylation leads to an improved water-solubility of proteins due to the hydrophilic sugar moieties. Without this modification, the high protein concentration of about 50-70 mg/ml in humans would likely not be possible. Interactions between glycans and amino acids also play an important role in the three-dimensional folding of the protein after synthesis in the ER. Improper folding leads to non-functional enzymes, which undergo degradation by the cellular machinery. Furthermore, *O*-GlcNAc glycosylation is a known molecular-switch, affecting a variety of processes in cells.

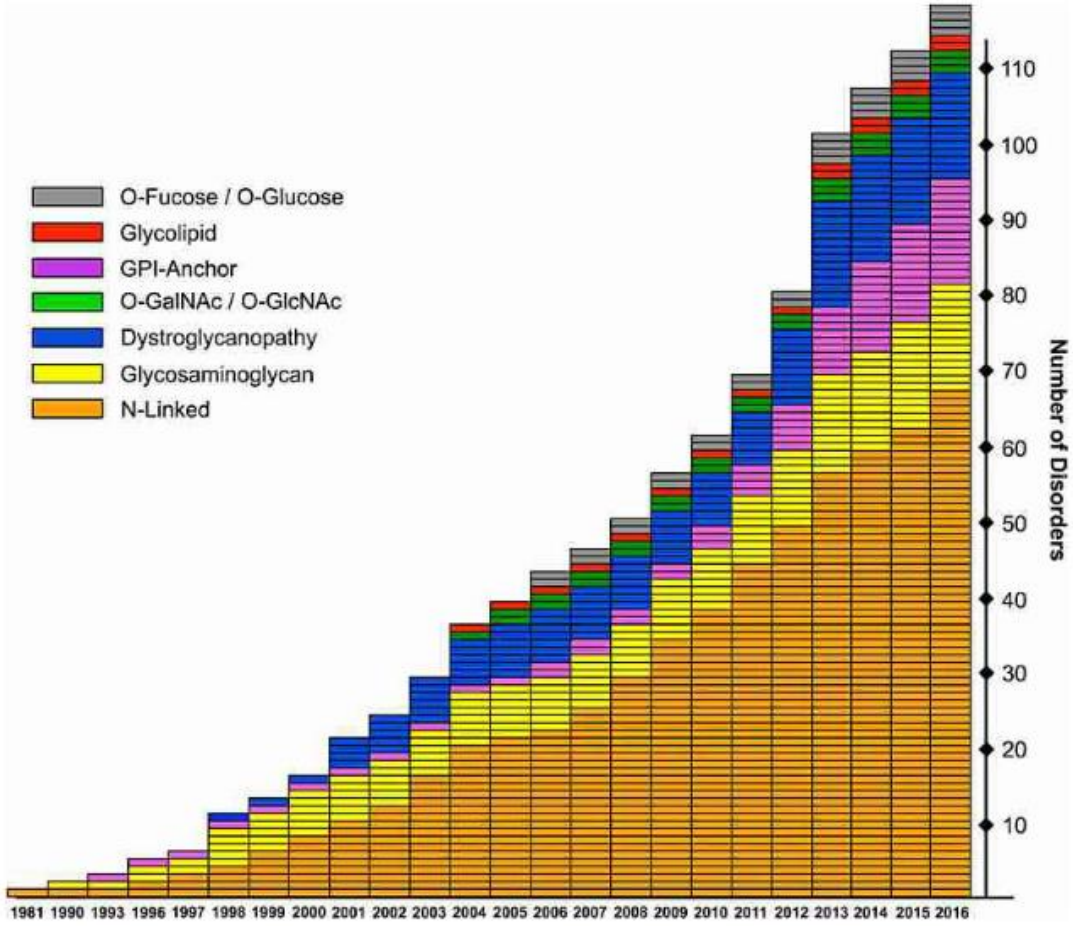


Figure 13: Number of known human glycosylation disorders. Reprinted from literature.[63]

Intrinsic recognition processes also include a variety of functions. Glycan recognising enzymes help trafficking glycoproteins to their intended location in the cells. The recognition of glycans can trigger endocytosis or phagocytosis, which is a way to degrade damaged cells or proteins. Furthermore, glycans of glycoproteins are often terminated by sialic acids which get intentionally cleaved during the aging process of the protein. This exposes the underlying glycan and can trigger degradation of the protein.

Extrinsic recognition processes are crucial for the interaction between different species and for the immune system. On the one hand, bacteria, fungi and viruses as well as bacterial and plant toxins often bind to glycans of the host cells. On the other hand, glycosylated surfaces of a pathogen are recognition sites for the immune system (**Figure 14**).

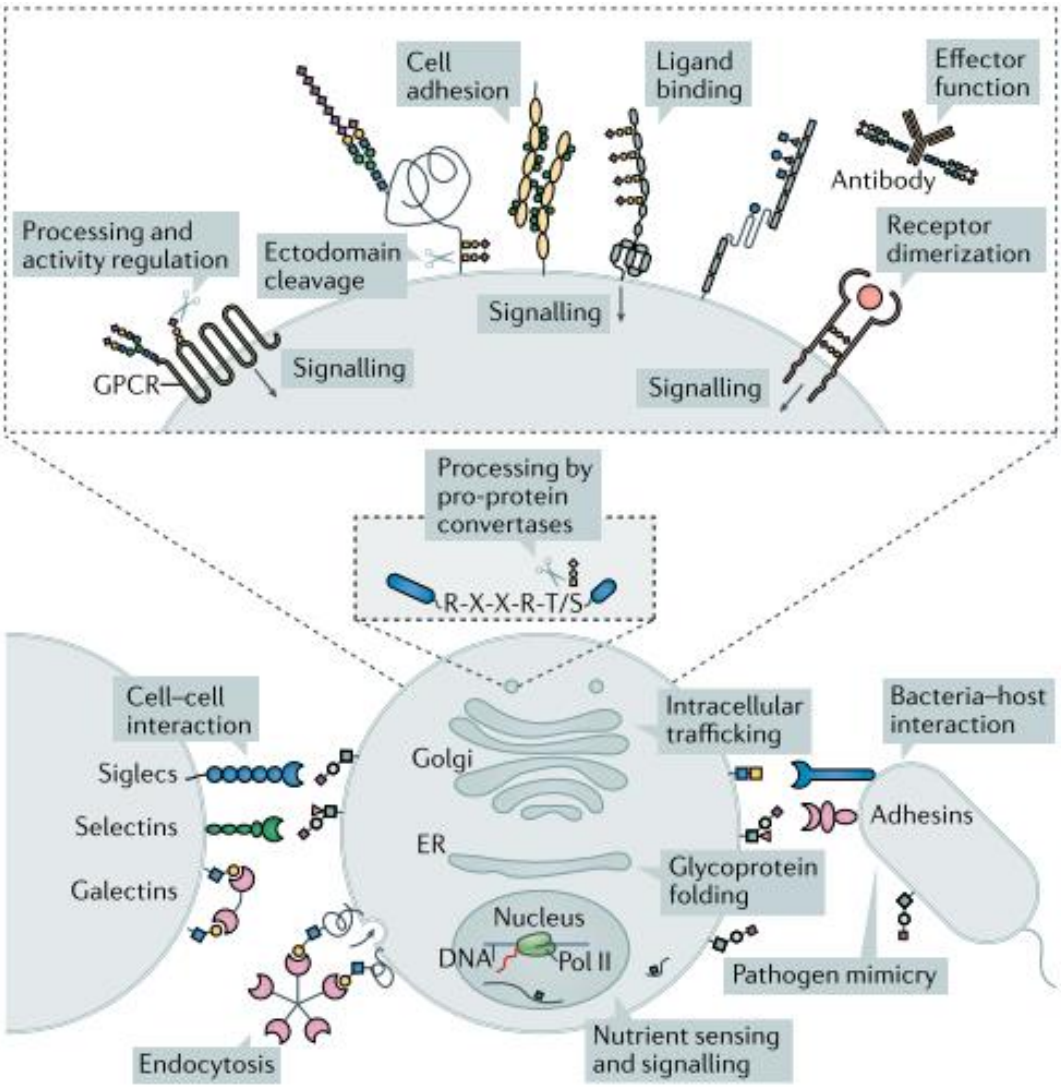


Figure 14: Illustration of different functions of glycoproteins in living organisms. Reprinted from literature.[60]

1.4 Amadori Rearrangement

1.4.1 History of the Amadori rearrangement

The Amadori rearrangement, a reaction between carbohydrates and amines, was thoroughly investigated by Mario Amadori at the beginning of the 20th century and is part of the Maillard reaction cascade, which was discovered by Louis-Camille Maillard in 1912. The Maillard reaction describes the non-enzymatic browning of food by the formation of melanoidins and other polymeric compounds. It is a complex reaction cascade, creating intermediates like furfural or acrylamide and has an impact on the taste and colour of many foods (**Figure 15**).[66–69] The Amadori

rearrangement, which was further investigated by Kuhn, Dansi, Weygand and Birkofer,[70–72] marks the beginning of the cascade. Until the 1950s, it was believed that the rearrangement can only occur with aromatic amines, which was proven wrong by Heyns and co-workers as well as by Carson. They showed that the rearrangement also takes place with ammonia, aliphatic amines as well as amino acids. The corresponding rearrangement of ketoses with amines to 2-aminodeoxy aldoses is nowadays known as the Heyns rearrangement.[73–75]

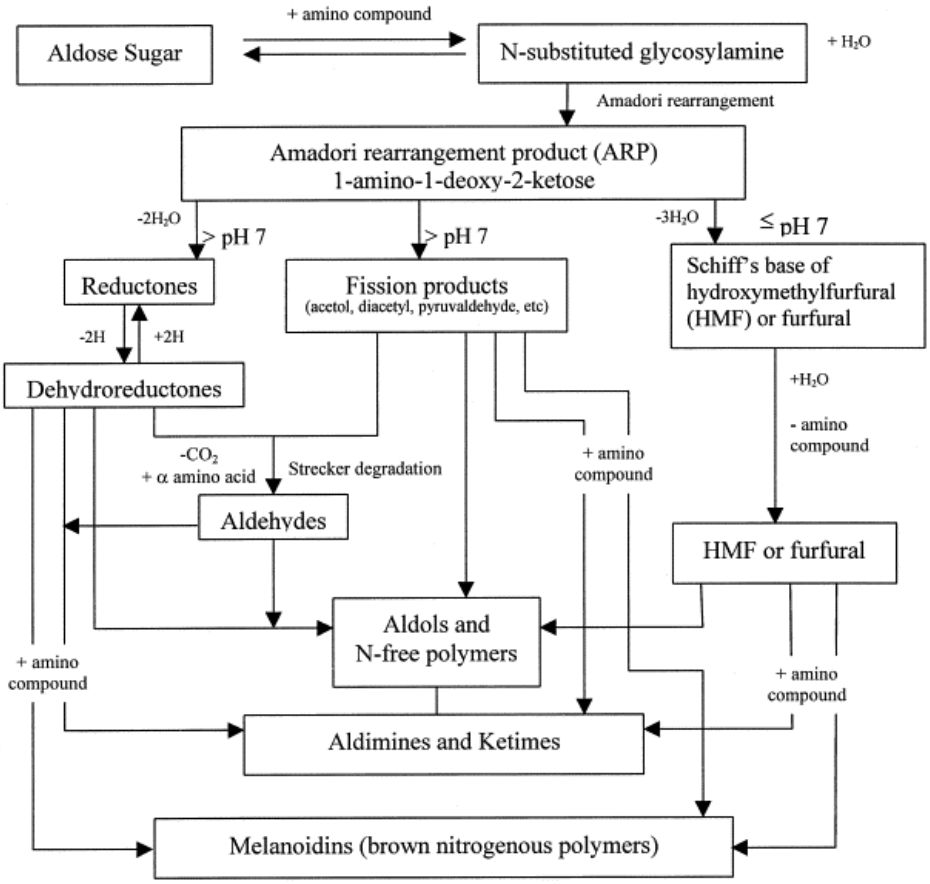
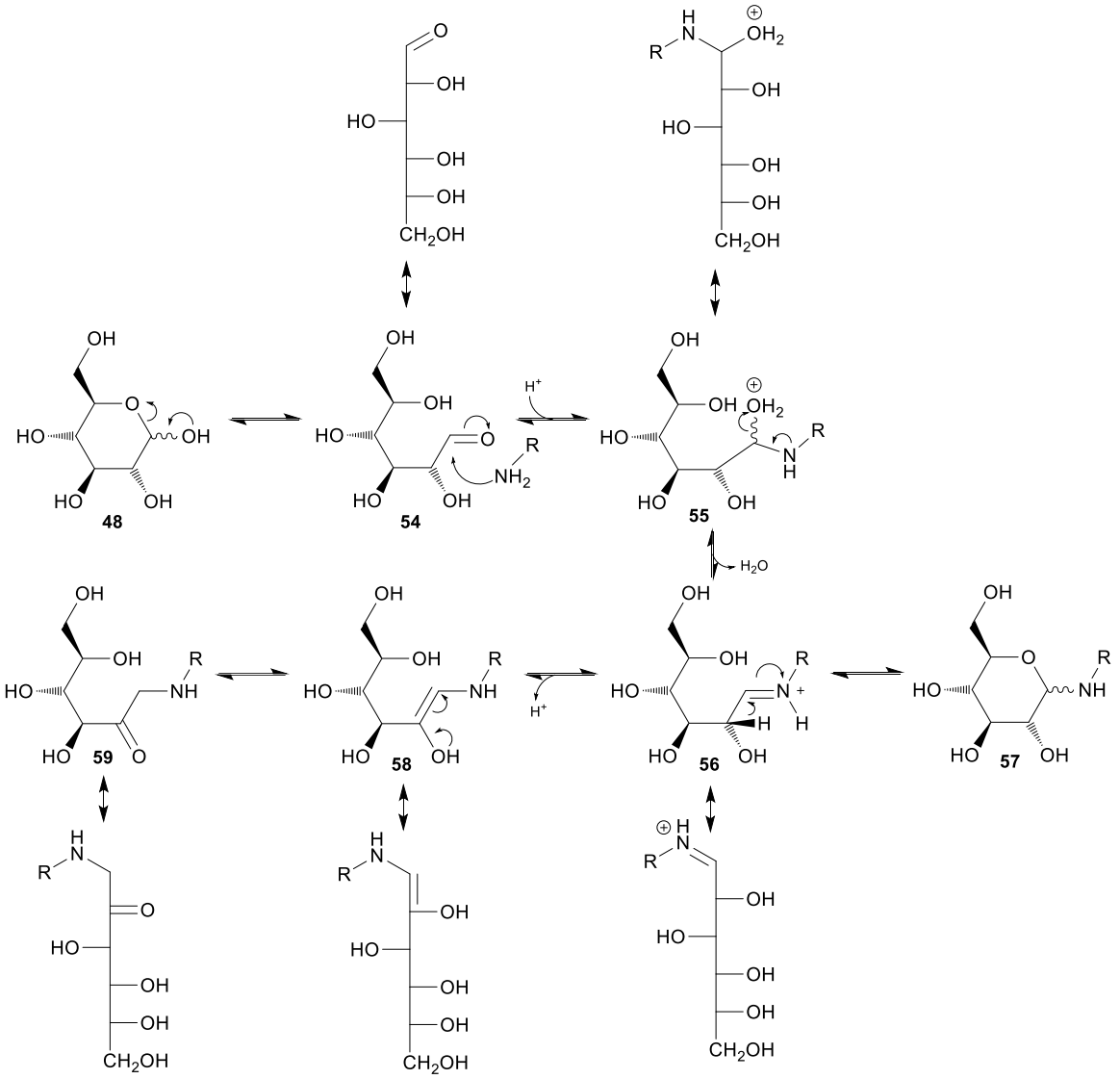


Figure 15: Maillard reaction cascade. Reprinted from literature.[76,77]

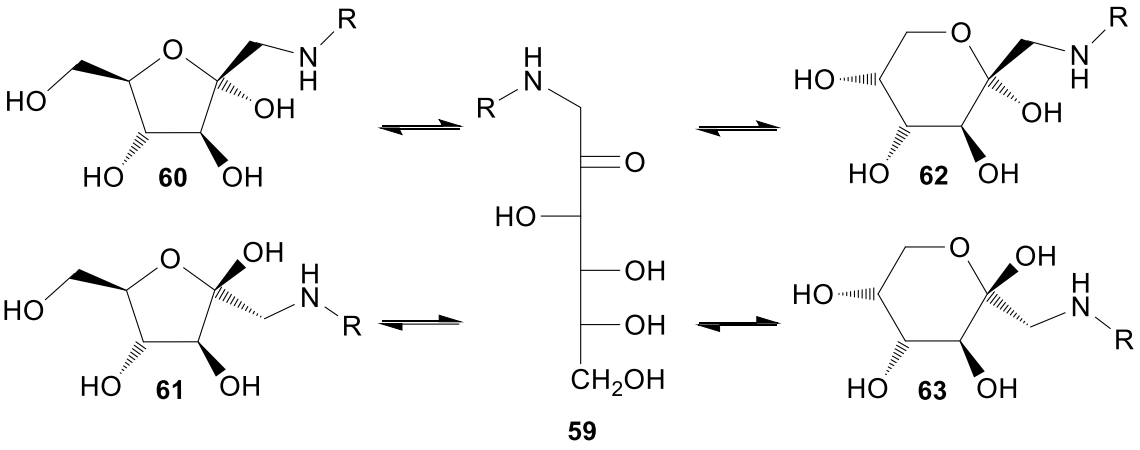
1.4.2 Mechanism

In the Amadori rearrangement, imines of aldoses isomerise to their corresponding 1-amino-1-deoxy-ketoses **59** under acid or base catalysis.[78,79] Prior to this rearrangement, the necessary Schiff's base is created by reaction of an aldose with an amine, which is also regarded as part of the Amadori reaction.[80] The mechanism of the Amadori rearrangement as proposed by Kuhn and Weygand is shown in **Scheme 9**.[72]



Scheme 9: Mechanism of the Amadori rearrangement with D-glucose (**48**) as an example.

