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GlcNAc analogues by Amadori Rearrangement of 3-acetamino-3-deoxyheptoses

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Abstract

Glycoproteins, proteins that consist of covalently bound carbohydrates, are ubiquitous in all forms of organisms where they are responsible for an enormously huge variety of important functions that are not yet fully understood. Compared to the naturally occurring *N*- and *O*-glycoproteins, synthetic *C*-glycosyl type glycoproteins typically show an increased stability, which is often very useful for biological studies. Hence a lot of effort has been invested in their synthesis. In this work, the Amadori rearrangement is described as conjugation method of *N*-acetyl-D-glucosamine to suitable amines and amino acids yielding *C*-glycosyl type glycoprotein precursor molecules. In order to obtain the D-GlcNAc motif in the respective glycoprotein the corresponding 3-NHAc-aldoheptose has to be synthesized and investigated on its applicability to serve as carbohydrate components in the Amadori rearrangement.

Kurzfassung

Glycoproteine, kovalent an Kohlenhydrate gebundene Proteine, sind in allen Lebensformen präsent wo sie für eine enorme Anzahl an wichtigen Funktionen verantwortlich sind, die noch nicht zur Gänze aufgeklärt sind. Aufgrund der, gegenüber natürlich vorkommenden Glycoproteinen, erhöhten Stabilität der synthetischen Analoga des *C*-glycosyl Typs, ist ihre Synthese von großem Interesse. In dieser Arbeit wird die Amadori Umlagerung als Schlüsselschritt für die Synthese von *C*-glycosyl Glycokonjugaten die das GlcNAc Motif enthalten untersucht. Als Ausgangsverbindung dient die entsprechende 3-NHAc-Aldoheptose welche durch C-Verlängerung von GlcNAc ausgehend synthetisiert wird. Die nach der Amadori Umlagerung erhaltenen Glycokonjugate können als Bausteine für den weiteren Aufbau von komplexeren Glycoproteinen verwendet werden.

Abbreviations

$[\alpha]_D^{20}$	specific optical rotation
alc. prim.	primary hydroxyl group
alc. sec.	secundary hydroxyl group
Asn	asparagine
Boc	*tert*-butyloxycarbonyl
C	cyclohexane
C-S	solvent for column chromatography
DBA	dibenzyl amine
DMAP	4-dimethylaminopyridine
EE	ethyl acetate
Et_3N	triethyl amine
EtOH	ethanol
excycl.	excyclic
Gal	galactose
GlcNAc	*N*-acetyl-D-glucosamine
HPLC	high performance liquid chromatography
Lys	lysine
Man	mannose
MeOD	methanol-d_4
MeOH	methanol
mRNA	messenger ribonucleic acid
NMR	nuclear magnetic resonance
OMe	methoxy
ppm	parts per million
R_f	retention factor
Ser	serine
THF	tetrahydrofurane
Thr	threonine
TLC	thin layer chromatography
TLC-S	solvent for thin layer chromatography
UV	ultraviolet
Z	benzyloxycarbonyl

1. Introduction

The term carbohydrate was originally derived in the nineteenth century from the French "hydrate de carbon", used for the family of compounds depicting the empirical formula $C_n(H_2O)_n$. However, subsequently, the term has been greatly extended to encompass many other structures containing heteroatoms for example N, S, Se,…

In general, carbohydrates are polyhydroxylated aldehydes or ketones containing 3 to 9 carbon atoms and are called triose, tetrose, pentose… respectively.

Produced by plants and cyanobacteria via photosynthesis, they do not only possess the function of energy storage (e.g. starch) and transport but also represent the major supporting tissue of plants (e.g. cellulose) and arthropods (e.g. chitin) (Figure 1).

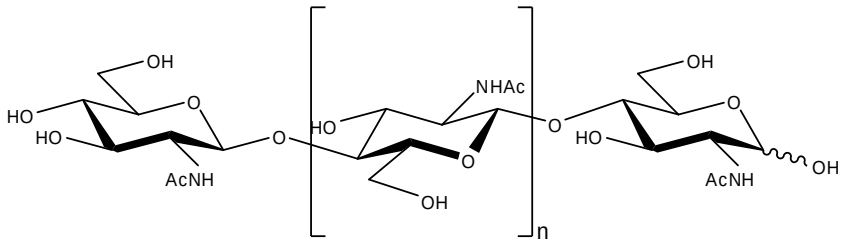


Figure 1: Chitin.

Moreover, carbohydrates and their derivatives play an important role as communication molecules in many intra- and intercellular processes. In particular, carbohydrates are of high importance in cell-cell recognition events[1] and are involved in related processes such as cellular differentiation[2], cell signalling regulation[3] and immune response[4].

The vast variety of missions fulfilled by carbohydrates may at least partly result from the huge structural and stereochemical diversity they exist in. The monosaccharides as building blocks of oligo- and polysaccharides in different configurations and substitutions can be connected in linear or branched form.

[1] Okajima, T.; Irvine, K.D. *Cell*, **2002**, *111*, 893-904.
[2] Nishiwaki, K.; Kubota, Y.; Chigira, Y.; Roy, S.K.; Suzuki, M; Scharzstein, M; Jigami, Y.; Hisamoto, N.; Matsumoto, K. *Nat. Cell. Biol.*, **2004**, *6*, 31-37.
[3] Helenius, A.; Aebi, M. *Science,* **2001**, *291*, 2364-2369.
[4] Lowe, J.B. *Cell*, **2001**, *104*, 804-812.