In the first step, D-glucose (**48**) in its open-chain form **54** gets attacked by an amine as a nucleophile to yield adduct **55**. Elimination of water leads to the formation of Schiff's base **56**, which Amadori has mistakenly called "labile isomer". Kuhn and Dansi[71] later showed that this "labile isomer" is *N*-glycoside **57**, which is in equilibrium with imine **56**. The first rearrangement step is the formation of enol **58** after protonation of Schiff's base **56**. Keto-enol tautomerism leads to ketose **59**, which can then form semi-ketals **60** - **63**. This variety of possible different products can make the workup of Amadori reactions challenging (**Scheme 10**).[81]

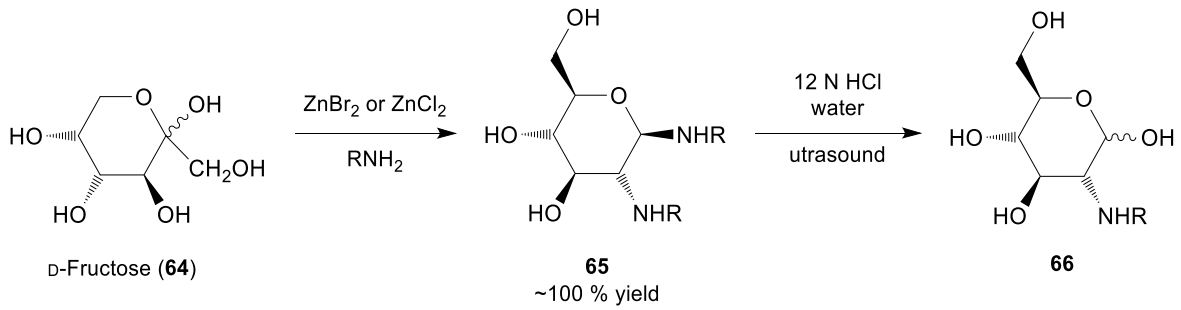


Scheme 10: Possible isomers of the Amadori rearrangement. Adapted from literature.[81]

1.4.3 Synthetic applications

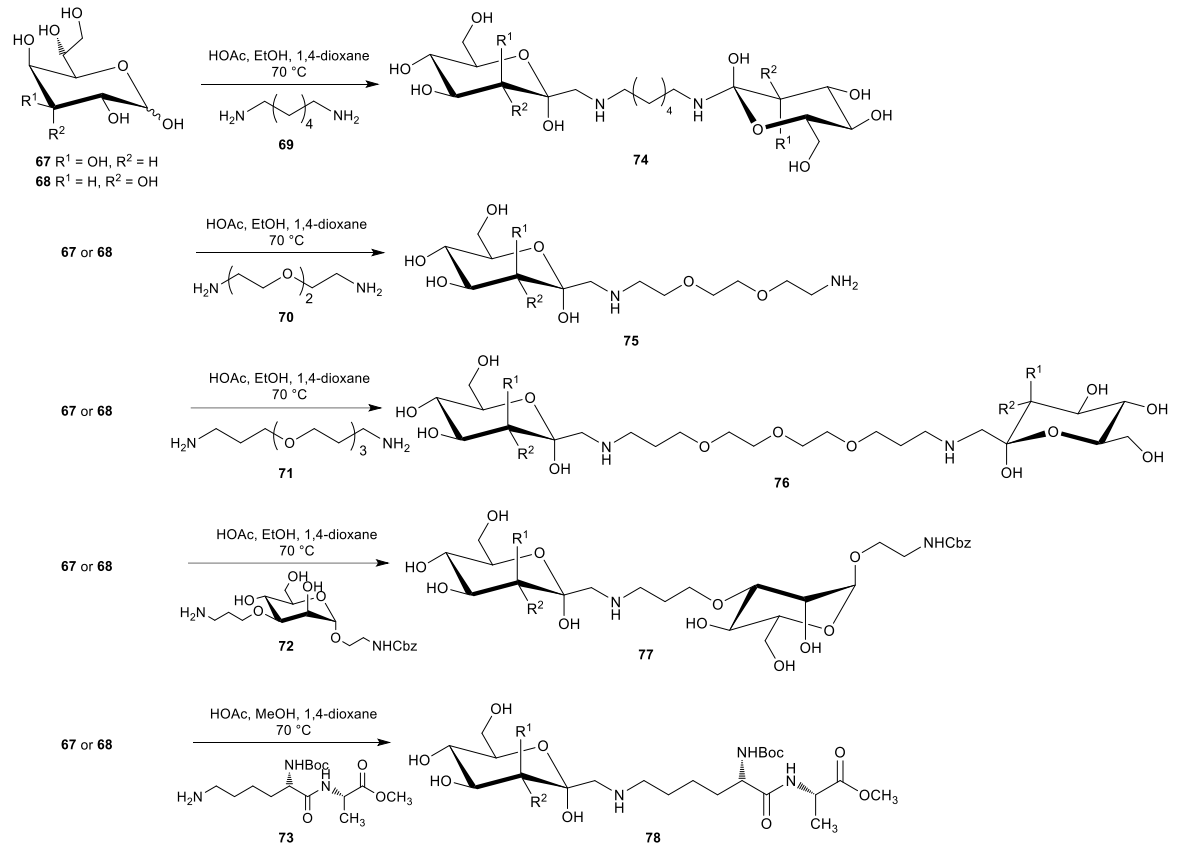
The Amadori rearrangement is known to be challenging regarding the workup due to many possible side products and the reaction conditions, such as temperature, duration, and the catalyst. However, by optimizing the reaction conditions, it is a versatile method for the synthesis of *C*-glycosyl-type sugar derivatives.[82]

Norin and co-workers utilised the Amadori and Heyns rearrangements to synthesize carbohydrate-based surfactants starting from D-fructose (**64**) using zinc halides as a catalyst.[83] This led to a Heyns and Amadori-like rearrangement, giving 1,2-dialkylamino-1,2-dideoxy-D-(*N*)-β-glucosides **65** in quantitative yields, which were hydrolysed to 2-alkylamino-2-deoxy-D-glucose **66** (**Scheme 11**). Recently, Nanishankar and co-workers further investigated the use of zinc salts and proposed zinc acetate as an efficient catalyst.[84]



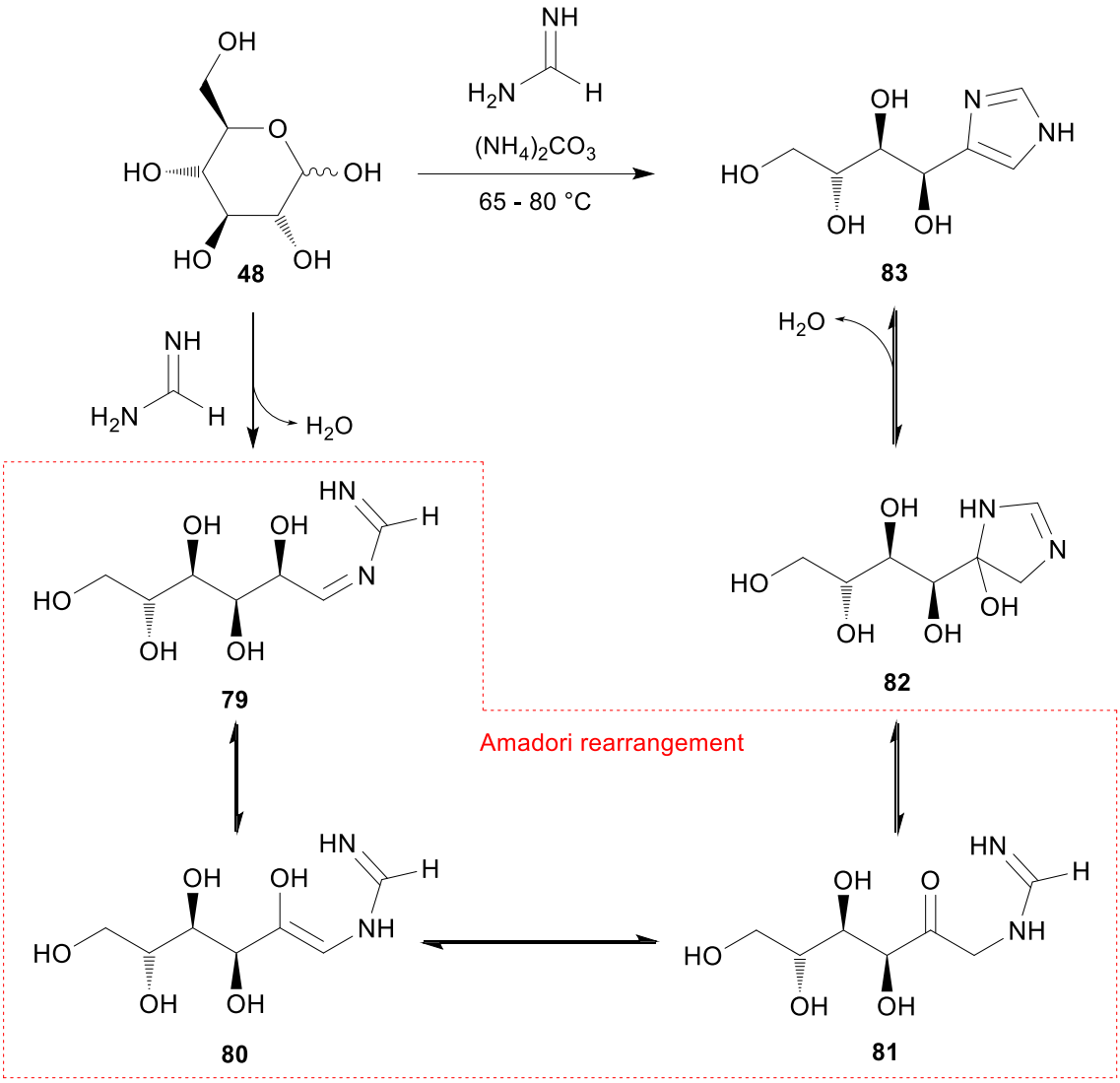
Scheme 11: Heyns and Amadori-like rearrangement of D-fructose (**64**).[83]

Wrodnigg, Lindhorst and co-workers investigated the Amadori rearrangement with different amines and D-*glycero*-D-*gulo* aldoheptose **67** as well as D-*glycero*-D-*galacto* aldoheptose **68**. Regarding the amines, they used for example diamines **69** - **71**, amino-functionalized glycoside **72** or amino acid **73**. A few selected examples of the employed reactions yielding glycosides **74** - **78** are illustrated in **Scheme 12**. Generally, the yields varied between 20 % and 80 %, demonstrating that the Amadori rearrangement is a flexible way to obtain *C*-glycosyl type bonds, although it strongly depends on the substrates and optimisation of the reaction conditions.[85]



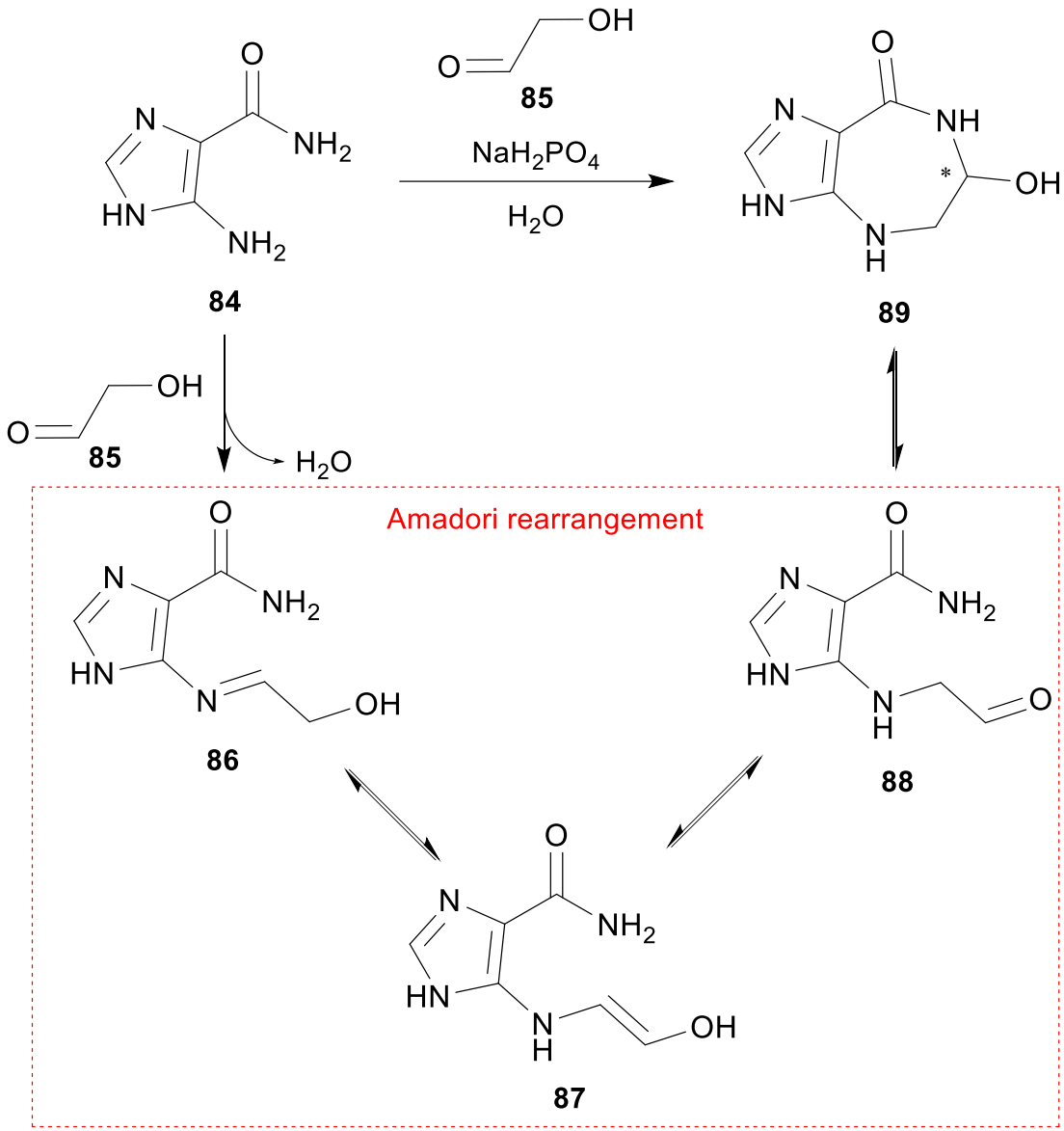
Scheme 12: Different products of the Amadori rearrangement investigated by Wrodnigg, Lindhorst and co-workers.[85]

Brust and Cuny[86] made use of the Amadori rearrangement in the synthesis of hydrophilic substituted imidazoles **83**. The reaction was carried out in a melt of $(NH_4)_2CO_3$ with an amidine source and with different sugars. **Scheme 13** shows the proposed mechanism through intermediates **79** - **82** with D-glucose (**48**) as an example.



Scheme 13: Synthesis of hydrophilic substituted imidazoles **83** by Brust and Cuny. Adapted from literature.[86]

Powner and co-workers[87] synthesised azepinomycin (**89**) without protecting groups and in water starting from 5-aminoimidazole-4-carboxamide (**84**) by employing glycolaldehyde (**85**). The formed imine **86** undergoes the Amadori rearrangement through intermediates **87** and **88** as shown in **Scheme 14**.



Scheme 14: Synthesis of azepinomycin (**89**) by Powner and co-workers. Adapted from literature.[87]

1.4.4 Biological aspects

As the Amadori rearrangement as well as further reactions of the Maillard reaction can take place under physiological conditions without enzyme catalysis, called glycation, they have an impact on biological systems.[88] The formation of Advanced Glycation End Products (AGEs), during the Maillard cascade, which is schematically shown in **Figure 16**, is believed to play a significant role in the pathogenesis of diabetes and Alzheimer's disease.[89]

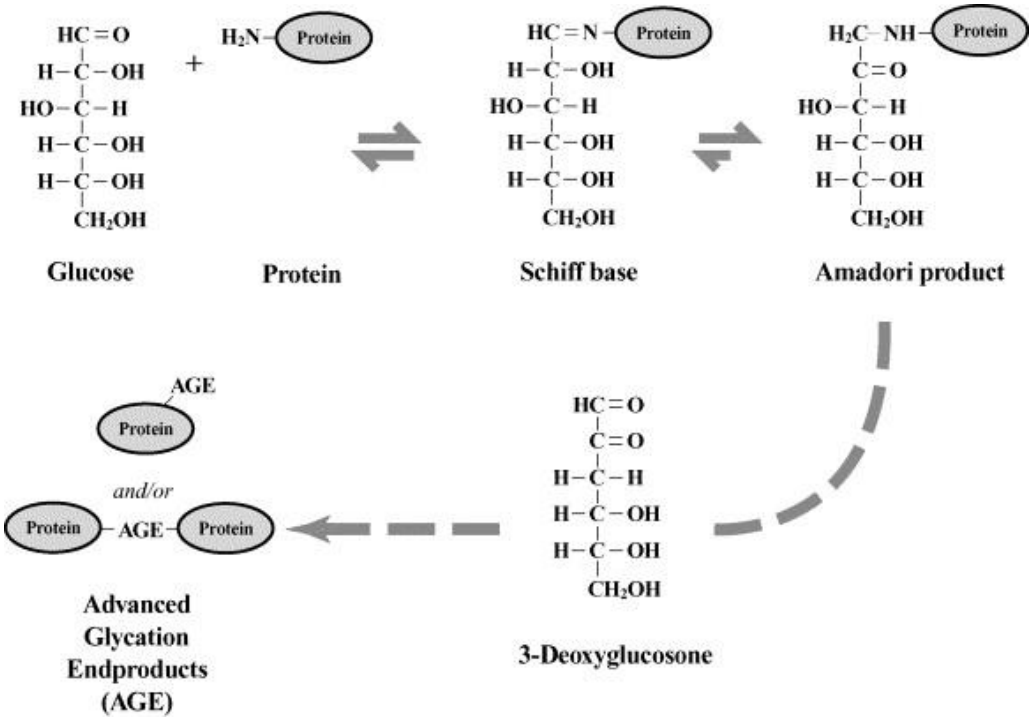


Figure 16: Schematic representation of the formation of AGEs. Reprinted from literature.[90]

Figure 17 shows structures for protein cross-linking AGEs **91** - **93** and non-cross-linking AGE **90**.

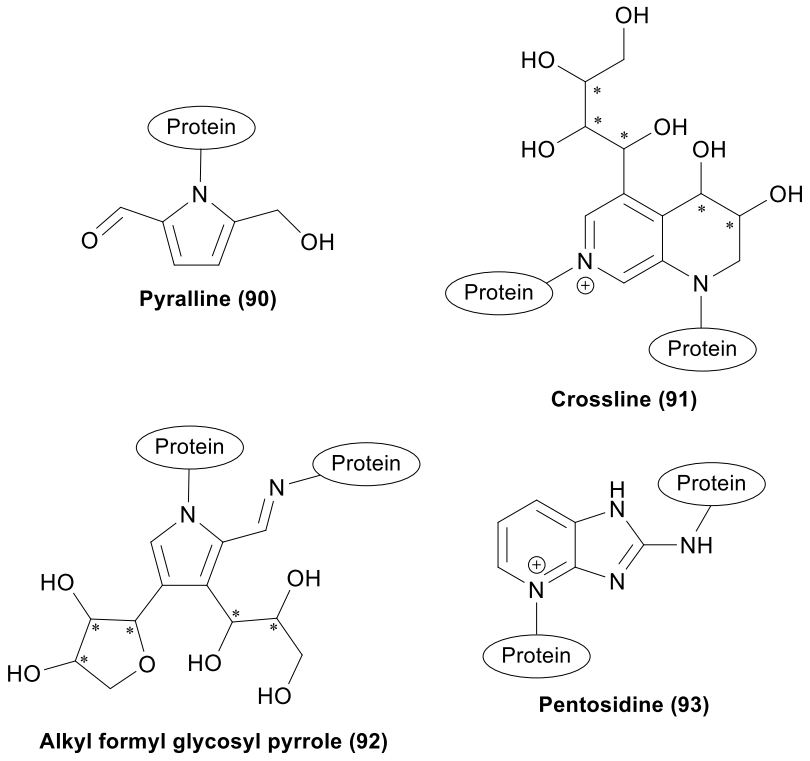
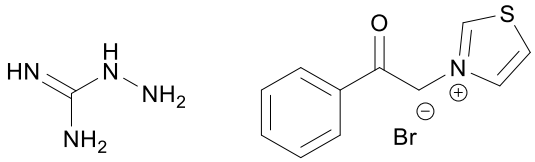


Figure 17: Structures of some examples of AGEs. Adapted from literature.[90]

One protein affected by diabetes is Human Serum Albumin (HSA), which contains several lysine residues suitable for an Amadori rearrangement with glucose. In consequence of the higher-than-average glucose levels in the blood of diabetes patients, glycation of HSA, which is the most abundant serum protein, can take place. The implications of this modification are manifold. For example, the structural change of this protein leads to altered drug binding and antioxidant properties. Furthermore, due to interactions with different receptors, glycated HSA can promote the formation of Reactive Oxygen Species (ROS) and it is believed to possibly lead to autoimmune diseases.[91] In this context, other proteins, such as haemoglobin and collagen, can also be affected by glycation, leading to similar harmful effects.[92,93]

The aggregation of proteins due to glycation-promoted crosslinking is also subjected to play a role in the pathogenesis of Alzheimer's disease. The formation of AGEs promotes the oligomerisation and polymerisation of the β-amyloid peptide, leading to the neurodegenerative disorder. It could also be shown that DNA can get glycated *in vitro*. Whether any correlation to cellular aging exists is still subject of research.[94]

Although glycation is non-enzymatic, inhibitors for this kind of reactions are subject to research. Compounds, such as aminoguanidine (**94**) or phenacylthiazolium bromide (**95**) (**Figure 18**), can target various sites in the pathway to AGEs (**Figure 19**). As these inhibitors have to react with the Maillard intermediates as soon as they are spontaneously formed, they have to be available in a high concentration over a long time, making such pharmaceutically active compounds challenging to design.[90,95]



94 **95**

Figure 18: Inhibitors for the formation of AGEs.[90]

Amadori rearrangement products and AGEs can also be used in the diagnostics of diseases. For example, in phenylketonuria, the phenylalanine levels increase to harmful concentrations due to a dysfunctional enzyme, leading to major health

issues. As the phenylalanine can undergo an Amadori rearrangement with glucose and other carbohydrates, these corresponding products can be used as biomarkers for the early recognition of the disease.[96]

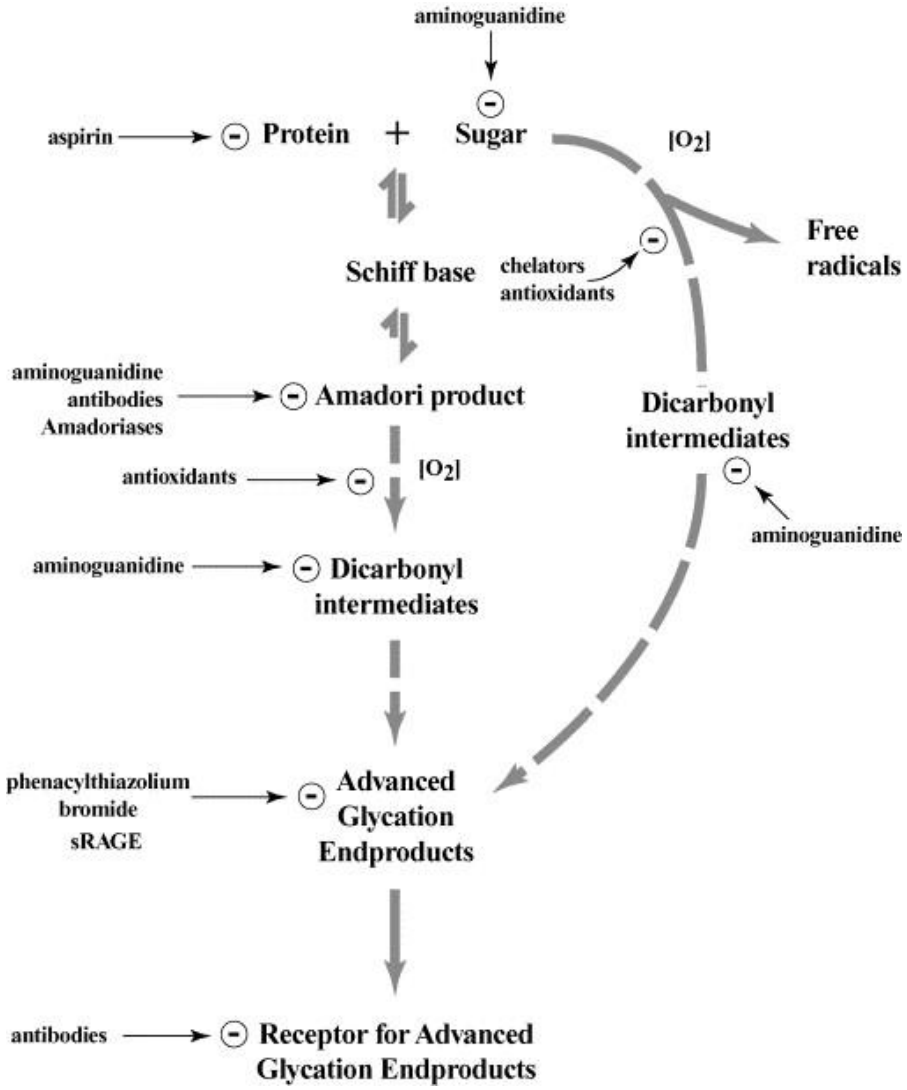


Figure 19: Potential sites for the inhibition of the formation of AGEs. Reprinted from literature.[90]

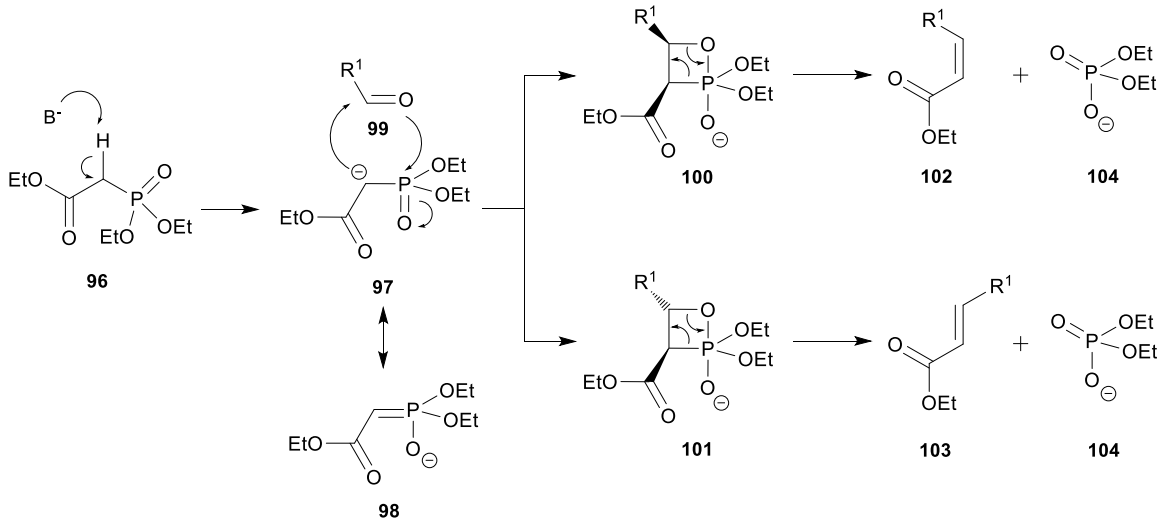
1.5 Horner-Wadsworth-Emmons Reaction

In 1954, Georg Wittig described a new carbon-carbon double bond formation reaction using tetramethyl phosphonium bromide and a carbonyl functionality.[97] This reaction is nowadays known as the Wittig reaction and has become an essential reaction in organic synthesis for different types of molecules. L. Horner, W. S. Wadsworth and W. D. Emmons have modified this reaction by using stabilised

phosphonates. In contrast to the Wittig reaction, this variation mainly yields (*E*)-configured analogues, although this outcome strongly depends on the substrate and the reaction conditions. The scope of this Horner-Wadsworth-Emmons (HWE) reaction has since then increased dramatically, making it a widely used method in natural product synthesis.[98]

1.5.1 Mechanism[99]

The reaction mechanism of the Wittig and HWE reaction are similar and still subject to research. For “Li-salt free” Wittig and HWE reactions, experimental and computational evidence strongly suggests a kinetically controlled reaction with an oxaphosphetane (OPA) **98** or **99** as the intermediate. The “Li-present” mechanism is still unknown. The proposed mechanism for a “Li-salt free” HWE-reaction is shown in **Scheme 15**.



Scheme 15: Proposed reaction mechanism of a “Li-salt free” HWE-reaction.

First, the phosphonate, here triethyl phosphonoacetate (**96**), gets deprotonated to the corresponding phosphorous-ylid **97**, which is in equilibrium with phosphorous-ylene **98**. The carbonyl funcytionality **99** can then react with the phosphorous compound **97** in a [2+2] cycloaddition to form *cis*-OPA **100** or *trans*-OPA **101**. Which of the isomers gets formed as the main product depends on the phosphorus-ylid. If stabilised ylids are used, which is often the case in HWE reactions, the cycloaddition results in the transition state shown in **Figure 20** (b). There, OPA formation passes

through a late transitionstate, meaning that the phosporus nearly rearranged to its trigonal bipyramidal conformation, which results in different sterical and electronical interactions between the carbonyl moiety and the phosphorous-ylid compared to classic Wittig-reactions, which mostly use non-stabilised ylids. In the case of non-stabilised ylids, an early transitionstate, shown in **Figure 20** (a), is found. Transitionstate (a) leads to the *cis*-isomer **100** , while transitionstate (b) gives the *trans*-isomer **101**.

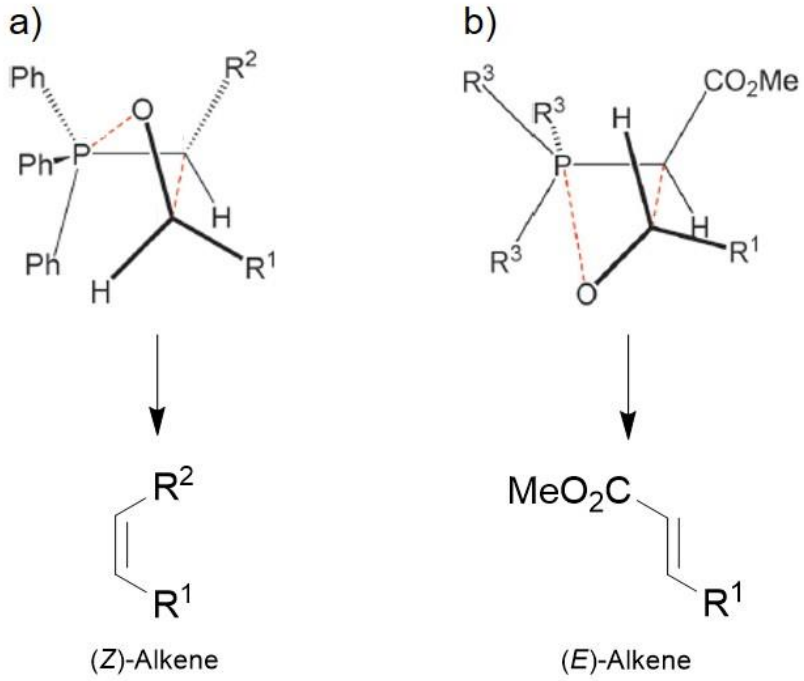


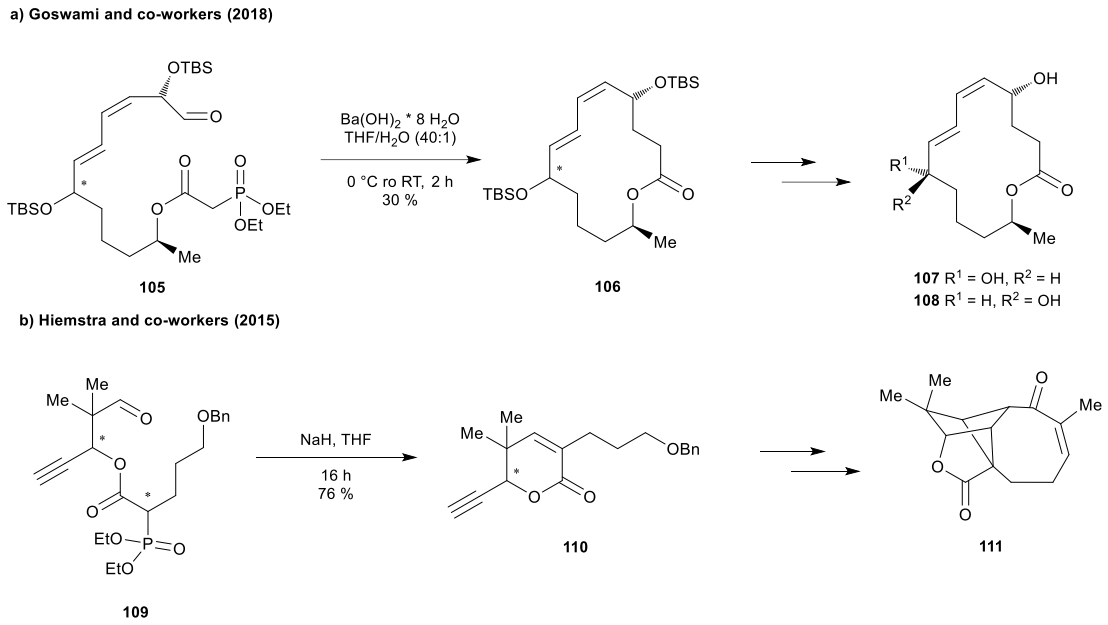
Figure 20: Proposed transition states for the formation of OPAs for (a) non-stabilised ylids (b) stabilised ylids. Adapted from literature.[99]

The conformation of the OPA defines the outcome of the following formation to alkenes **102** or **103**. *Cis*-OPAs **100** react to the corresponding (*Z*)-alkenes **102** while the *trans*-isomers **101** decompose to (*E*)-alkenes **103**. This explains the (*Z*)-selectivity for classical non-stabilised Wittig reactions and (*E*)-selectivity for HWE-reactions.

1.5.2 Synthetic applications

The scope and application of the HWE-reaction in organic synthesis has been reviewed several times.[100–102] Most recently, Beemelmanns and co-worker have

investigated the applications of the HWE-reaction in natural product synthesis.[103] Some paradigmatic examples out of this review will be shown in the following.

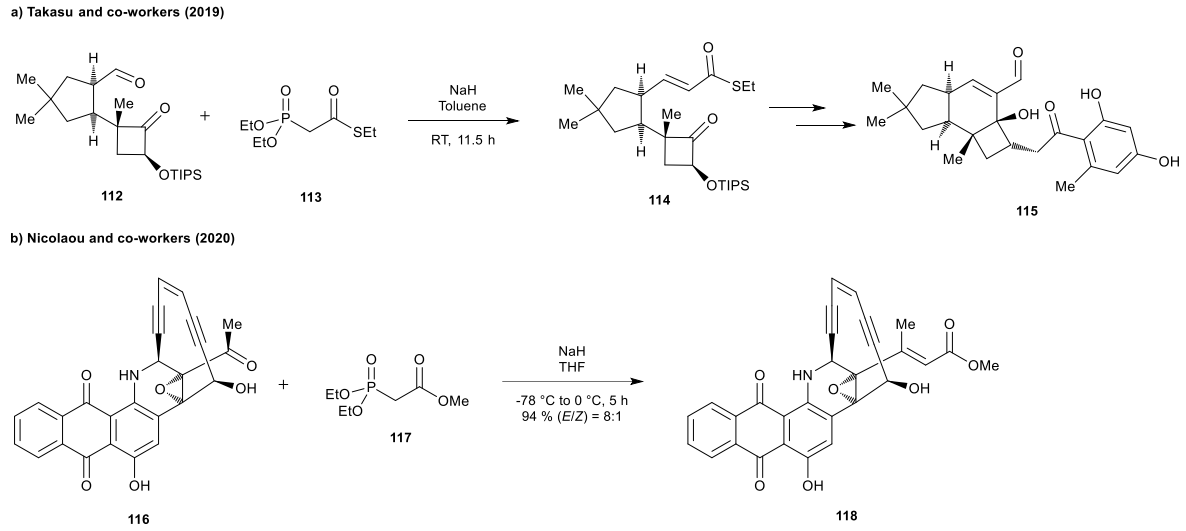


Scheme 16: Applications of the HWE-reaction by (a) Goswami and co-workers and (b) Hiemstra and co-workers.

Goswami and co-workers[104] used an intramolecular HWE-reaction for the synthesis of the 14-membered rings pestalotioprolide E (**107**) and pestalotioprolide F (**108**). They investigated different conditions for the cyclisation and found $Ba(OH)_2 * 8\ H_2O$ in THF/H_2O (40:1) as the most suitable, giving 30 % of the desired compound **106** after two hours starting from substance **105** (**Scheme 16a**). Hiemstra and co-workers[105] synthesised aquatolide (**111**) in 16 steps, including an intramolecular HWE-reaction to create a six-membered ring **110**. They have used classical conditions with sodium hydride as base and THF as solvent starting from compound **109** (**Scheme 16b**).

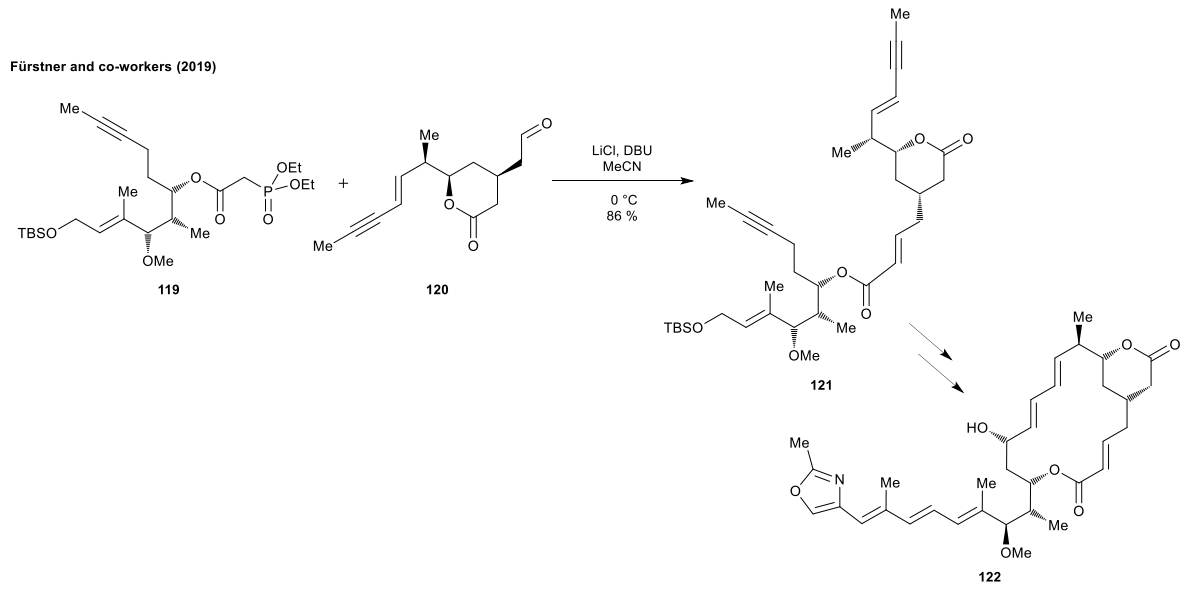
Takasu and co-workers[106] employed the HWE-reaction in the total synthesis of melleolide (**115**) to create an α,β-unsaturated thioester **114** starting from compound **112** with phosphonate **113** (**Scheme 17a**). As the HWE-reaction is selective for carbonyls and does not undergo major side reactions, it is also suitable for late-stage modifications of bigger molecules. Nicolaou and co-workers[107] used this approach in the synthesis of tiancimycin B (**118**), where an α,β-unsaturated ester is introduced on compound **116** with phosphonate **117** late in the reaction sequence (**Scheme**

17b). It is noteworthy that this reaction can take place besides an epoxide and the carbonyl functions of the anthraquinone moiety do not react.



Scheme 17: Applications of the HWE-reaction by (a) Takasu and co-workers and (b) Nicolau and co-workers.

A HWE-reaction can also be used to couple bigger fragments. Fürstner and co-workers[108] used this approach in their total synthesis of rhizoxin D (**122**) to couple the fragments **119** and **120** to intermediate **121** (**Scheme 18**).



Scheme 18: Application of the HWE-reaction by Fürstner and co-workers.

1.6 Surface Modifications

Surfaces that come in contact with biological systems, for example the surfaces of implants, need to have several special properties. Most obvious, they must be biocompatible. Furthermore, they must be resistant against the accumulation of biological foulants, for example bacteria or proteins. If incompatible materials are used, they can lead to mechanical damage, bacterial colonisation or can get attacked by the immune system, leading to inflammation. Polysaccharides like cellulose or cellulose acetate possess an excellent biocompatibility but are susceptible to fouling. To overcome this, different surface modifications are possible, depending on the desired application. Surfaces can be prepared to regulate the adsorption of molecules or the adhesion of cells, but it is also possible to use them for a controlled release of molecules, like drugs, or even to employ reactions on them (**Figure 21**).[109]

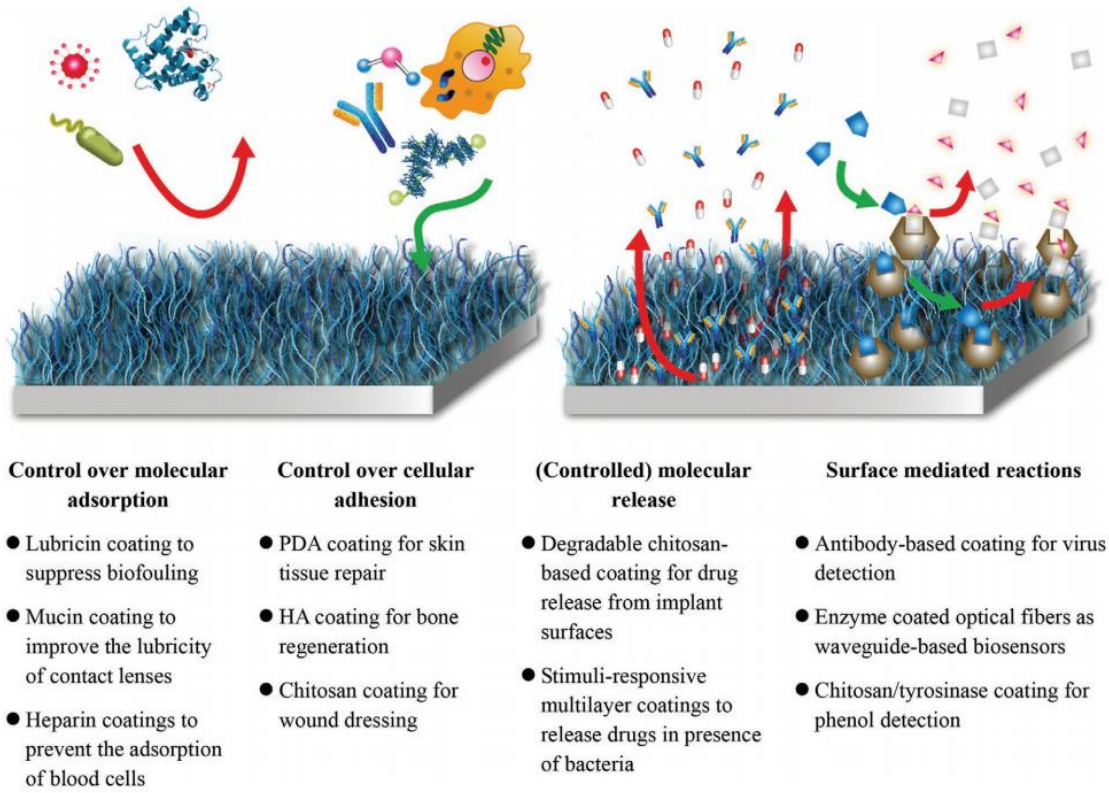
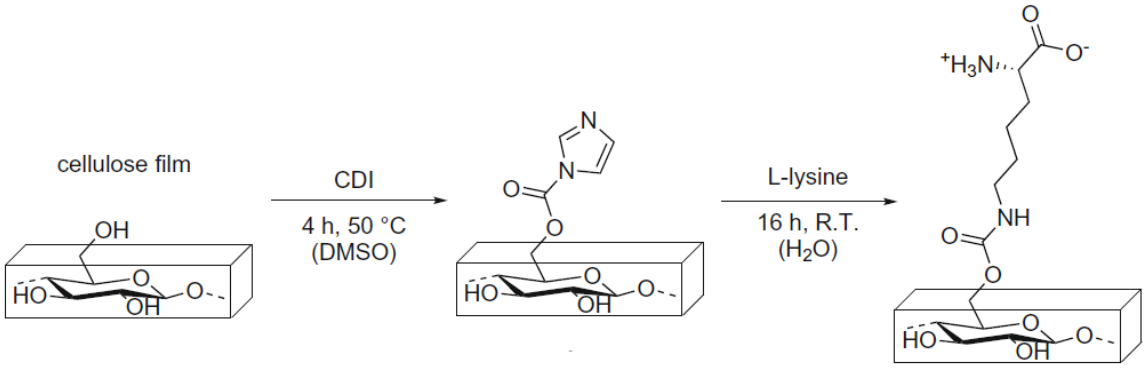


Figure 21: Different surface coatings for various applications. Reprinted from literature.[109]

Other methods to improve the antifouling properties of cellulose derivatives are coatings with polysaccharide multilayers or coupling of amino acids such as lysine. Regarding the polysaccharide multilayers, an alternating coating of carboxymethyl cellulose and chitosan on partially deacylated cellulose acetate thin films led to a 55 % reduced adsorption of bovine serum albumin (BSA).[110] A mixed coating of chitosan and TEMPO oxidised cellulose nanofibril (TOCN) on regenerated cellulose led to a 99.9 % reduction of bacterial colonies in comparison with a TOCN coating without chitosan.[111] The covalent coupling of lysine to a cellulose thin film as a carbamate with *N*,*N*'-carbonyldiimidazole (CDI) gave surfaces which significantly reduced the BSA adsorption and also reduced the adsorption of fibrinogen by six times (**Scheme 19**).[112]



Scheme 19: Coupling of lysine to a cellulose thin film as a carbamate. Adapted from literature.[112]

2 Aims and Synthetic Targets

The aim of this master's thesis is to synthesise a *C*-glycosyl-type as well as a *C*-glycosyl sugar-lysine glycoconjugate for application on spin coated thin-film chitosan surfaces. **Figure 22** shows the two target molecules **123** and **124** for this thesis.