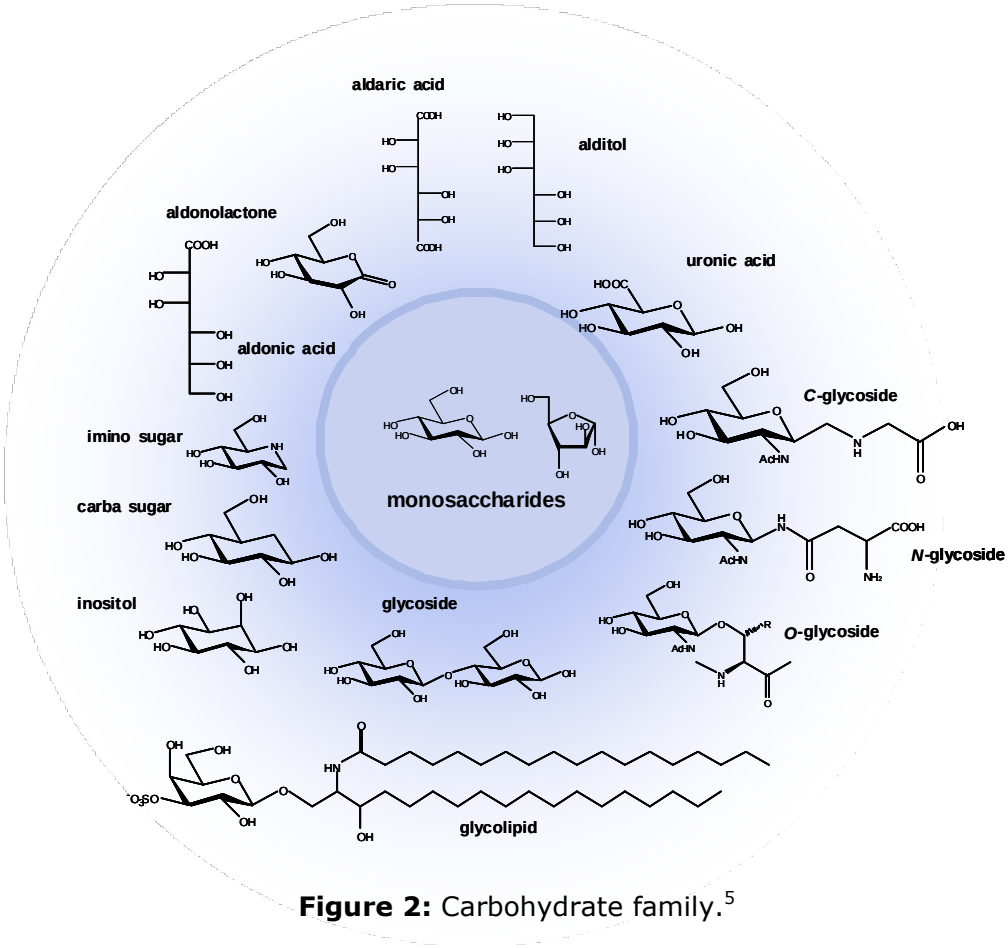


Figure 2: Carbohydrate family.[5]

Since each monosaccharide carries several hydroxyl groups and potentially functional groups, the carbohydrate family consists of numerous members. Not only the "classical" 5- or 6- membered heterocyclic rings are to be mentioned here but also open chain alditols, aldaric and aldonic acids and conjugations of carbohydrates with other substance classes such as peptides or proteins (Figure 2).

The chemistry of simple sugars was described by Emil Fischer and the ring structures defined by Hartworth and coworkers[6] in the interwar years. During the 1960s, especially based on the investigation of blood determinants, it became clear that naturally occurring carbohydrates are commonly conjugated to proteins and lipids forming glycoproteins and glycolipids, respectively. Since these glycoconjugates occur in a great variability of structures and are responsible for a wide array of biological functions, they are of enormous interest for chemists.

[5] Adapted from: Lindhorst, T.K. *Essentials of Carbohydrate Chemistry and Biochemistry*, Wiley-VCH, Weinheim, **2007**, p.2.

[6] Charlton, W.; Haworth, W.N.; Peat, S. *J. Chem. Soc.*, **1926**, 89-101; Haworth, W.N.; Hirst, E.L. *J. Chem. Soc.*, **1926**, 1858-1868.

1.1. Glycolipids

Membranes of all classes of organisms from bacteria to mammals are composed of glycolipids. These consist of a hydrophobic part such as acylglycerol, ceramide or prenyl phosphane moieties that are glycosidically linked to a carbohydrate compound of one or more saccharide units. Glycolipids are not only important factors in membrane stabilization and cell surface rigidization but also play an essential role in a broad variety of biological processes. Examples are photosynthetic electron transport in plants, cell-cell communication, receptor modulation and signal transduction.[7]