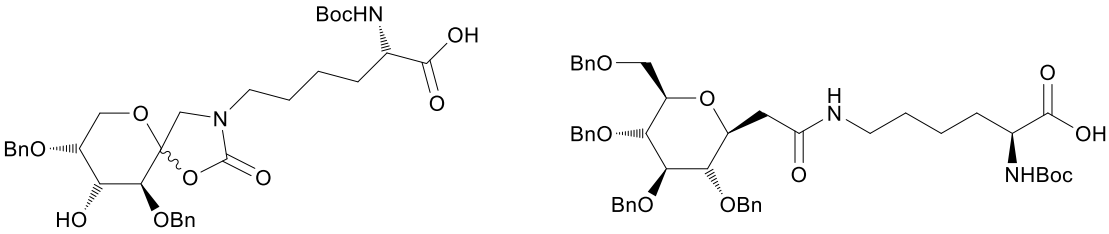


Figure 22: Target molecules for this thesis.

These compounds can be used for the chemical modification of surfaces for biological applications and create the possibility to build up glycoproteins on them (**Figure 23**). The surfaces are prepared by Tadeja Katan in her master's thesis.[113]

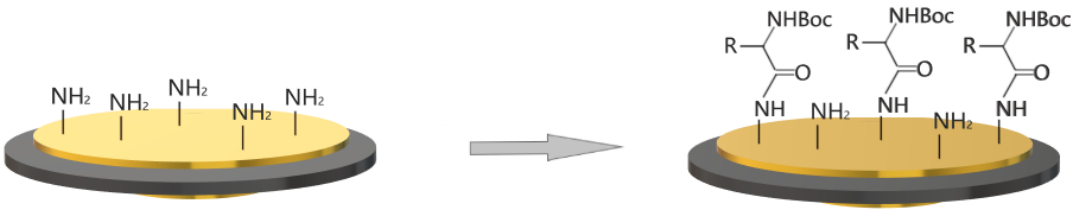
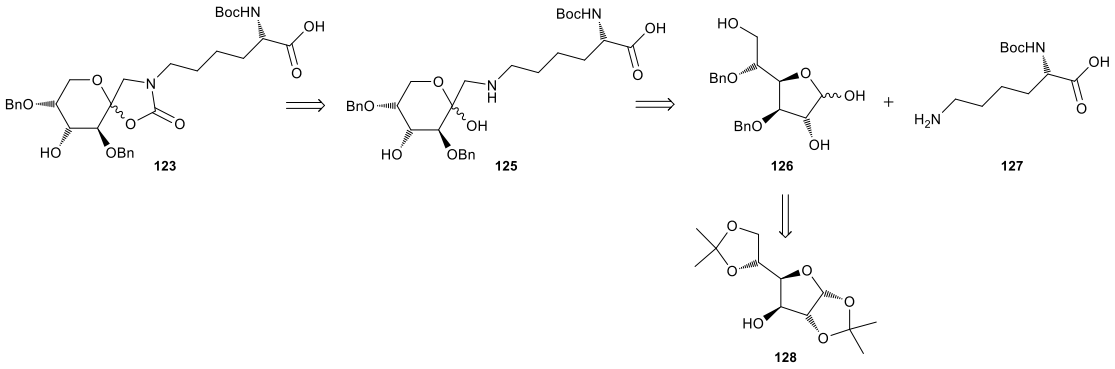


Figure 23: Schematic representation of the coupling of amino acids to chitosan thin-film surfaces. Reprinted from literature.[114]

The requirements are a sufficient amount and purity for a covalent amide coupling to the chitosan as well as an appropriate protecting group strategy for further peptide synthesis on the surface.

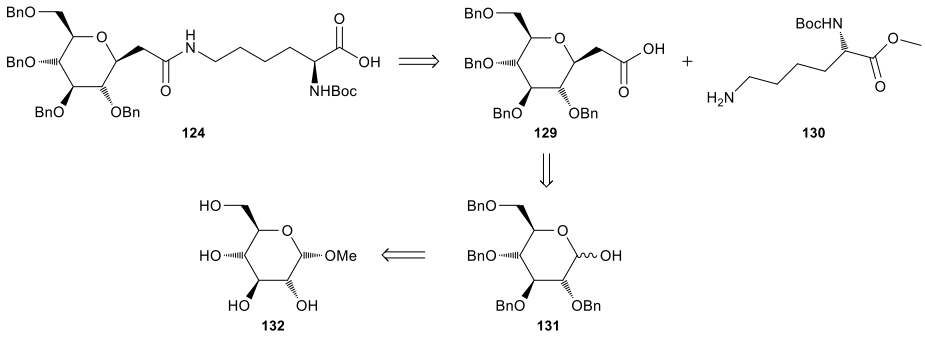
The first target molecule **123**, a *C*-glycosyl type lysine conjugate, can be synthesised by an Amadori rearrangement of key intermediate **126** with Boc-Lys-OH **127**. To reduce the amount of side products formed in the Amadori reaction and to make workup easier by decreasing the polarity of the product, it is desirable to protect most

of the hydroxyl groups of the sugar moiety. The required sugar derivative **126** can be obtained from the readily available 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (**128**) (**Scheme 20**).[115]



Scheme 20: Retrosynthetic analysis of Amadori target molecule **123**.

For the second target molecule **124**, a *C*-glycosidic lysine conjugate, the coupling of amino acid **130** with sugar moiety **129** can be achieved by an amide coupling. Therefore, key intermediate **129** can be synthesised by an HWE-reaction followed by a spontaneous intramolecular Michael addition[116,117] starting from readily available methyl α-D-glucopyranoside (**132**) (**Scheme 21**).

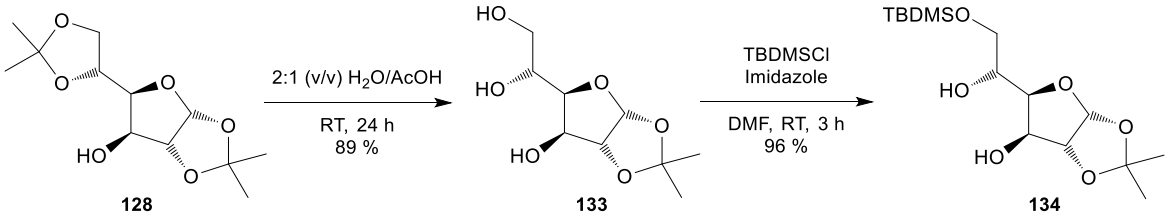


Scheme 21: Retrosynthetic analysis of *C*-glycosyl target molecule **124**.

3 Results and Discussion

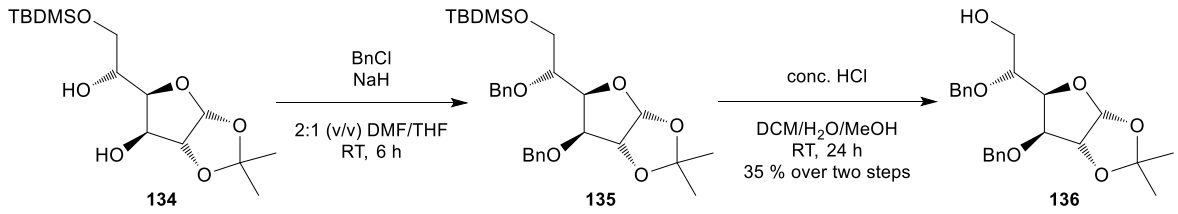
3.1 Amadori Product

For the Amadori rearrangement, the necessary sugar intermediate **126** is prepared starting from commercially available 1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (**128**). Sugar **128** is treated with acetic acid in aqueous solution to regioselectivity remove the terminal isopropylidene group to yield sugar **133**. Subsequent treatment of compound **133** with *tert*-butyldimethylsilylchloride (TBDMSCl) and imidazole as a base gives derivative **134** with the silylated alcohol exclusively at position C-6 (**Scheme 22**). The hydroxyl groups at positions C-3 and C-5 are not getting attacked by the bulky TBDMSCl as these secondary alcohols are sterically more hindered and therefore less reactive than the primary alcohol at position C-6.



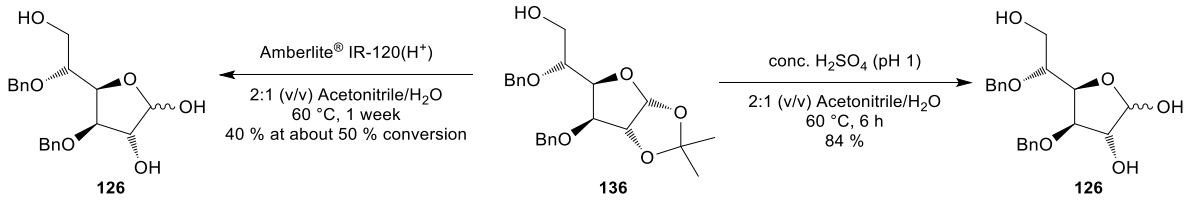
Scheme 22: Synthesis of compounds **133** and **134.**

To protect the hydroxyl groups at positions C-3 and C-5, compound **134** is deprotonated with sodium hydride and subsequently reacted with benzyl chloride. The resulting fully protected sugar derivative **135** is then treated with conc. HCl to remove the silyl protecting group at position O-6 to yield compound **136** (**Scheme 23**).



Scheme 23: Synthesis of compounds **135** and **136**.

The last step before the Amadori rearrangement is the removal of the 1,2-*O*-isopropylidene group. Therefore, sugar **136** is dissolved in a 2:1 (v/v) mixture of acetonitrile and water and acidic ion-exchanger Amberlite® IR-120(H^+) is added. It turns out that under these conditions the reaction proceeds very slowly, taking one week for 50 % conversion at 60 °C. To decrease the reaction time, different reaction conditions are investigated. Therefore, compound **136** is treated with concentrated H_2SO_4 in a 2:1 (v/v) mixture of acetonitrile and water. After 6 hours at 60 °C, TLC indicates completed conversion of the starting material. Neutralisation with $NaHCO_3$ and removal of the solvents under reduced pressure gives compound **126** mixed with salts. The resulting residue is taken up in DCM, whereupon a suspension is formed. The desired product is soluble in DCM thus it can get decanted and separated from the salts. This procedure gives key intermediate **126** in a yield of 84 % (**Scheme 24**). The advantage of using the ion-exchanger procedure would be the quick workup by simple filtration of the ion-exchanger. However, the shorter reaction time makes the sulfuric acid-approach more feasible.

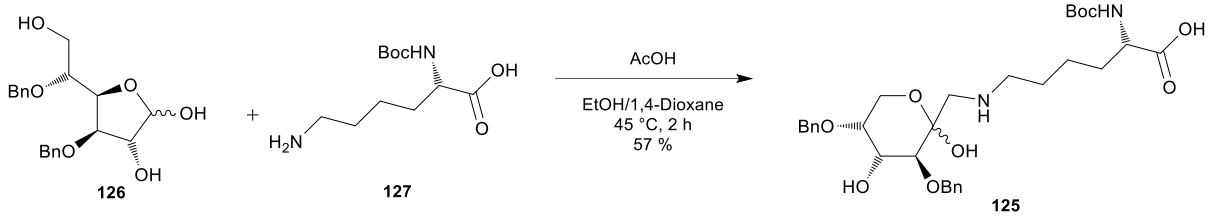


Scheme 24: Synthesis of sugar derivative **126** for use in the Amadori rearrangement.

This protecting group strategy is necessary for several reasons. First, the free hydroxyl group at position C-6 is needed for the formation of the desired pyranoses during the Amadori rearrangement. Second, to prevent formation of undesired furanoses, the hydroxyl group at position C-5 needs to be blocked. Third, the unprotected anomeric centre is mandatory for the Amadori rearrangement. Additionally, the benzyl groups decrease the polarity of the product, making column chromatography more feasible. While the Amadori rearrangement would also take place on a completely unprotected sugar, the resulting mixture of products would lower the yield and make purification more challenging.

With sugar **126** in hand and commercially available protected amino acid Boc-Lys-OH **127**, the Amadori rearrangement can be carried out as shown in **Scheme 25**. As

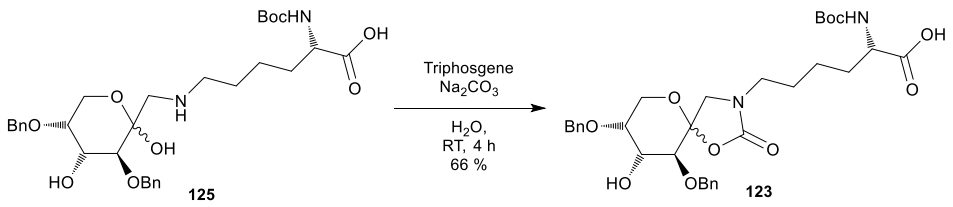
it is known that the formation of side products during the rearrangement strongly depends on the temperature, different reaction conditions are investigated. It is found that temperatures higher than 50 °C produce more side products while temperatures lower than 30 °C increase the reaction time significantly or lead to no reaction at all. Therefore, 45 °C are chosen as the most suitable option.



Scheme 25: Amadori rearrangement of key intermediate **126** with Boc-Lys-OH **127**.

Although product **125** contains a carboxylic acid, column chromatography is feasible. The formed side products are less polar compared to product **125** and can be separated quite easily. The product is isolated as a α/β-mixture. As explained before, due to the protected C-5 hydroxyl group, the formation of furanoses was prevented and only the pyranose α- and β-anomers are possible products.

In contrast to “real” *C*-glycosides, the Amadori products are less stable due to the fact that the rearrangement is reversible and because of the still existing hemiketal moiety, enabling ring opening and anomerisation. To circumvent this issue, the anomeric position of compound **125** is stabilised as a cyclic carbamate. By employing triphosgene, the anomeric hydroxyl group in connection with the ***ε***-nitrogen of the lysine forms an oxazolidinone ring (**Scheme 26**) in product **123**.[118] This also enables separation of the two anomers of glycoconjugate **123** on TLC. Separating the anomers by column chromatography is not possible and as it is not necessary for the further application on the chitosan surface to separate them, they are left as a mixture.



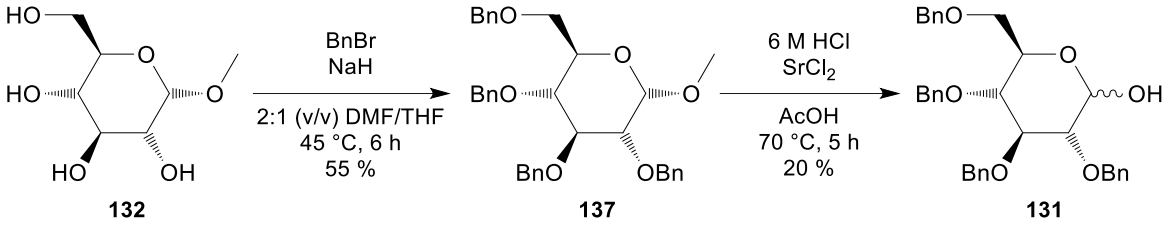
Scheme 26: Stabilisation of Amadori product **125** using triphosgene.

During the workup procedure of this reaction, which includes extraction of the aqueous solution with ethyl acetate, sometimes more polar products appear on the TLC and the yield drops significantly. Although the solution is kept at basic pH the whole time by using an excess of Na_2CO_3 to neutralise the HCl getting formed by the reaction of triphosgene, it appears that the Boc-group has been cleaved. Adding Boc_2O to the solution results in reappearance of the product spots. However, the yield is still lower than usual. It is supposed that triphosgene, which was used in excess and is insoluble in water, reacts with glycoconjugate **123** and cleaves the Boc-group as soon as it gets dissolved in ethyl acetate while extracting. Triphosgene is capable of many different kinds of reactions, leading to unwanted side reactions, for instance with the carboxylic acid or the free hydroxyl group.[119] Filtering off excess triphosgene before extracting the reaction prevents the excessive formation of side products and the loss of yield.

Due to limited solubility of dried target molecule **123** in common solvents, characterisation by NMR turns out to be difficult, which was also discovered for the *N*-Cbz analogue by Wrodnigg and co-workers.[118]

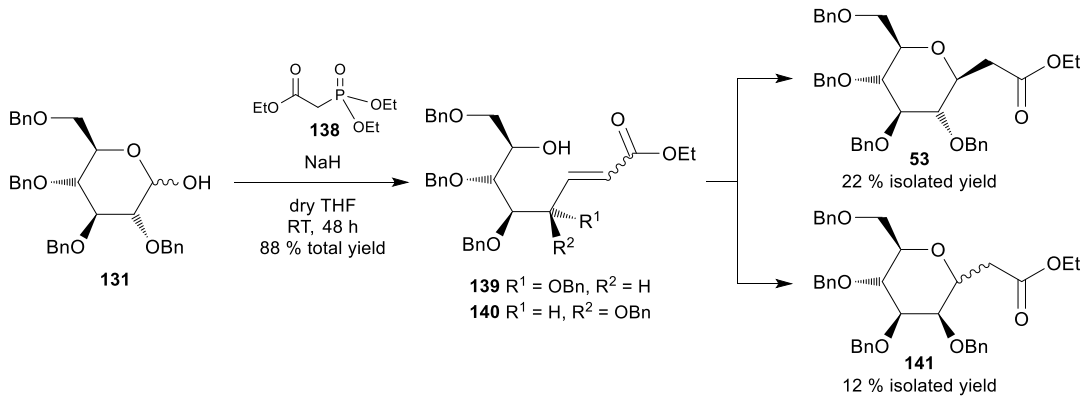
3.2 HWE Product

For the second target molecule **124**, the necessary sugar derivative is synthesized starting from commercially available methyl α-D-glucopyranoside (**132**). Treatment of sugar **132** with benzyl bromide after deprotonation with sodium hydride gives per-*O*-benzylated methyl glucopyranoside **137**. For the HWE-reaction, the anomeric hydroxyl group needs to be deprotected, which is conducted by acidic treatment of *O*-glycoside **137**. Therefore, two different conditions for this cleavage are investigated. For the first approach, methyl glucopyranoside **137** is dissolved in acetic acid and 4 M H_2SO_4 is added.[120] For the second one, educt **137** is also dissolved in acetic acid, but 6 M HCl and 0.1 mol% $SrCl_2$ are added.[121] Both reactions are heated up to 70 °C. The latter approach turns out to be more convenient regarding the workup procedure due to a bigger difference between the polarity of the formed side products compared to the desired sugar **131** (**Scheme 27**).



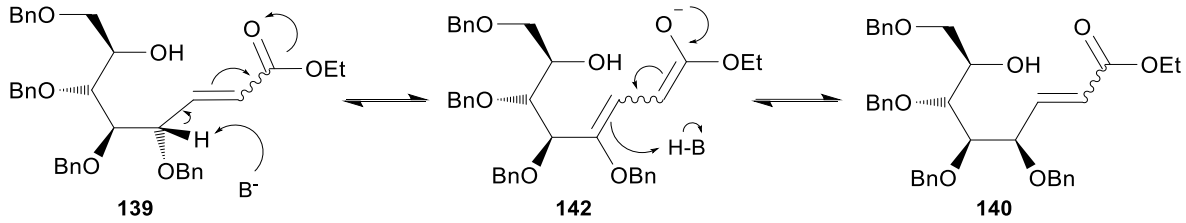
Scheme 27: Synthesis of protected sugar **131**.

The HWE-reaction followed by a spontaneous intramolecular Michael-addition is carried out as shown in **Scheme 28**. First, triethyl phosphonoacetate is deprotonated by an excess of sodium hydride in dry THF. Other bases, such as potassium *tert*-butanolate or sodium methanolate, lead to no reaction in the next step. Tetra-*O*-benzylglucopyranose **131** is dissolved in dry THF and transferred to the previously prepared solution of deprotonated triethyl phosphonoacetate (**138**). After 48 hours, completed conversion of the starting material to main products **53** and **141** is indicated by TLC.



Scheme 28: Horner-Wadsworth-Emmons reaction on compound **131**.

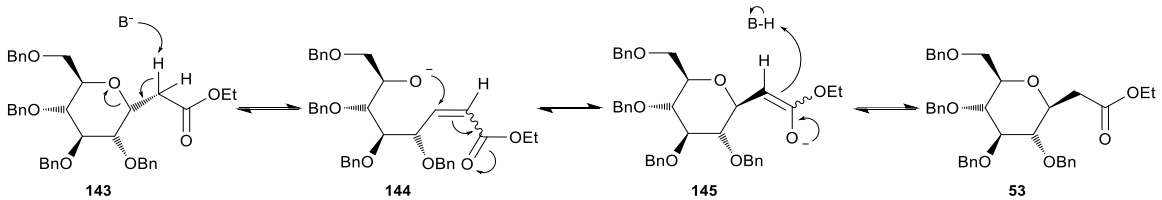
After saponification of ester **53**, which is shown later, XRD-analysis has proven sugar **53** to be the desired "*gluco"*-configured product. Product **141** cannot be crystallised. Comparison with literature indicates the formation of a "*manno"*-configured isomer.[116,117] It is suggested that the isomerisation at the former position C-2 to the "*manno"*-configuration takes place via the open-chain intermediate **142** as illustrated in **Scheme 29**.



Scheme 29: Epimerisation of **139** to the "*manno*"-configuration.

When non-dried THF is used for the HWE-reaction, it does not reach full conversion. Additionally, mainly the open-chain intermediates **139** and **140** are formed, which can be identified by TLC by their distinct purple colour when charred with vanillin/H_2SO_4 and by NMR.

For the "*gluco"*-configured product, only the β-anomer **53** is formed. Allevi and co-workers investigated the behaviour of some *C*-glucopyranosides in basic conditions. It was found that compound **143** isomerises to an α:β ratio of 4:96 in 24 hours through the intermediates **144** and **145** under treatment with sodium hydride in THF while weaker bases had no effect on this system. The proposed mechanism is shown in **Scheme 30**. They also showed that under this reaction conditions, 10 % of the β-"*manno"*-configured product is formed, again indicating that the isomerisation takes place via the open-chain intermediate.[122] We could additionally show that, when the HWE-reaction is stopped before full conversion is reached, NMR-analysis indicates that likely also α-anomer **143** is formed, which isomerises to β-anomer **53**, as the reaction conditions include excess sodium hydride. The isomerisation from the α- to the β-anomer likely takes place because in the β-anomer all substituents are in an equatorial position.



Scheme 30: Proposed mechanism for the isomerisation of **143** to the β-anomer **53** as proposed by Allevi and co-workers.[122]

The next step is the saponification of ester **53** to carboxylic acid **129**, which is necessary for the following amide coupling. This is accomplished by treatment with

potassium hydroxide in a 2:1 (v/v) mixture of 1,4-dioxane and water. Neutralisation with acidic ion exchanger Amberlite® IR-120(H^+) and recrystallization from cyclohexane gives carboxylic acid **129** as colourless needles. They can be analysed by XRD, conforming the desired β-"*gluco"*-configuration of product **129** (**Figure 24**).

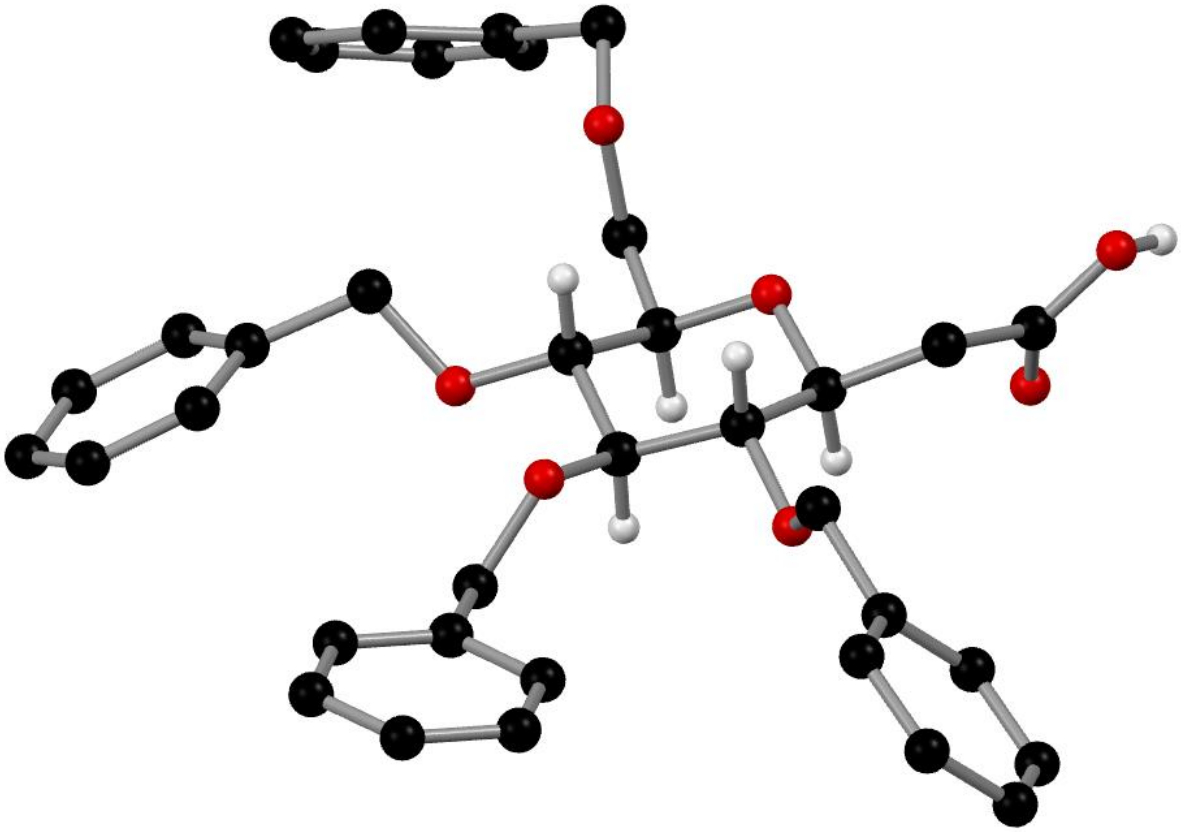
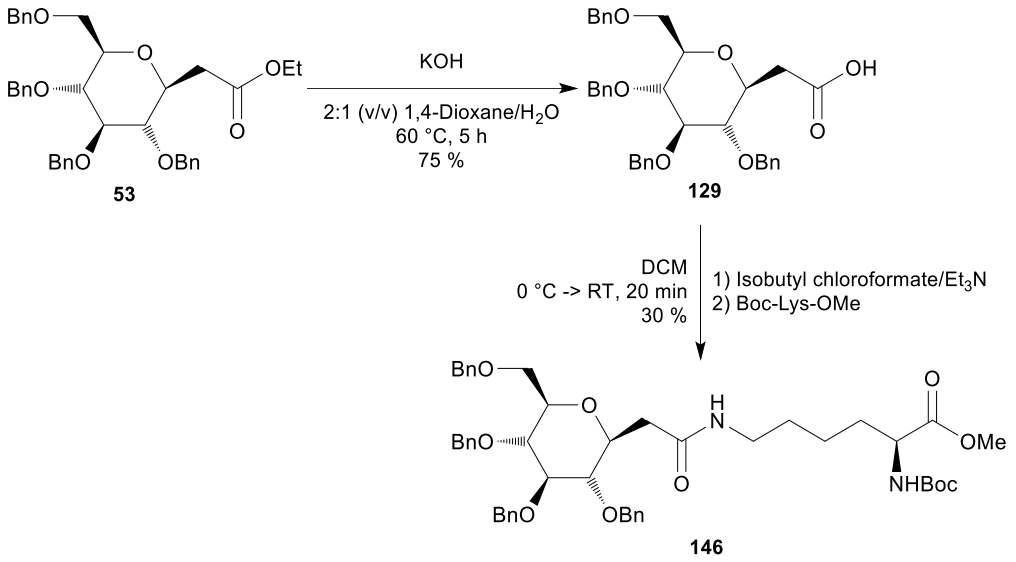


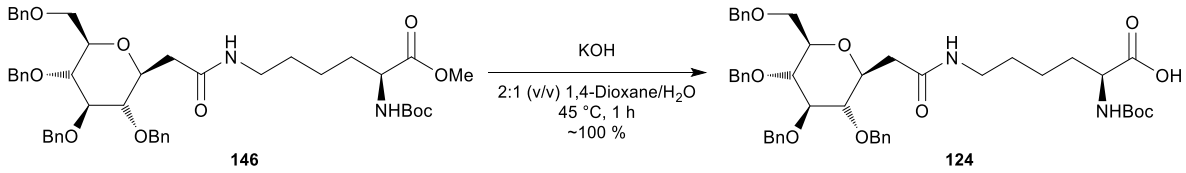
Figure 24: Crystal structure of **129** confirming the β-"*gluco"* configuration. Some hydrogen atoms are hidden for clarity.

For the amide coupling of sugar **129** and Boc-Lys-OMe via the ε-amino group, the mixed anhydride method is employed. Acid **129** is first reacted with isobutyl chloroformate to get the mixed anhydride *in situ* and protected amino acid Boc-Lys-OMe is then added to get glycoconjugate **146** (**Scheme 31**).



Scheme 31: Synthesis of sugar-lysine conjugate **146** via an amide coupling.

As the coupling of target molecule **124** to the chitosan film should also be done by an amide formation, the ester of compound **146** needs to be saponified. This is performed in a 2:1 (v/v) mixture of 1,4-dioxane and water employing potassium hydroxide as a base, followed by neutralisation with acidic ion exchanger Amberlite® IR-120(H^+), yielding target molecule **124** as a colourless solid after recrystallization from CH/EA (**Scheme 32**).



Scheme 32: Saponification of ester **157** to give target molecule **124**.

3.3 Surface Modification

Coupling of the two target molecules of this thesis to chitosan thin-films is under investigation at the time of writing. Preliminary studies of the coupling and following analysis of protected amino acid Boc-Gly-OH to the surfaces by Katan[113] gave promising results. An increased contact angle could be detected by water contact angle measurements as well as an increased mass by QCM-D, both indicating surface bound amino acids (**Figure 25**).

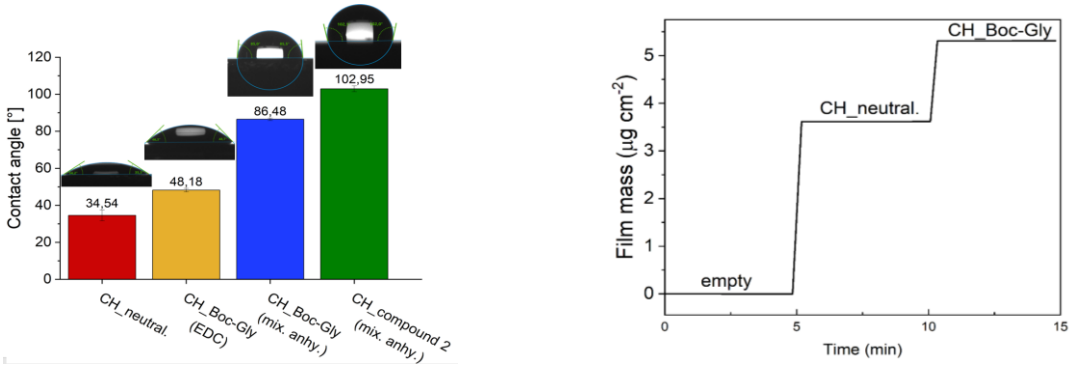


Figure 25: Water contact angle and QCM-D measurements of Boc-Gly coupled to a chitosan thin-film. Reprinted from literature.[114]

Additionally, dansylated lysine **147** synthesised in the line of this master's thesis was coupled to the surface and investigated by fluorescence microscopy, which visually confirmed the presence of amino acid **147** (**Figure 26**).

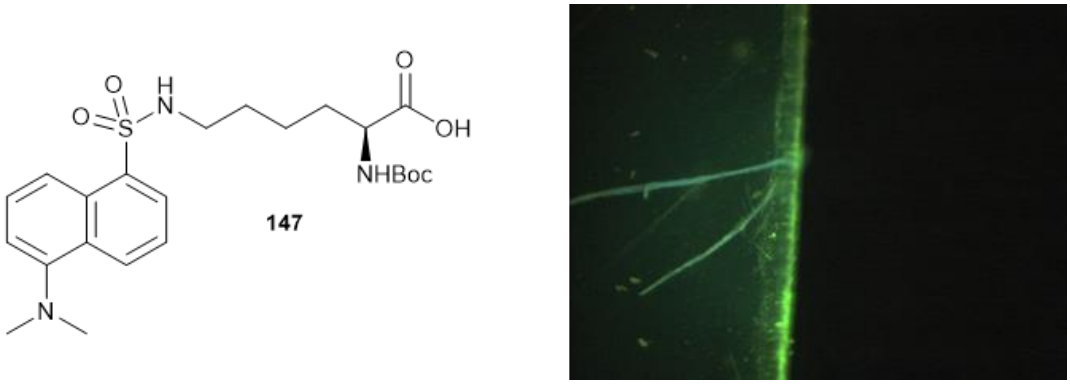


Figure 26: Structure of amino acid **147** and fluorescence microscopy image of **147** coupled to a chitosan thin-film. Adapted from literature.[114]

4 Conclusion and Outlook

Both target molecules **123** and **124** could be synthesised in sufficient amount and purity.

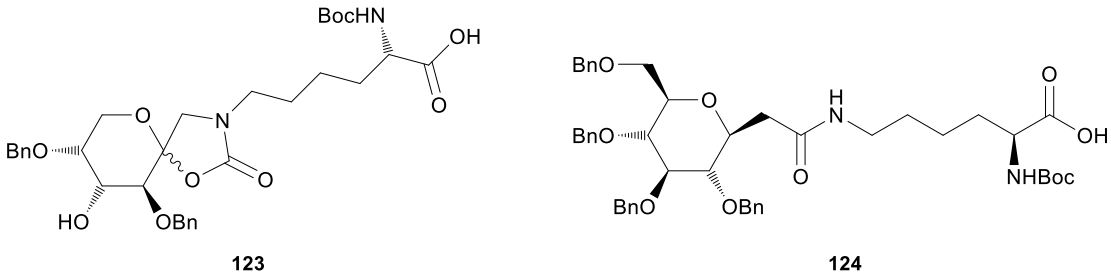
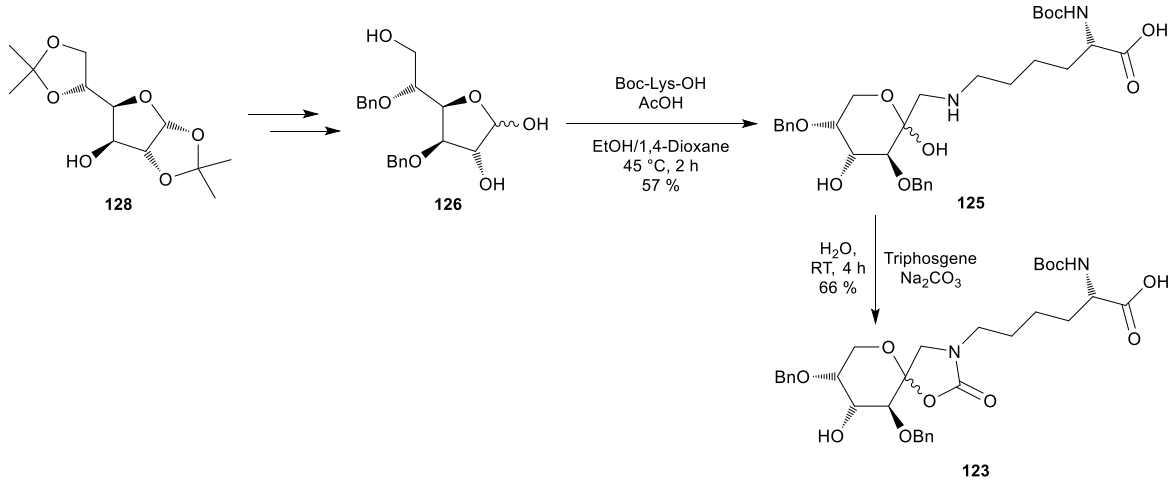


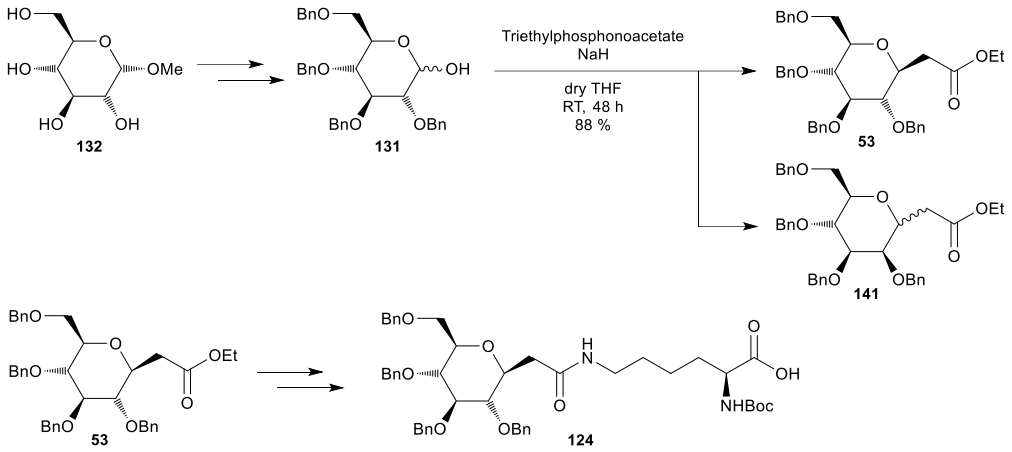
Figure 27: Target molecules of this thesis.

For the first target molecule **123**, the synthesis of the required protected sugar moiety **126** was straightforward by literature known protecting group manipulations starting from commercially available sugar **128**. The key-step in this reaction sequence, the Amadori rearrangement to glycoconjugate **125**, could be optimised to give a yield of 57 % after two hours. The workup procedure for the carbamate formation to target molecule **123** using triphosgene could be improved after an unexpected formation of side products during the workup was discovered. Characterisation appeared to be challenging, as the product was not soluble in common solvents.



Scheme 33: Synthesis of target molecule **123**.

For the second target molecule **124**, the HWE-reaction gave key intermediate **53** in a good yield starting from sugar **132**, although more than expected "*manno*"-configured product **141** was formed. Further formation of the target molecule **124** by saponification and amide coupling with Boc-Lys-OMe was straightforward, although the amide coupling can still be improved. The yields for the coupling were generally low and other reaction conditions or coupling methods could be tried out.



Scheme 34: Synthesis of target molecule **124**.

Preliminary studies of the surface modification gave promising results. In the future, coupling of the target molecules to thin film chitosan surfaces will be further investigated. Optimisation of this surface modification and selective cleavage of the amino acid protecting groups enables the possibility to grow peptides on the surface,[123] which creates the ability to specifically modify the properties and make it suitable for medical applications like implants. There is also the possibility to alter the sugar moiety, clearing the way to a variety of different glycosylated surfaces and glycoproteins.

5 Experimental Section

5.1 General Methods

5.1.1 Thin layer chromatography (TLC)

Analytical TLC was performed on precoated aluminium plates with silica gel 60 and the fluorescence indicator F_{254} (Supelco 1.05554). Detection was by UV light (254 nm) and staining with the solutions mentioned below.

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 (100 g) 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).

5.1.2 Column chromatography

Column chromatography was performed on silica gel 60 (0.04 – 0.063 mm, Macherey-Nagel, 815380.25). The used solvent mixture is given with the product.