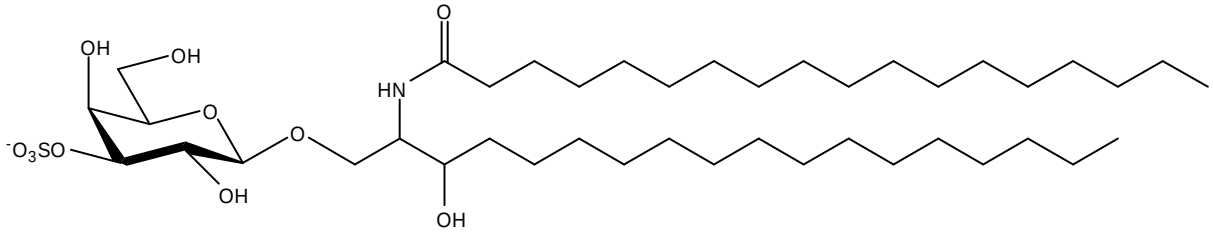


Figure 3: 3-*O*-Sulfogalactosylceramide: An acidic, sulfated glycosphingolipid, often known as sulfatide, which occurs in membranes of various cell types, and is also found in particularly high concentrations in myelin where it constitutes 3-4% of total membrane lipids. Its synthesis primarily takes place in the oligodendrocytes in the central nervous system. Accumulation of this lipid in the lysosomes is a characteristic of metachromatic leukodystrophy, a lysosomal storage disease.[8]

1.2. Glycoproteins

Glycoproteins, proteins that content covalently bound carbohydrates, comprise one of the most important classes of biological compounds. They are ubiquitous in all forms of life, where they serve a variety of functions as antigenes, enzymes, hormones, toxins and transport proteins.[9] The exteriors of cells are particularly rich in glycoproteins, which are known to be involved in a number of cell-surface reactions. Their carbohydrate content varies from less than 1% in some collagens to over 99% in glycogen. The carbohydrate moiety may be attached to the protein as monosaccharide, oligo- or polysaccharide and is a major determinant of cell surfaces as it can change dramatically during development and between species. These carbohydrate chains are classified

[7] Holst, O. In *Glycoscience- Chemistry and Chemical Biology*; Fraser-Reid, B.O.; Tatsuta, K.; Thiem, J., Ed.; Springer: Berlin Heidelberg, **2001**, 2083-2097.
[8] http://www.hmdb.ca/metabolites/HMDB00024.
[9] Wittmann, V. In *Glycoscience- Chemistry and Chemical Biology*; Fraser-Reid, B.O.; Tatsuta, K.; Thiem, J., Ed.; Springer: Berlin Heidelberg, **2001**, 2253-2287.

according to the linkage between the sugar moiety and amino acid depending on whether they are conjugated to the amino acid via a nitrogen atom (always supplied by asparagine) or an oxygen atom (usually from serine or threonine). These *N*- or *O*-glycans, respectively do not only differ in structure but do also fulfil different functions.

1.2.1. *N*-Glycans

N-Glycosylation is a modification conducted during the translation of mRNA to proteins and is available to all secreted or membrane-bound proteins containing the triplet amino acid sequence AsnXaa[10]Ser/Thr. The asparagine residue of this sequence binds covalently to the so called "core region" which all *N*-glycoproteins have in common (Figure 4).[9,11]

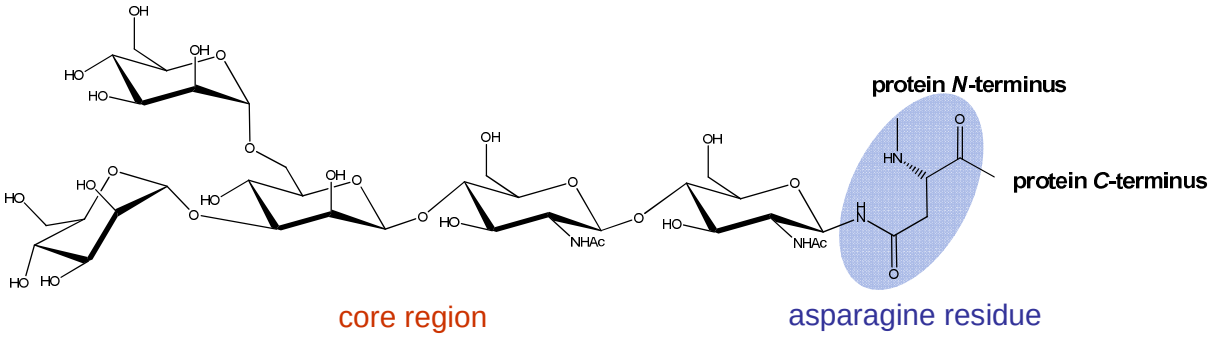


Figure 4: Peptide linked core pentasaccharide Man(α1-6)[Man(α1-3)]Man(β1-4)GlcNAc(β1-4)GlcNAc.