5.1.3 NMR

NMR spectra were recorded on a Bruker Ultrashield spectrometer at 300.36 MHz for ^{1}H spectra and 75.53 MHz for ^{13}C. The used deuterated solvent is given with the NMR-data. Chemical shifts are listed in delta (δ), using the residual protons of the non-deuterated solvent as reference. Coupling constants J are given in Hertz (Hz). Structures of crucial intermediates have been unambiguously assigned by APT, COSY and HSQC spectroscopy.

Abbreviations:

s	singlet	**d**	doublet
bs	broad singlet	**dd**	double doublet
t	triplet	**ddd**	double double doublet
m	multiplet	**b.m.**	broad multiplet

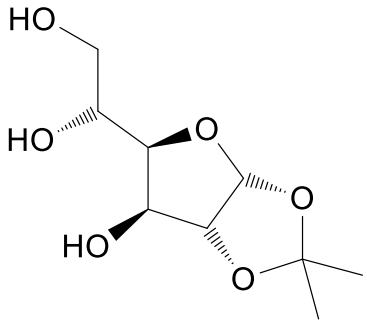
5.1.4 Polarimeter

Optical rotations $[\alpha]_D^{20}$ were measured at 20 °C on a Perkin Elmer 341 polarimeter at a wave length of 589 nm and a path length of 10 cm.

5.2 Procedures

1,2-*O*-Isopropylidene-α-D-glucofuranose (133)[115]

1,2;5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**128**) (10 g, 38.4 mmol, 1 eq.) is dissolved in a mixture of 2:1 (v/v) water and acetic acid (300 ml) and stirred at RT for 24 hours. After TLC shows completed conversion of the starting material, the solvents are evaporated under reduced pressure. Column chromatography gives product **133** (7.53 g, 34.2 mmol, 89 %) as a colourless oil. It is immediately used for the next synthetic step.

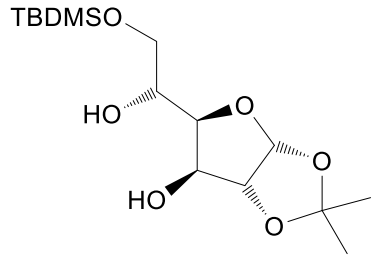


$C_9H_{16}O_6$
MW = 220.22 g/mol
TLC: CAM
R_f = 0.25 (EA)
Column chromatography: DCM → EA → MeOH

6-*O*-*tert*-Butyldimethylsilyl-1,2-*O*-isopropylidene-α-D-glucofuranose (134)[115]

Compound **133** (10 g, 45.4 mmol, 1 eq.) is dissolved in DMF. Imidazole (7.3 g, 113 mmol, 2.5 eq.) and TBDMSCl (8.2 g, 54.4 mmol, 1.2 eq.) are subsequently added.

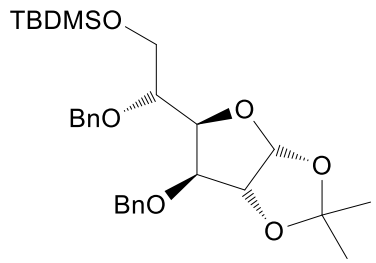
The colourless solution is stirred at RT for 3 hours. After TLC shows completed conversion of the starting material, 20 ml MeOH are added and the solution is stirred for further 15 minutes. Next, the solvents are removed under reduced pressure. The resulting residue is taken up in DCM and washed with 2 M HCl and sat. $NaHCO_3$ solution. The combined organic layers are dried over Na_2SO_4, filtered off and concentrated under reduced pressure. This gives crude product **134** (29.1 g, 86.9 mmol, 96 %) as a pale-yellow oil. It is immediately used for the next synthetic step.



$C_{15}H_{30}O_6Si$
MW = 334.48 g/mol
TLC: UV, CAM
R_f = 0.35 (CH/EA 2/1 v/v)

3,4-Di-*O*-*benzyl*-6-*O*-*tert*-butyldimethylsilyl-1,2-*O*-isopropylidene-α-D-glucofuranose (135)[115]

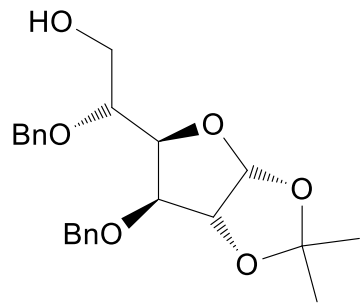
Compound **134** (29.1 g, 90.8 mmol, 1 eq.) is dissolved in 550 ml of a 10:1 (v/v) mixture of DMF and THF. The solution is cooled with an ice bath and sodium hydride (11.2 g, 281 mmol, 3 eq., 60 w% in mineral oil) is slowly added. After 15 minutes, benzyl chloride (29.5 ml, 256 mmol, 2.8 eq.) is slowly added. The round-bottom flask is equipped with a drying tube filled with anhydrous $CaCl_2$ and the ice bath is removed. The reaction is allowed to stir for 6 hours at RT. After TLC shows completed conversion of the starting material, 20 ml MeOH are added and the yellow solution is stirred for further 15 minutes. Next, the solvents are removed under reduced pressure. The resulting orange residue is taken up in DCM and consecutively washed with 2 M HCl and sat. $NaHCO_3$ solution. The combined organic layers are dried over Na_2SO_4, filtered off and evaporated under reduced pressure to give crude product **135** as an orange oil. It is immediately used for the next synthetic step.



$C_{29}H_{42}O_6Si$
MW = 514.73 g/mol
TLC: UV, CAM
R_f = 0.26 (CH/EA 3/1 v/v)

3,4-Di-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose (136)

Crude compound **135** is dissolved in 400 ml DCM and 50 ml water are added. MeOH is added until a homogenous solution is achieved. Concentrated HCl is added until the pH is adjusted to one. The yellow solution is stirred for 24 hours at room temperature. After TLC shows completed conversion of the starting material, DCM is added until the organic and aqueous layers separate. The layers are separated and the aqueous layer is washed with DCM. The combined organic layers are washed with sat. $NaHCO_3$ solution, dried over Na_2SO_4, filtered off and the solvents are evaporated under reduced pressure to yield a yellow oil. Silica gel chromatography gives product **136** (12.7 g, 31.7 mmol, 35 % over two steps) as a colourless oil.



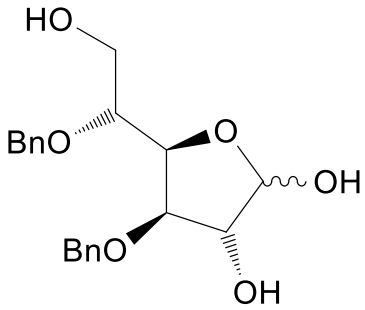
$C_{23}H_{28}O_6$
MW = 400.47 g/mol
TLC: UV, CAM
R_f = 0.43 (CH/EA 2/1 v/v)
Column chromatography: CH/EA 20:1 → 8:1 → 3:1 → 1:2

NMR data identical to literature.[124,125]

3,4-Di-*O*-benzyl-D-glucofuranose (126)

Compound **136** (1 g, 2.5 mmol, 1 eq.) is dissolved in a 2:1 (v/v) mixture of acetonitrile and water. A few drops of conc. H_2SO_4 are added until pH = 1 is adjusted. The colourless solution is stirred for 6 hours at 60 °C. After TLC shows completed conversion of the starting material, the pale-yellow solution is allowed to cool to RT. $NaHCO_3$ is slowly added to neutralise the solution. When a pH of seven is adjusted,

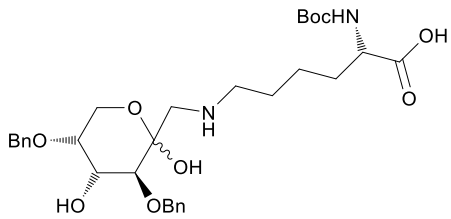
the solvents are removed under reduced pressure. The resulting residue is suspended in DCM and the solvent is separated from the colourless solid by decanting. The organic layer is evaporated to dryness under reduced pressure. Column chromatography gives product **126** (0.75 g, 2.1 mmol, 84 %) as a colourless oil. It is immediately used for the next synthetic step.



$C_{20}H_{24}O_6$
MW = 360.41 g/mol
TLC: UV, CAM
R_f = 0.46 (EA)
Column Chromatography: CH/EA 2:1 → 1:1 → EA

1-(*N*-(*5S*-*tert*-Butyloxycarbonyl)amino-6-carboxypentyl)amino-3,5-di-*O*-benzyl-1-deoxy-D-fructopyranose (125)

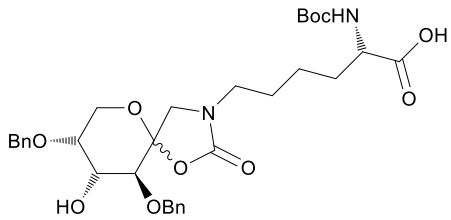
Compound **126** (220 mg, 0.61 mmol, 1 eq.) is dissolved in EtOH and 10 drops of 1,4-dioxane are added. Next, Boc-Lys-OH (150 mg, 0.61 mmol, 1 eq.) and AcOH (35 µl, 0.61 mmol, 1 eq.) are consecutively added, which gives a colourless suspension. The reaction is stirred at 45 °C. After a few minutes, the suspension starts to turn pale yellow and clears up. After 2 hours TLC shows completed conversion of the starting material. The solvents are removed under reduced pressure. Column chromatography of the resulting residue gives product **125** (205 mg, 0.35 mmol, 57 %) as a pale-yellow oil. It is immediately used for the next synthetic step.



$C_{31}H_{44}N_2O_9$
MW = 588.70 g/mol
TLC: UV, CAM, NIP
R_f = 0.59 ($CHCl_3$/MeOH/NH_3 3/1/1% v/v/v%)
Column chromatography: $CHCl_3$/MeOH/NH_3 3/1/1% (v/v/v%)

1-(*N*-(*5S*-*tert*-Butyloxycarbonyl)amino-6-carboxypentyl)-amino-3,5-di-*O*-benzyl-1-*N*,2-*O*-carbonyl-1-deoxy-D-fructopyranose (123)

Compound **125** (205 mg, 0.35 mmol, 1 eq.) is suspended in water and ten drops of 1,4-dioxane are added. Na_2CO_3 (554 mg, 5.2 mmol, 15 eq.) is added, resulting in a pale-yellow solution. The solution is cooled with an ice bath. Next, triphosgene (165 mg, 0.56 mmol, 1.6 eq.) is added. The suspension is allowed to come to RT and stirred for 4 hours. After TLC shows completed conversion of the starting material, the solution is filtered to remove excess triphosgene and then washed with EA. The organic layer is separated, dried over Na_2SO_4, filtered and the solvents are removed under reduced pressure. Column chromatography gives an α/β-mixture of product **123** in favour of the β-anomer (142 mg, 0.23 mmol, 66 %).



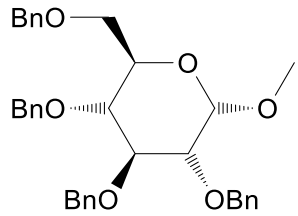
$C_{32}H_{42}N_2O_{10}$
MW = 614.69 g/mol
TLC: UV, CAM, NIP
R_f = 0.53 ($CHCl_3$/MeOH/NH_3 2/1/1% v/v/v%)
Column chromatography: $CHCl_3$/MeOH/NH_3 3/1/1% (v/v/v%)

^{1}H-NMR (300 MHz, CD_3OD): Signals were found in the expected region.
^{13}C-NMR (75 MHz, CD_3OD) δ in ppm for the β-anomer: 179.4 (C-6'), 158.4, 157.9 (2C, 2x *C*OON), 139.7 (2C, Ph-ipso), 129.6 – 128.6 (10C, benzyl), 104.4 (C-2), 80.3 (Boc-C_q), 78.9, 78.6 (2C, C-3, C-4), 76.2, 73.0 (2C, PhCH_2), 72.4 (C-5), 63.8 (C-6), 56.1 (C-5'), 53.8 (C-1), 44.3 (C-1'), 33.0 (C-4'), 28.8 (3C, Boc-CH_3), 27.8 (C-2'), 23.8 (C-9').

Methyl 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranoside (137)

Methyl α-D-glucopyranoside (**132**) (10 g, 52 mmol, 1 eq.) is suspended in a mixture of 2:1 (v/v) DMF/THF and cooled with an ice bath. Sodium hydride (9.9 g, 247 mmol, 4.8 eq., 60 w% in mineral oil) is slowly added. The suspension is stirred for 15 minutes. Next, benzyl bromide (29 ml, 242 mmol, 4.7 eq.) is slowly added. The reaction is stirred at 45 °C for 6 hours. After TLC indicates completed conversion of the starting material, the reaction is quenched with MeOH and the solvents are removed under reduced pressure. The resulting yellow-orange residue is taken up

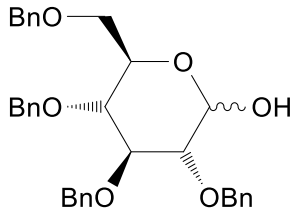
in DCM and consecutively washed with 2 M HCl and sat. $NaHCO_3$ solution. The combined organic layers are dried over Na_2SO_4, filtered and the solvents are removed under reduced pressure. Column chromatography gives product **137** (16 g, 29 mmol, 55 %) as a pale-yellow oil.



$C_{35}H_{38}O_6$
MW = 554.68 g/mol
TLC: UV, CAM
R_f = 0.43 (CH/EA 4:1 v/v)
Column chromatography: CH/EA (v/v) 15:1 → 10:1 → 5:1

2,3,4,6-Tetra-*O*-benzyl-D-glucopyranose (131)[121]

Compound **137** (3 g, 5.4 mmol, 1 eq.) is dissolved in AcOH (15 ml) and $SrCl_2$*6 H_2O (144 mg, 0.54 mmol, 0.1 eq.) is added. Next, 6 M HCl (2.2 ml) is added and the reaction is stirred at 70 °C for 5 hours. After TLC shows completed conversion of the starting material, the solution is cooled to RT. Ice water is added and the product is extracted with DCM. The organic layer is consecutively washed with sat. $NaHCO_3$ solution and sat. NaCl solution, dried over Na_2SO_4, filtered and the solvents are removed under reduced pressure. Column chromatography gives product **131** (612 mg, 1.1 mmol, 20 %) as a colourless solid.



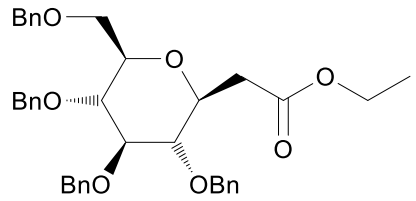
$C_{34}H_{36}O_6$
MW = 540.66 g/mol
TLC: UV, CAM
R_f = 0.58 (CH/EA 2/1 v/v)
Column chromatography: CH/EA (v/v) 15/1 → 10/1 → 3/1 → 1/1

NMR data identical to literature[126].

Ethyl 3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-2-deoxy-D-*glycero*-D-*gulo*-octulonate (53)[121]

Ethyl 3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-2-deoxy-D-*glycero*-D-*galacto*-octulonate (141)[121]

Triethyl phosphonoacetate (**138**) (9.2 ml, 46 mmol, 5 eq.) is dissolved in dry THF. The colourless solution is cooled with an ice bath. Sodium hydride (1.85 g, 46 mmol, 5 eq., 60 w% in mineral oil) is slowly added. The round-bottom flask is equipped with a drying tube filled with anhydrous $CaCl_2$ and the ice bath is removed. The solution is stirred for 5 hours. Next, compound **131** (5 g, 9.2 mmol, 1 eq.) is dissolved in dry THF and added to the previously prepared solution. The reaction is stirred for 48 hours at RT. After TLC shows completed conversion of the starting material, the solvents are removed under reduced pressure. The resulting orange residue is taken up in DCM and consecutively washed with 2 M HCl and sat. $NaHCO_3$ solution. The combined organic layers are dried over Na_2SO_4, filtered and the solvents are removed under reduced pressure. Column chromatography gives products **53** (1.25 g, 2.0 mmol, 22 %), **141** (0.69 g, 1.1 mmol, 12 %) and a mixture of **53** and **141** (3.11 g, 5.0 mmol, 54 %) as colourless oils.



Compound 53

$C_{38}H_{42}O_7$

MW = 610.75 g/mol

TLC: UV, CAM, VAN

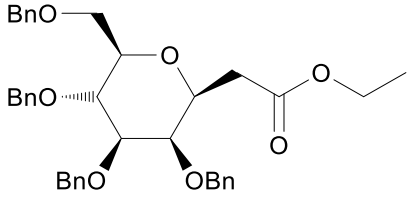
R_f = 0.80 (CH/EA 2/1 v/v)

Column chromatography: CH/EA (v/v) 10/1 → 8/1

$[\alpha]_D^{20}$ = - 0.58 (c = 1.01, $CHCl_3$)

^{1}H-NMR (300 MHz, $CDCl_3$) δ in ppm: 7.31 – 7.05 (m, 20H, aromatic), 4.89 – 4.70 (m, 4H, PhC*H*$_2$), 4.59 – 4.39 (m, 4H, PhC*H*$_2$), 4.00 (q, $J_{1',2'}$ 7.1 Hz, 2H, -OC*H*$_2$CH$_3$), 3.73 – 3.54 (m, 5H, H-3, H-5, H-6, 2 x H-8), 3.38 (dt, $J_{6,7}$ 9.1, $J_{7,8}$ 3.1 Hz, 1H, H-7), 3.29 (dd, $J_{3,4}$ = $J_{4,5}$ 9.0 Hz, 1H, H-4), 2.66 (dd, $J_{2a,2b}$ 15.3, $J_{2a,3}$ 3.7 Hz, 1H, H-2a), 2.39 (dd, $J_{2a,2b}$ 15.3, $J_{2b,3}$ 8.3 Hz, 1H, H-2b), 1.12 (t, $J_{1',2'}$ 7.1 Hz, 3H, -OCH$_2$C*H*$_3$)

^{13}C-NMR (75 MHz, $CDCl_3$) δ in ppm: 171.1 (C-1), 138.6, 138.3, 138.2, 138.1 (4C, Ph-ipso), 128.7 – 127.6 (20C, benzyl), 87.3 (C-5), 81.4 (C-4), 79.3 (C-7), 78.6 (C-6), 76.1 (C-3), 75.7, 75.2, 75.1, 73.5 (4C, PhCH$_2$), 68.8 (C-8), 60.6 (-OCH$_2$CH$_3$), 37.7 (C-2), 14.3 (-OCH$_2$CH$_3$).



Compound 141

$C_{38}H_{42}O_7$

MW = 610.75 g/mol

TLC: UV, CAM, VAN

R_f = 0.74 (CH/EA 2/1 v/v)

Column chromatography: CH/EA (v/v) 10/1 → 8/1

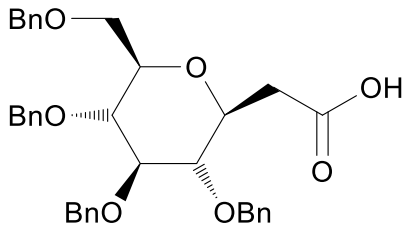
$[\alpha]_D^{20}$ = + 5.54 (c = 0.99, $CHCl_3$)

^{1}H-NMR (300 MHz, $CDCl_3$) δ in ppm: 7.39 – 7.04 (m, 20H, aromatic), 4.99 – 4.39 (m, 8H, PhC*H*$_2$), 4.04 – 3.91 (m, 2H, -OC*H*$_2$CH$_3$), 3.88 – 3.77 (m, 2H, H-4, H-7), 3.71 (t, *J* 6.7 Hz, H-3), 3.68 – 3.55 (m, 3H, 2 x H-8, H-5/6), 3.44 – 3.36 (m, 1H, H-5/6), 2.63 (dd, $J_{2a,2b}$ 16.3, $J_{2a,3}$ 6.1 Hz, 1H, H-2a), 2.51 (dd, $J_{2a,2b}$ 16.3, $J_{2b,3}$ 7.3 Hz, 1H, H-2b), 1.13 (t, $J_{1',2'}$ 7.1 Hz, 3H, -OCH$_2$C*H*$_3$).

^{13}C-NMR (75 MHz, $CDCl_3$) δ in ppm: 171.3 (C-1), 138.6, 138.5, 138.4 (4C, Ph-ipso), 128.6 – 127.5 (20C, benzyl), 85.1, 79.9 (2C, C-5, C-6), 75.3, 75.1, 74.8, 74.7, 74.5, 73.5, 72.7 (7C, 4 x Ph*C*H$_2$, C-3, C-4, C-7), 69.6 (C-8), 60.6 (C-1'), 36.3 (C-2), 14.3 (C-2').

3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-2-deoxy-D-*glycero*-D-*gulo*-octuloic acid (129)

Compound **53** (1.3 g, 2.0 mmol, 1 eq.) is dissolved in 1,4-dioxane (60 ml) and KOH (1 g, 18 mmol, 9 eq.) dissolved in water (30 ml) is added. The reaction is stirred at 60 °C for 5 hours. After TLC shows completed conversion of the starting material, the solution is allowed to cool to RT and neutralised with the acidic ion-exchanger Amberlite® IR-120(H^+). The ion exchanger is filtered off and the solvents are removed under reduced pressure. Recrystallisation from CH gives product **129** (0.89 g, 1.5 mmol, 75 %) as a colourless solid.