Apart from this core unit, *N*-linked glycans display an extraordinary diversity and even *N*-glycans of the same protein may have different structures. These can be further classified into three groups depending on the so to say "higher substructure" of the carbohydrate moiety. High mannose oligosaccharides have two to up to six mannose residues conjugated to the core, whereas complex oligosaccharides contain varying numbers of sialylated *N*-acetyllactosamine units as well as a fucose residue attached to the *N*-acetylglucosamine (GlcNAc) and a bisecting GlcNAc residue linked to the β-linked mannose residue. Hybrid

[10] Xaa can be any of the 20 natural amino acids except proline.
[11] Helenius, A.; Aebi, M. *Science*, **2001**, *291*, 2364-2369; Stick, R.V.; Williams, S.J. In *Carbohydrates- The Essential Molecules of Life,* Elsevier: Oxford Amsterdam, **2009**, *11*, 369-412.

oligosaccharides represent a combination of both groups and usually contain the bisecting GlcNAc (Figure 5).[12]

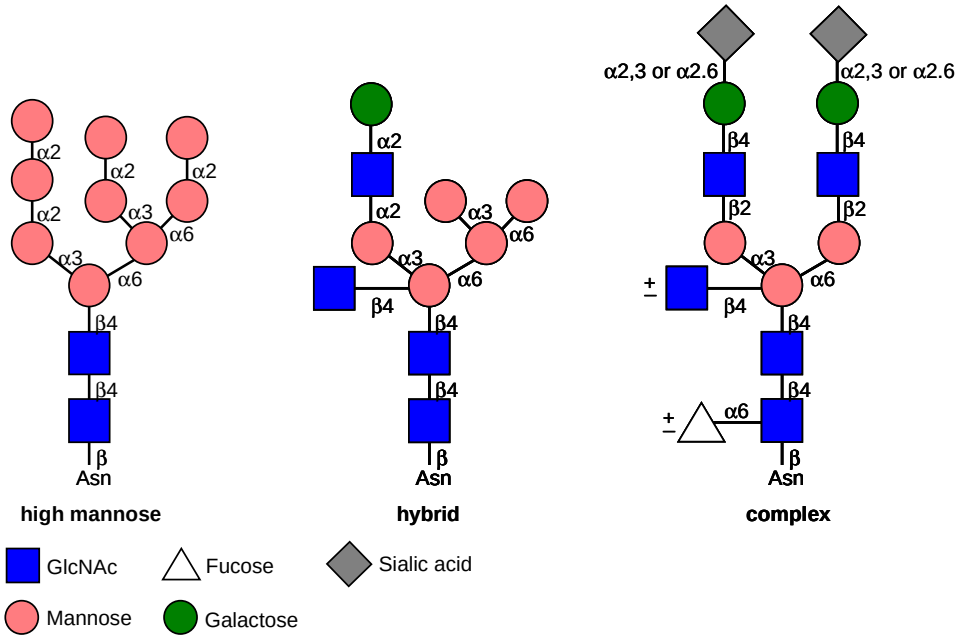


Figure 5: Three groups of "higher substructures".

Biologically, *N*-glycoproteins are synthesized cotranslationally in the rough endoplasmatic reticulum where the synthesis is initiated with the transfer of a triantennary tetradecasaccharide from a dolichol phosphate glycosyl donor to the growing nascent peptide chain.

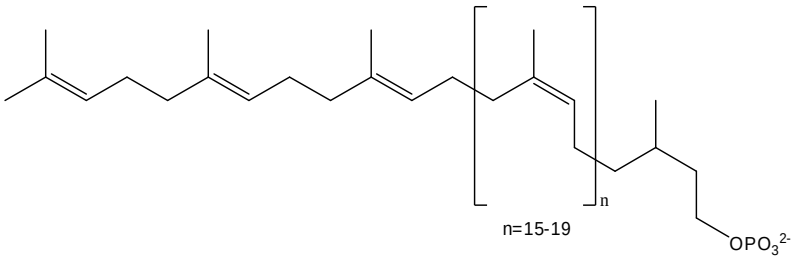


Figure 6: Dolichol phosphate.

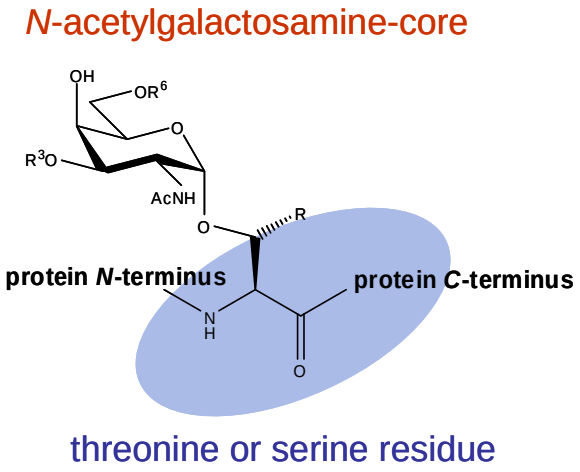
This initiate core is subsequently trimmed and processed by several glycosylhydrolases and glycosyltransferases resulting in the above described common pentasacharide core.

As in eukaryotes the most important function of *N*-glycosylation is the promotion of proper folding of newly synthesized polypeptides, the necessity of cotranslocational glycan-addition clearly emerges.

[12] Schachter, H. *Clin. Biochem.*, **1984**, *17*, 3-14.

1.2.2. *O*-Glycans

O-Glycolsylated proteins are the second major group of biologically important glycoproteins. In eukaryotes, the most abundant glycoproteins are of the mucin-type, where *N*-acetyl-α-galactosamine is linked to the β-hydroxyl group of either threonine or serine via an *O*-glycosidic linkage. Contrary to *N*-glycoconjugates, these mucin-type-*O*-glycans show a higher structural diversity. They can be classified into eight "core-groups" (Figure 7),[13] however core 1 is the most abundant.[11]



serine: R=H
threonine: R=CH_3

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

Figure 7: Mucin-type core structures.