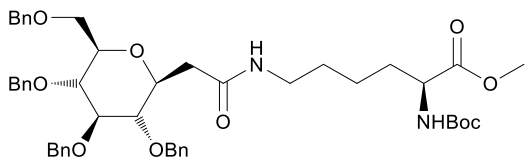
$C_{36}H_{38}O_7$
MW = 582.69 g/mol
TLC: UV, CAM, VAN
R_f = 0.37 (CH/EA 1/1 v/v + three drops AcOH)
m.p. = 102 - 104 °C
$[\alpha]_D^{20}$ = + 3.92 (c = 1.00, $CHCl_3$)

^{1}H-NMR (300 MHz, $CDCl_3$) δ in ppm: 7.36 – 7.12 (m, 20H, aromatic), 4.96 – 4.77 (m, 4H, Ph-C*H*$_2$), 4.67 – 4.45 (m, 4H, Ph-C*H*$_2$), 3.78 – 3.61 (m, 5H, H-3, H-5, H-6, 2 x H-8), 3.50 – 3.43 (m, 1H, H-7), 3.36 (dd, $J_{3,4}$ = $J_{4,5}$ 9.0 Hz, 1H, H-4), 2.75 (dd, $J_{2a,2b}$ 15.7, $J_{2a,3}$ 3.4 Hz, 1H, H-2a), 2.48 (dd, $J_{2a,2b}$ 15.8, $J_{2b,3}$ 8.2 Hz, 1H, H-2b).

^{13}C-NMR (75 MHz, $CDCl_3$) δ in ppm: 176.1 (C-1), 138.5, 138.1, 138.1, 137.9 (4C, Ph-ipso), 128.7 – 127.6 (20C, benzyl), 87.2 (C-5), 81.0 (C-4), 79.1 (C-7), 78.4 (C-6), 75.7, 75.7, 75.3, 75.1, 73.5 (5C, 4 x Ph*C*H$_2$, C-3), 68.6 (C-8), 37.3 (C-2).

N-(*5S*-(*tert*-Butyloxycarbonyl)amino-6-methoxycarboxypentyl)-3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-2-deoxy-D-*glycero*-D-*gulo*-octonamide (146)

Compound **129** (600 mg, 1 mmol, 1 eq.) is dissolved in DCM and cooled with an ice bath. Triethylamine (0.71 ml, 5.1 mmol, 5 eq.) and isobutyl chloroformate (0.16 ml, 1.2 mmol, 1.2 eq.) are subsequently added and the solution is stirred for 10 minutes. Next, Boc-Lys-OMe acetate (0.46 g, 1.4 mmol, 1.4 eq.) dissolved in DCM is added to the previously prepared solution. The ice bath is removed and the reaction is stirred for 30 minutes at RT. After TLC shows completed conversion of the starting material, the solution is consecutively washed with 2 M HCl and sat. $NaHCO_3$ solution. The combined organic layers are dried over Na_2SO_4, filtered and the solvents are removed under reduced pressure. Column chromatography gives product **146** (250 mg, 0.3 mmol, 30 %) as a colourless solid.



$C_{48}H_{60}N_2O_{10}$
MW = 825.01 g/mol
TLC: UV, CAM, VAN, NIP
R_f = 0.43 (CH/EA 1/1 v/v)
Column chromatography: CH/EA (v/v) 4/1 → 3/1 → 2/1
m.p. = 87 - 90 °C
$[\alpha]_D^{20}$ = + 15.1 (c = 1.02, $CHCl_3$)

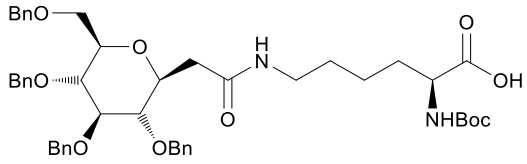
^{1}H-NMR (300 MHz, $CDCl_3$) δ in ppm: 7.38 – 7.04 (m, 20H, aromatic), 6.62 (t, *J* 5.1 Hz, amide-N*H*), 4.99 (d, *J* 8.2 Hz, 1H, carbamate-N*H*), 4.88 – 4.35 (m, 8H, PhC*H*$_2$), 4.15 (dd, *J* 12.1, *J* 7.1 Hz, 1H, H-5'), 3.70 – 3.57 (m, 5H, -OC*H*$_3$, H-5, H-8a), 3.57 – 3.37 (m, 4H, H-3, H-6, H-7, H-8b), 3.23 (dd, $J_{3,4}$ = $J_{4,5}$ 9.2 Hz, 1H, H-4), 3.08 – 2.84 (m, 2H, 2 x H-1'), 2.63 (dd, $J_{2a,2b}$ 15.4, $J_{2a,3}$ 2.3 Hz, 1H, H-2a), 2.32 (dd, $J_{2a,2b}$ 15.5, $J_{2b,3}$ 8.3 Hz, 1H, H-2b), 1.68 – 1.40 (m, 2H, 2 x H-4'), 1.36 (s, 9H, 3 x Boc-C*H*$_3$), 1.28 – 1.07 (m, 4H, 2 x H-2', 2 x H-3').

^{13}C-NMR (75 MHz, $CDCl_3$) δ in ppm: 173.4, 170.6 (2C, C-1, C-6'), 155.6 (Boc-*C*OON), 138.4, 138.0, 137.9, 137.9 (4C, Ph-ipso), 128.7 – 127.8 (20 C, benzyl), 86.9 (C-5), 81.3 (C-4), 80.0 (Boc-*C*$_q$), 78.4 (2C, C-6, C-7), 76.1 (C-3), 75.8, 75.4, 75.2, 73.5 (4C, Ph*C*H$_2$), 69.3 (C-8), 53.4 (C-5'), 52.3 (-O*Me*), 39.0, 38.8 (2C, C-1', C-2), 32.3 (C-4'), 29.2 (C-2'), 28.5 (3C, Boc-*C*H$_3$), 22.7 (C-3').

N-(*5S*-(*tert*-Butyloxycarbonyl)amino-6-carboxypentyl)-3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-2-deoxy-D-*glycero*-D-*gulo*-octonamide (124)

Compound **146** (250 mg, 0.3 mmol, 1 eq.) is dissolved in 1,4-dioxane (20 ml) and KOH (170 mg, 3 mmol, 10 eq.) dissolved in water (10 ml) is added. The solution is stirred at 45 °C for 1 hour. After TLC shows completed conversion of the starting

material, the 1,4-dioxane is removed under reduced pressure. The aqueous solution is neutralised with 1 M HCl and then extracted with EA. The combined organic layers are dried over Na_2SO_4, filtered and the solvents are removed under reduced pressure. Recrystallisation from EA/CH gives product **124** (266 mg, 0.3 mmol, 100 %) as a colourless solid.



$C_{47}H_{58}N_2O_{10}$
MW = 810.99 g/mol
TLC: UV, CAM, VAN, NIP
R_f = 0.10 (CH/EA 1/1 v/v + three drops AcOH v/v)
m.p. = 130 - 131 °C
$[\alpha]_D^{20}$ = + 35.7 (c = 0.975, $CHCl_3$)

^{1}H-NMR (300 MHz, $CDCl_3$) δ in ppm: 7.36 – 7.06 (m, 20H, aromatic), 6.76 (t, *J* 5.9 Hz, amide-N*H*), 5.21 (d, *J* 7.5 Hz, 1H, carbamate-N*H*), 4.90 – 4.34 (m, 8H, PhC*H*$_2$), 4.16 (dd, *J* 12.9, *J* 7.4 Hz, 1H, H-5'), 3.69 – 3.58 (m, 2H, H-5, H-8a), 3.57 – 3.45 (m, 3H, H-3, H-6, H-8b), 3.44 – 3.36 (m, 1H, H-7), 3.24 (dd, $J_{3,4}$ = $J_{4,5}$ 9.2 Hz, 1H, H-4), 3.13 – 2.84 (m, 2H, 2 x H-1'), 2.65 (dd, $J_{2a,2b}$ 15.4, $J_{2a,3}$ 2.1 Hz, 1H, H-2a), 2.38 (dd, $J_{2a,2b}$ 15.4, $J_{2b,3}$ 7.1 Hz, 1H, H-2b), 1.74 – 1.44 (m, 2H, 2 x H-4'), 1.35 (s, 9H, 3 x Boc-C*H*$_3$), 1.26 – 1.05 (m, 4H, 2 x H-2', 2 x H-3').

^{13}C-NMR (75 MHz, $CDCl_3$) δ in ppm: 174.9, 171.1 (2C, C-1, C-6'), 155.7 (Boc-*C*OON), 137.9, 137.9, 137.7 (4C, Ph-ipso), 128.7 – 127.9 (20C, benzyl), 87.0 (C-5), 81.0 (C-4), 79.8 (Boc-*C*$_q$), 78.3, 78.0 (2C, C-3, C-7), 76.1, 76.0, 75.4, 75.2, 73.6 (5C, 4 x Ph*C*H$_2$, C-6), 69.0 (C-8), 53.4 (C-5'), 39.0 (C-1'), 38.5 (C-2), 32.1 (C-4'), 29.1 (C-2'), 28.5 (3C, Boc-*C*H$_3$), 22.3 (C-3').

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7 Appendix

7.1 Abbreviations

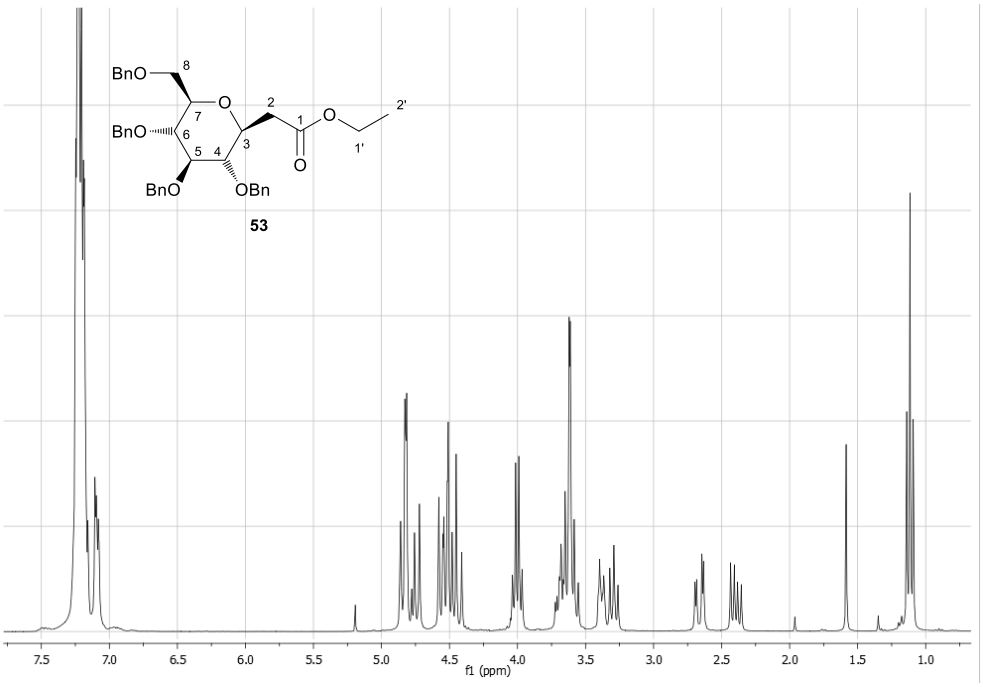
$[\alpha]_D^{20}$	specific optical rotation
Ac	acetyl
AcOH	acetic acid
AGEs	advanced glycation end products
APT	attached proton test
aq.	aqueous solution
Bn	benzyl
Boc	*tert*-butyloxycarbonyl
Boc_2O	di-*tert*-butyl dicarbonate
BSA	bovine serum albumin
CAM	ceric sulfate, ammonium molybdate
cat.	catalytic
CBz	carboxybenzyl
CDI	*N*,*N*'-carbonyldiimidazole
CH	cyclohexane
CN	nitrile
conc.	concentrated
COSY	correlation spectroscopy
DCM	dichloromethane
DEAD	diethyl azodicarboxylate
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EA	ethyl acetate
ER	endoplasmic reticulum
Et	ethyl
EtOH	ethanol
HCl	hydrochloric acid
HPLC	high performance liquid chromatography

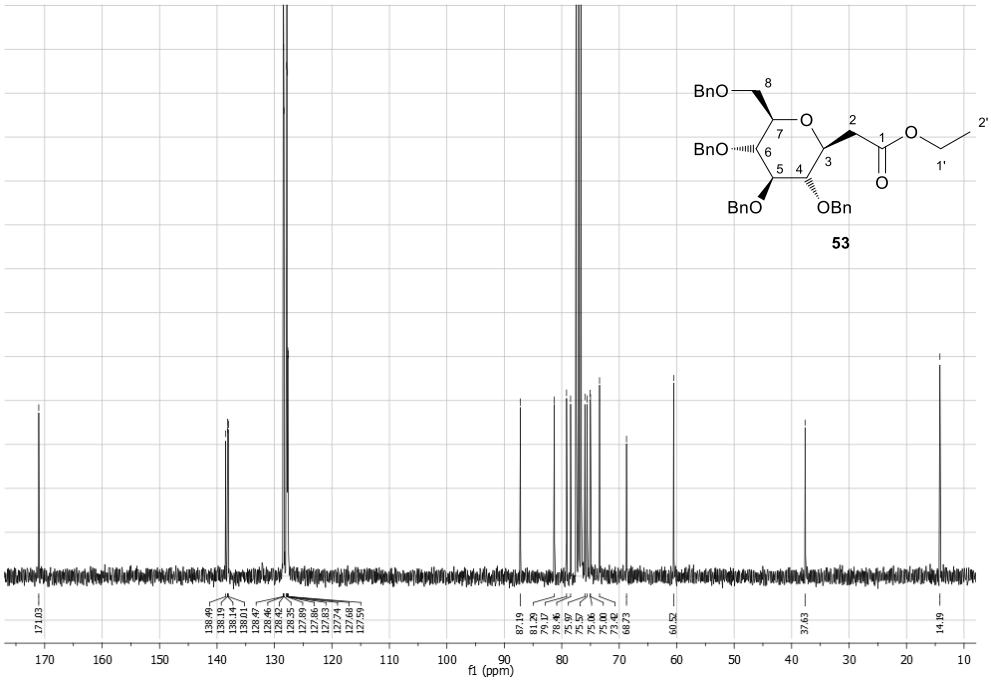
HSA	human serum albumin
HSQC	heteronuclear single quantum coherence
HWE	Horner-Wadsworth-Emmons reaction
^{i}Pr	isopropyl
m.p.	melting point
Me	methyl
MeCN	acetonitrile
MeOH	methanol
MS	mass spectroscopy
MW	molecular weight
NMR	nuclear magnetic resonance
OPA	oxaphosphetane
PG	protecting group
Ph	phenyl
Rf	retardation factor
sat.	saturated solution
TBDMS	*tert*-butyl-diemthylsilyl
t-BuOK	potassium *tert*-butoxide
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilane
TOCN	TEMPO oxidised cellulose nanofibril
Ts	tosyl
v/v	volume/volume