O-Gylcoproteins differ from their *N*-analogues also in terms of biosynthesis which is a predominantly posttranslational and postfolding event here. It starts with the transfer of *N*-acetyl-α-galactosamine to the side chain of serine or threonine catalyzed by *N*-acetygalactosaminyltransferase. Exposed regions of the protein surface like β-turns or regions with extended conformations are preferentially *O*-glycolsylated whereas serine, threonine and proline are commonly found before and after occupied positions. The exact location where *O*-glycolsylation is initiated is still discussed and might depend on the type of *N*-acetygalactosaminyltransferase of which at least nine forms exist.[14]
Other carbohydrates *O*-glycosidically linked to an amino acid are α-L-fucose, β-D-glucose found in the epidermal growth factor domains of different proteins as well as α-mannose, α/β-galactose and *N*-acetyl-β-glucosamine. The latter has

[13] Hölemann, A.; Seeberger, P.H. *Curr. Op. Biotech.*, **2004**, *15*, 615-622.
[14] Spiro, R.G. *Glycobiol.*, **2002**, *12*, 43R-56R.

been found to be widely spread in intracellular glycoproteins being a prevalent posttranslational modification.

1.2.3. *C*-Glycans

The bewilderingly huge variety of functions of glycoproteins is not fully understood yet. Hence, a lot of effort has been invested in the synthesis of homogenous and structurally defined products that potentially serve as model for biological studies. Among these, *C*-glycosyl type glycoconjugates are of high interest due to their increased resistance against hydrolysis and *in vivo* stability.[15] Moreover, *C*-linked glycopeptides have been found to possess inhibitory activity against glycoamidases.[16]

This class of glycopeptides only rarely occurs in nature. However, a few examples can be mentioned with vineomycin[17] and showdomycin[18] as antibiotic representatives. A group of compounds called enkastines (Figure 8) has been isolated while screening for enkephalinase inhibitors.[19] Enkephalinase catalyzes the hydrolysis of enkephalines which, being a natural ligand for opiate receptors, play an important role in the physiological suppression of pain.[20] Actually, these structures are products of an Amadori rearrangement between D-glucose and respective dipeptides.

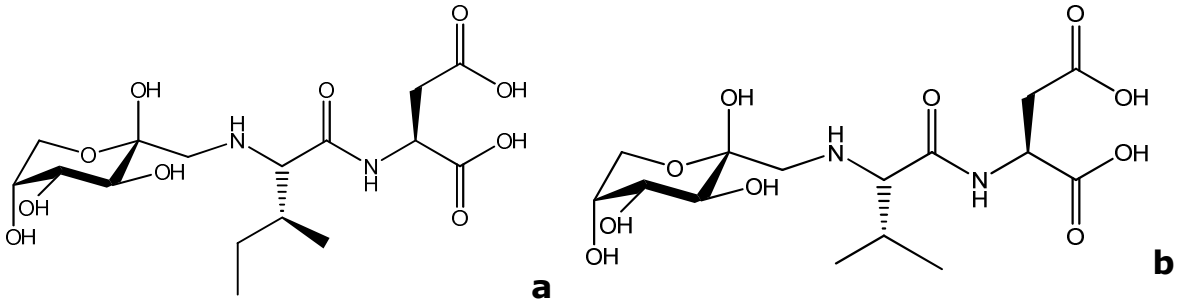


Figure 8: *N*-(β-1-Deoxyfructos-1-yl)-isoleucyl-aspartate **a**; *N*-(β-1-Deoxyfructos-1-yl)-valyl-aspartate **b**; enkastines.

[15] Paulsen, H.; Hoffmann, M.; Kessler, H. *Angew. Chem*., **1997**, *109*, 1240-1241; Bridiau, N.; Cabanel, S.; Maugard, T. *Tetrahedron,* **2009**, *65*, 531-535.
[16] Wang, L.-X.; Tang, M.; Suzuki, T.; Kitajima, K.; Inoue, Y.; Inoue, S.; Fan, J.-Q.; Lee, Y. C. *J. Am. Chem. Soc*., **1997**, *119*, 11137-11146.
[17] Darnall, K.R.; Townsend, L.B.; Robins, R.K. *Procl. Natl. Acad. Sci. U. S. A.*, **1967**, *57*, 548-553.
[18] Imamura, N.; Kakinuma, K.; Ikekawa, N. *J. Antibiot*., **1982**, *25*, 602-608; Imamura, N.; Kakinuma, K.; Ikekawa, N. *J. Antibiot*., **1981**, *24,* 1517-1518.
[19] Vértesy, L.; Fehlhaber, H.-W.; Kogler, H.; Schindler, P.W. *Liebigs Ann*., **1996**, 121-126.
[20] Hughes, J.; Smith, T.W.; Kosterlitz, H.W.; Fothergill, L.; Morgan, B.A.; Morris, H.R. *Nature*, **1975**, *258*, 577-579.

1.3. Amadori rearrangement

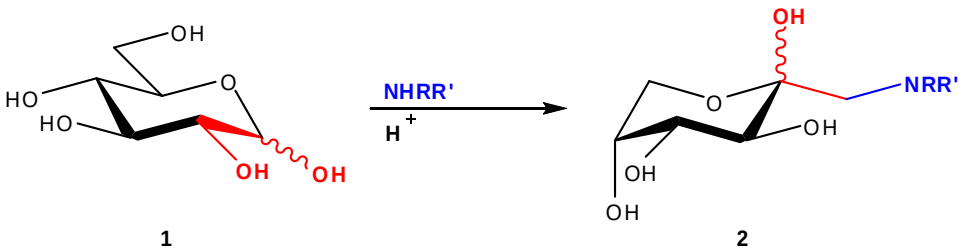
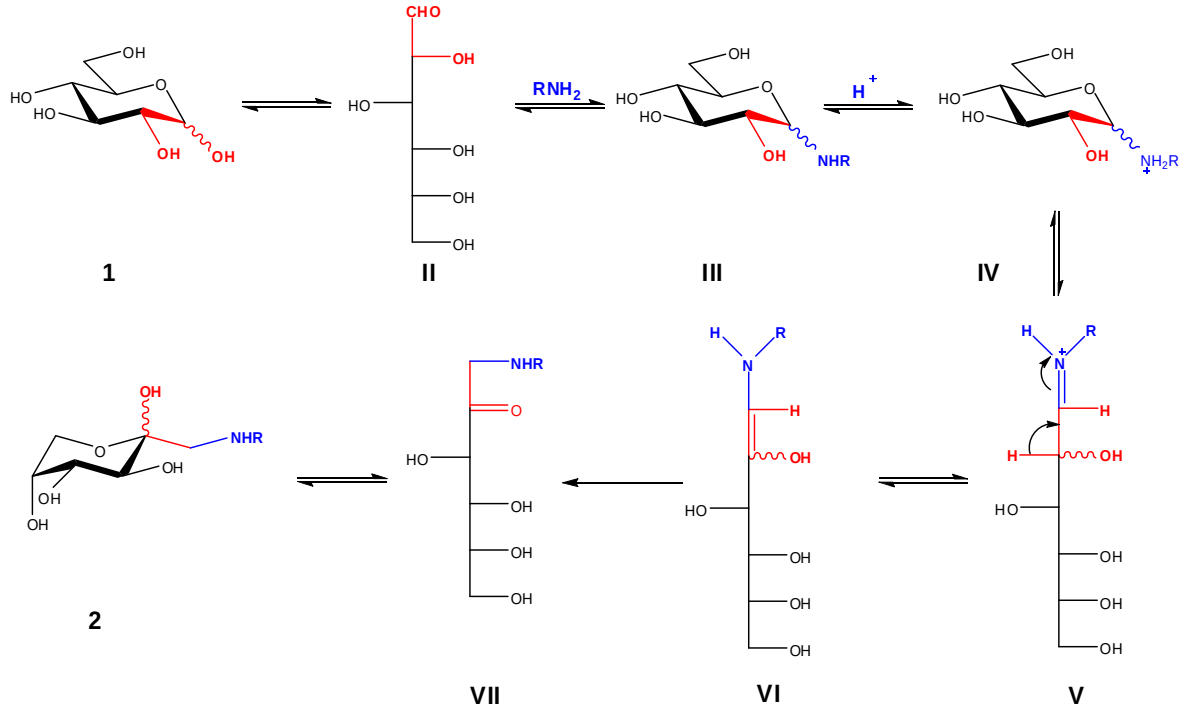


Figure 9: Amadori rearrangement from D-glucose to 1-amino-1-deoxy-D-fructose.

First reported by and named after Mario Amadori[21], this reaction represents the key step of the Maillard reaction cascade, the non-enzymatic browning of foods.[22,23,24] This reaction also takes place during cooking and baking processes leading to flavoured compounds. The Amadori rearrangement is initiated by the condensation of α–hydroxy-aldehydes such as D-glucose **1** with suitable amines forming *N*-substituted glycosylamines **III** (Scheme 1).[25] In the following an acid catalyzed rearrangement to the corresponding α-aminoketose (Amadori product) takes place. The still widely accepted mechanism was proposed by Kuhn and Weygand in 1937.[26]

The nitrogen of the *N*-substituted aldosylamine **III** is protonated forming the ammonium ion **IV** which is in equilibrium with the aldimine **V**. Tautomerization of this aldimine to the corresponding enaminol **VI** finally results in the stabilizing formation of a ketose **VII** and further ring closure gives product **2**. The loss of the hydrogen at C-2 in Schiff base **V** is considered to be the rate determining step.[27]

[21] Amadori, M. *Atti Real. Accad. Naz. Lincei*, **1925**, *2*, 337-342; Amadori, M. *Atti Real. Accad. Naz. Lincei*, **1929**, *9*, 68-73; Amadori, M. *Atti Real. Accad. Naz. Lincei*, **1929**, *9*, 226-230; Amadori, M. *Atti Real. Accad. Naz. Lincei*, **1931**, *13*, 72-77.
[22] Hodge, J.E. *Adv. Carb. Chem.,* **1955**, *10*, 169-203.
[23] Wrodnigg, T.M.; Eder, B. In *Glycoscience- Epimerisation, Isomerisation and Rearrangement Reactions of Carbohydrates*, Stütz, A.E., Ed.; Springer: Berlin, Heidelberg, New York, **2001**, 115-152.
[24] Maillard, M.L.-C. *C. R. Acad. Sci.*, **1912**, *2*, 66-68; Maillard, M.L.-C. *C. R. Acad. Sci.*, **1912**, *2*, 1554-1556.
[25] Hodge, J.E. *Agric. Food Chem*., **1953**, *1*, 928-943.
[26] Kuhn, R.; Weygand, F. *Ber*., **1937**, *70*, 769-772.
[27] Paulsen, H.; Pfulghaupt, K.-W. In *The Carbohydrates- Chemistry and Biochemistry*; Pigman, W.; Horton, D., Ed.; Academic Press: New York, **1980**, 881-921.