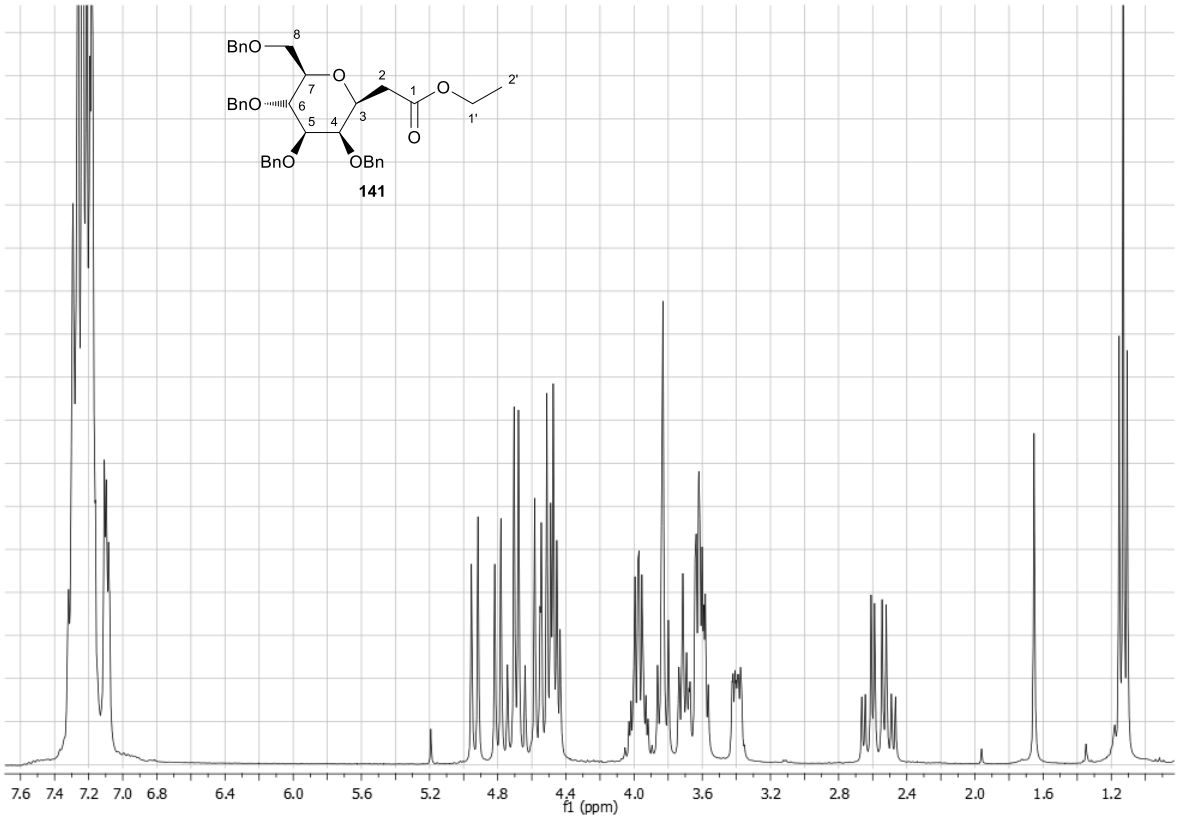
7.2 List of Figures

7.3 List of Schemes

7.4 Selected NMR-Spectra



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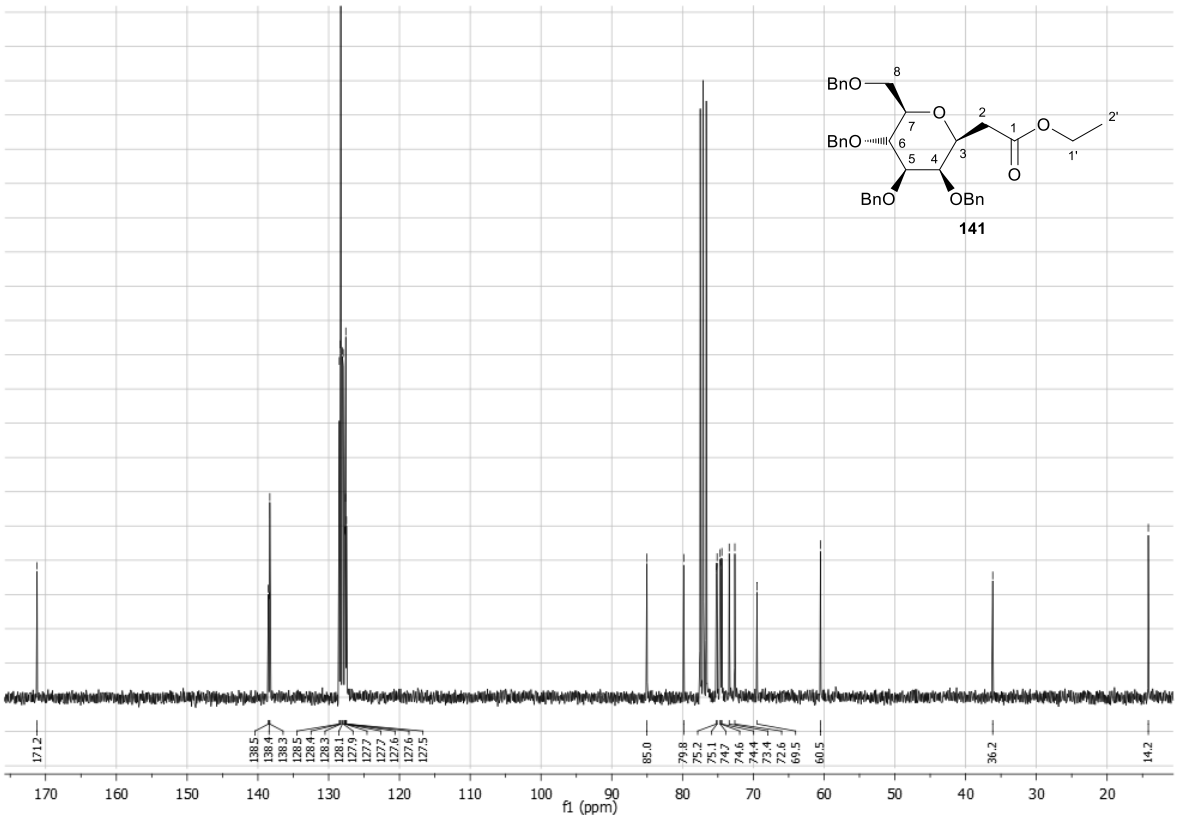
f1 (ppm)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127.9
127.7
127.7
127.6
127.6
127.5
85.0
79.8
75.2
75.1
74.7
74.6
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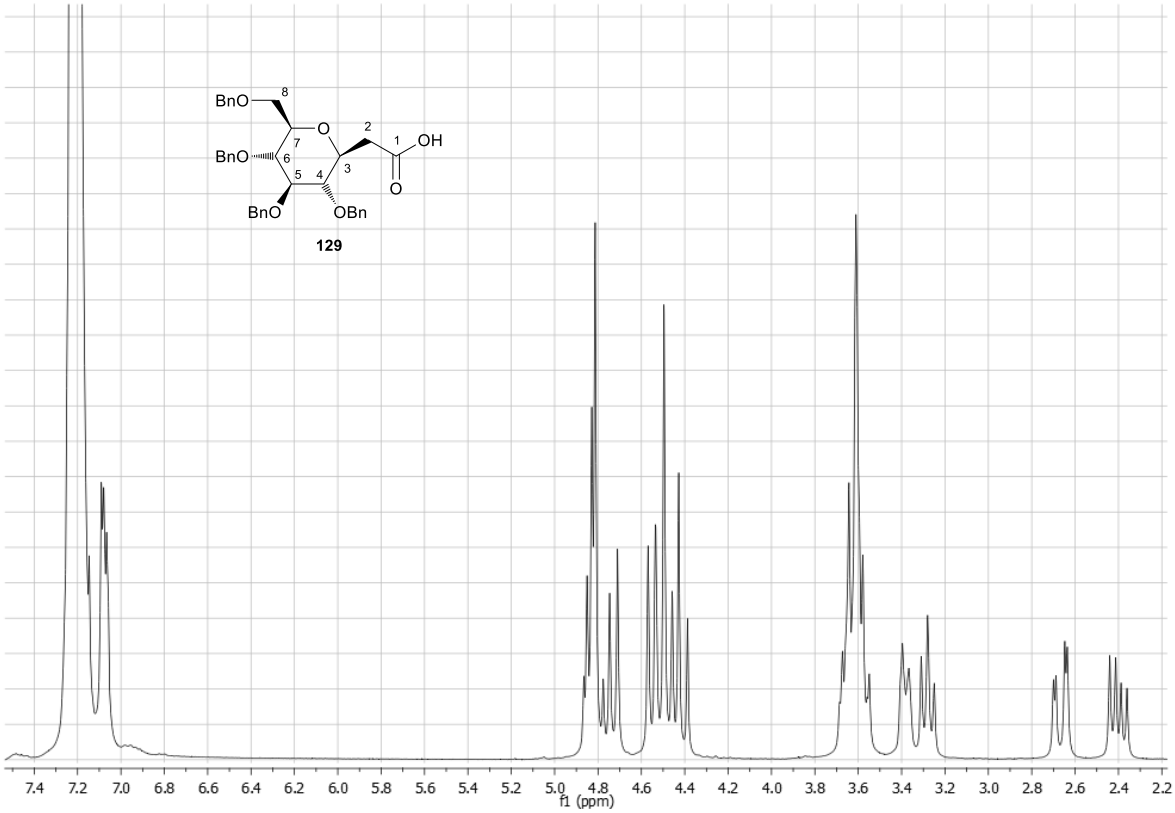
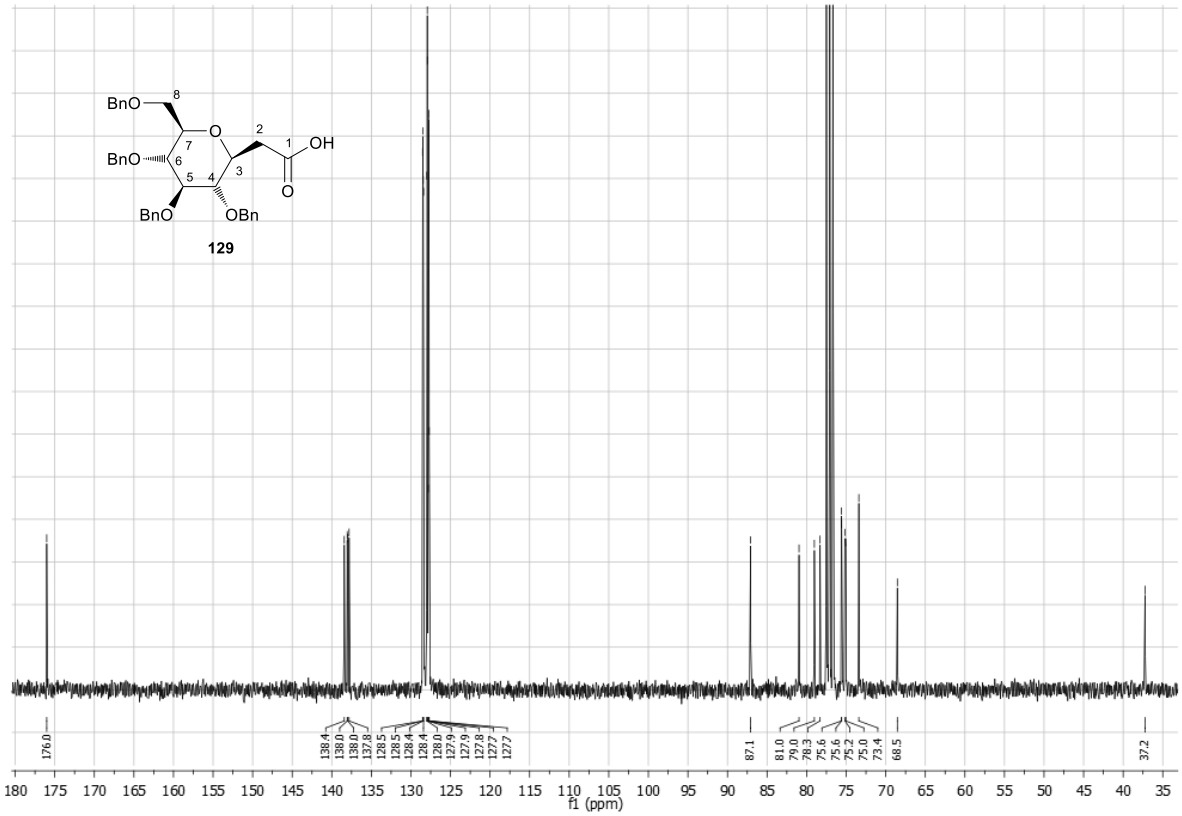
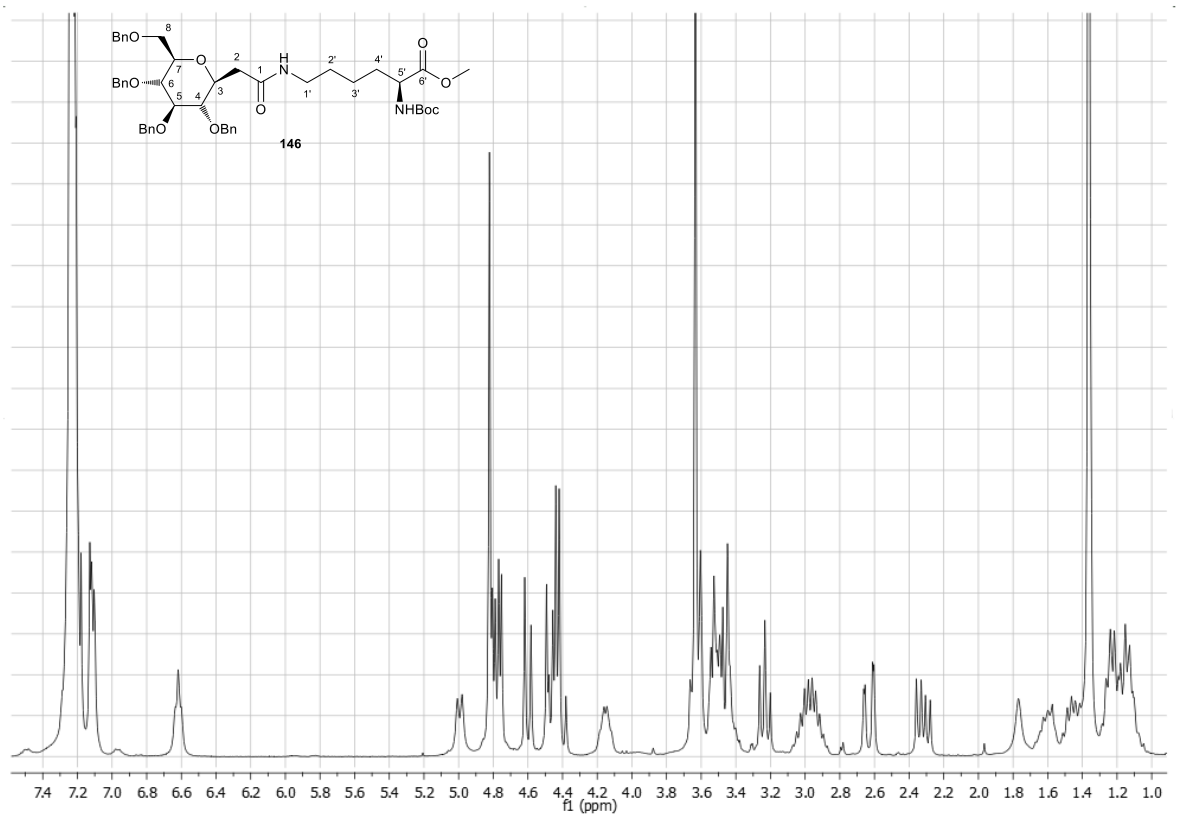
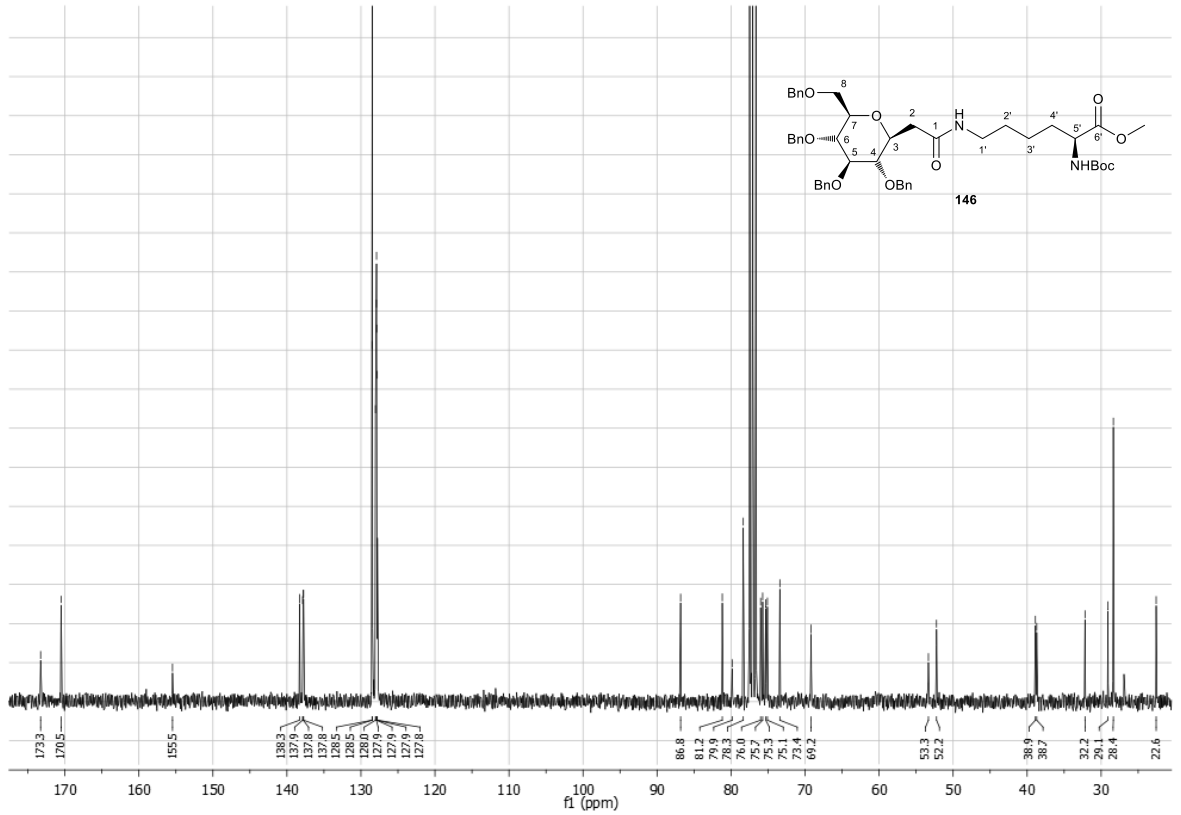
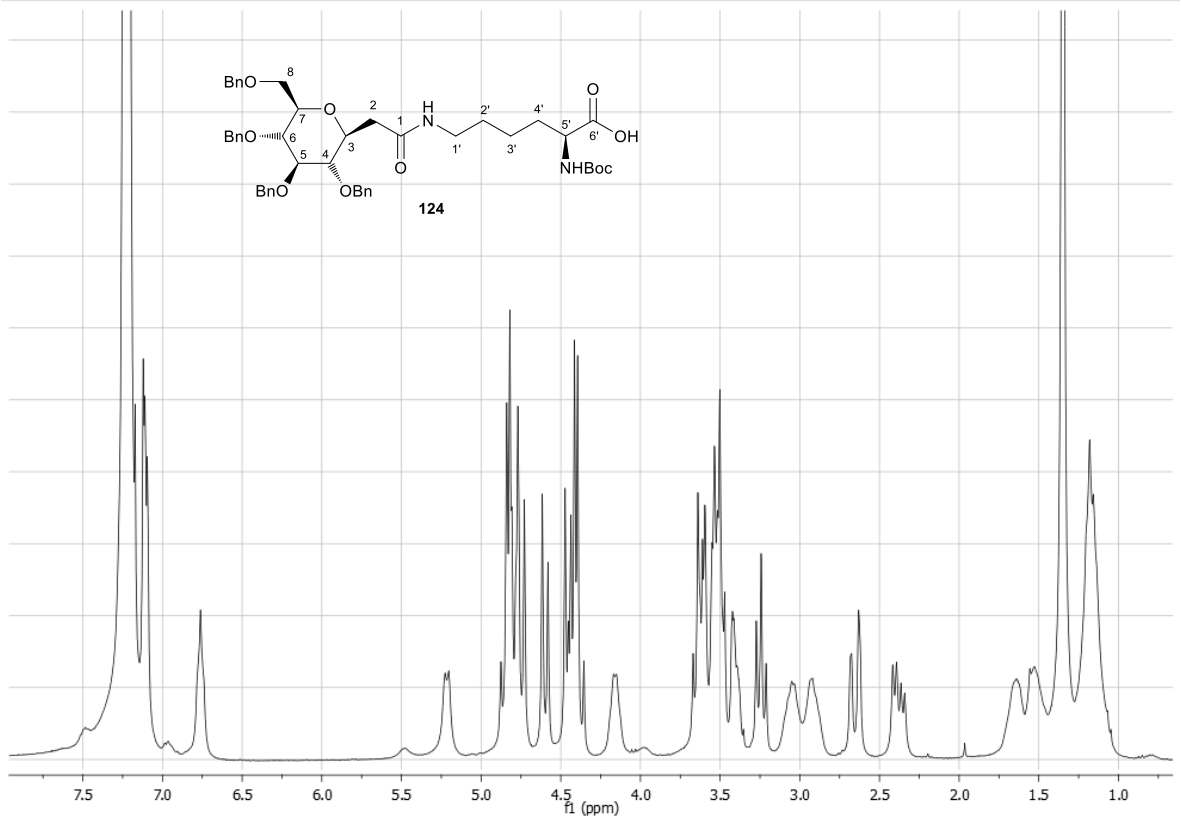
f1 (ppm)

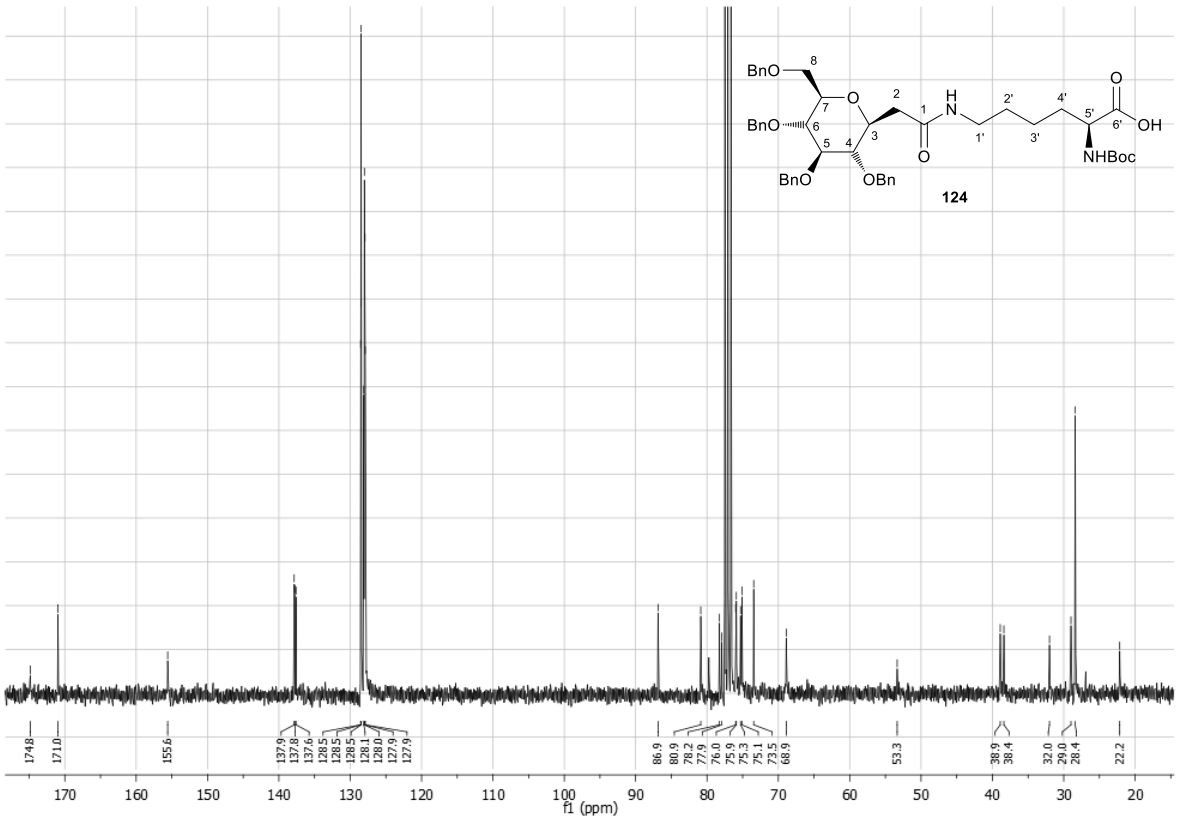

BnO
BnO
BnO
OBn
OH
O
129
f1 (ppm)


BnO
BnO
BnO
OBn
OH
O
129
176.0
138.4
138.0
138.0
137.8
128.5
128.4
128.4
128.0
127.9
127.9
127.8
127.7
127.7
87.1
81.0
79.0
78.3
75.6
75.6
75.2
75.0
73.4
68.5
37.2
f1 (ppm)


BnO
BnO
BnO
OBn
NHBoc
146
7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0
f1 (ppm)


BnO
BnO
BnO
OBn
NHBoc
146
173.3
170.5
155.5
138.3
137.9
137.8
128.5
128.0
127.9
127.9
127.8
86.8
81.2
79.9
78.3
76.0
75.7
75.3
75.1
73.4
69.2
53.3
52.2
38.9
38.7
32.2
29.1
28.4
22.6
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30
f1 (ppm)


BnO
BnO
BnO
OBn
NHBoc
OH
124
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


BnO
BnO
BnO
OBn
NHBoc
OH
124
174.8
171.0
155.6
137.9
137.8
137.6
128.5
128.5
128.5
128.1
128.0
127.9
127.9
86.9
80.9
78.2
77.9
76.0
75.9
75.3
75.1
73.5
68.9
53.3
38.9
38.4
32.0
29.0
28.4
22.2
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)