Scheme 1: Mechanism of the Amadori rearrangement.

Amadori was able to isolate two compounds from the reaction of D-glucose with 4-methylaniline, 4-methoxyaniline and 4-ethoxyaniline. One of them labile and one stable towards hydrolysis, which presumably were the *N*-substituted aldosylamine **III** (labile compound) and the Amadori compound **2**, respectively.[22] He was not able to analyze the latter correctly but it has later been indentified as such by Kuhn ad Weygand.[27] Kuhn and Dansi[28] had already postulated before that a novel rearrangement must have taken place in Amadori's experiments.

In 1940, Weygand was the first to realize the remarkable positive effect when catalytic amounts of acid are added on yields as well as on purity.[29] In contrast to aromatic amines, which were originally used by Amadori, *N*-alkylamines were believed to be non reactive under Amadori conditions so far known.[30]

This assumption lasted until Hodge and Rist published their results obtained from the reaction of D-glucose with *N*-alkylamines in the presence of malonic acid.[31] At the same time, Gottschalk managed to prove that the Amadori rearrangement is possible with amino acids[32] and that physiologically important mucoproteins

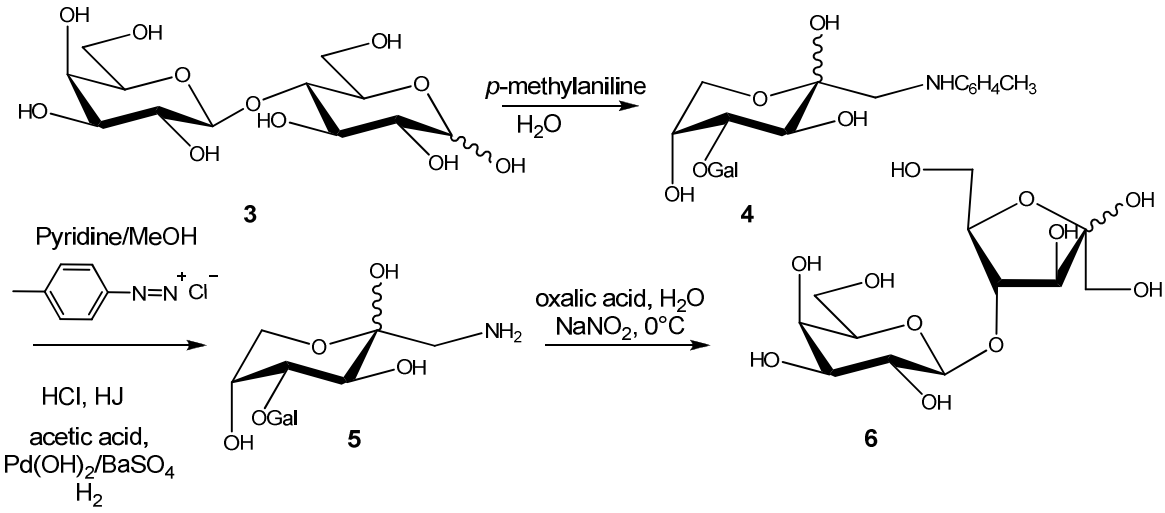
[28] Kuhn, R.; Dansi, A. *Ber.*, **1936**, *69*, 1745-1754.
[29] Weygand, F. *Ber.*, **1940**, *73*, 1284-1291.
[30] Mitts, E.; Hixon, R.M. *J. Am. Chem. Soc.*, **1944**, *66*, 483-486.
[31] Hodge, J.E.; Rist, C.E. *J. Am. Chem. Soc.*, **1952**, *75*, 316-322.
[32] Gottschalk, A.; Partridge, S.M. *Nature*, **1950**, *165*, 684-685.

contain an Amadori-like linkage between amino acids and sugar moieties.[33] In the following, a lot of emphasis was placed on the synthesis and the characterization of *in vivo* occurring Amadori compounds.[34]

Once the Amadori products are formed, they can eventually undergo the Maillard reaction cascade involving rearrangement and decomposition reactions leading to strongly coloured and flavoured compounds including pyrroles, imines, furans, pyridines and imidazoles as well as their polymerization products.[25,35,36]

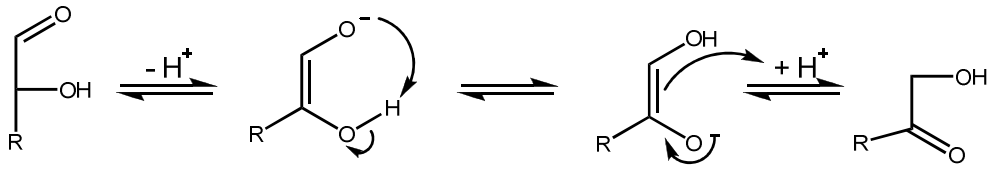
It has been found that Maillard reaction products are involved in Alzheimer's disease[35] as well as diabetic cataract formation[37] and diabetic and age-related postsynthetic glycosylation of hemoglobin[38] and collagen.[39]

One of the first applications of the Amadori reaction was the synthesis of lactulose **6** (Scheme 2)[40,41] as alternative to the Lobry de Bruyn – Alberda van Ekenstein rearrangement (Scheme 3).[42]



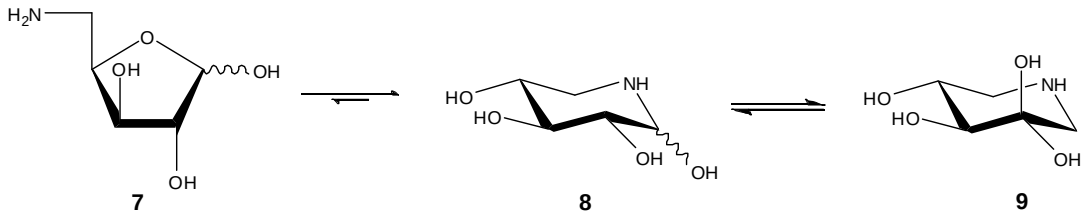
Scheme 2: Synthesis of lactulose **6** via Amadori rearrangement.[41]

[33] Gottschalk, A. *Biochem. J.*, **1952**, *52*, 455-460.
[34] Mossine, V.V.; Glinsky, G.V.; Feather, M.S. *Carb. Res.*, **1994**, *262*, 257-270; Day, J.F.; Thorpe, S.R.; Baynest, J.W. *J. Biol. Chem.,* **1979**, *254*, 595-597.
[35] Smith, M.A.; Taneda, S.; Richey, P.L.; Miyata S.; Yan, S.-D; Stern, D.; Syre, L.M.; Monnier, V.M.; Perry, G. *Proc. Natl. Acad. Sci.U.S.A.*, **1994**, *91*, 5710-5714.
[36] Reynolds, T.M. *Adv. Food. Res.*, **1963**, *12*, 1-52.
[37] Stevens, V.J.; Rouzer, C.A.; Monnier, V.M.; Cerami, A. *Proc. Natl. Acad. Sci. U.S.A.,* **1978**, *75*, 2918-2922.
[38] Koenig, R.J.; Blobstein, S.H.; Cerami, A. *J. Biol. Chem.*, **1977**, *252*, 2992-2997.
[39] Schnider, S.L.; Kohn, R.R. *J. Clin. Invest.*, **1980**, *66*, 1179-1181; Monnier, V.M.; Cerami, A. *Science,* **1981**, *211*, 491-493.
[40] Kuhn, R; Krüger, G; Seelinger, A. *Ann. Chem.*, **1959**, *628*, 240-255.
[41] Kuhn, R.; Krüger, G.; Seeliger, A., *Liebigs Ann. Chem.*, **1959**, *628*, 240-255.
[42] Montgomery, E.M.; Hudson, C.S. *J. Am. Chem. Soc.*, **1930**, *52*, 2101-2106; Speck, J.C. *Adv. Carb. Chem*. **1958**, *13*, 63-103.

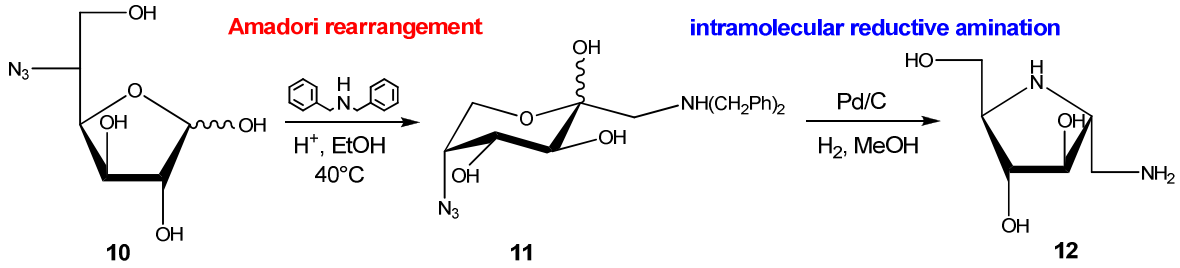


Scheme 3: Mechanism of the Lobry de Bruyn – Alberda van Ekenstein rearrangement.

In the late 60s, it has been found that suitable amino sugars can undergo an intramolecular Amadori rearrangement leading to iminosugars (Scheme 4).[43] These carbohydrate analogues with a basic nitrogen instead of oxygen in the ring, such as 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol **(12)** (Scheme 5), have been found to possess high inhibitory activity against glycosidases.[44]



Scheme 4: First reported intramolecular Amadori rearrangement.



Scheme 5: Synthesis of 1-amino-1,2,5-trideoxy-2,5-imino-D-mannitol.

Although being a highly useful synthetic tool, the Amadori rearrangement is accompanied with some limitations. If the reaction is conducted at excessive temperatures and time, the easily dehydrated Amadori ketose can enter the Maillard reaction cascade. Tautomerization to the 1,2 enolic structure of the ketose and further dehydration gives the aldimine. The latter is subsequently partly liberated by hydrolysis to give the hydroxymethylfurfural or the furfural depending on the decomposed carbohydrate compound.[33] During the final stage

[43] Paulsen, H.; Todt, K. *Adv. Carb. Chem. Biochem.*, **1968**, *23*, 115-232, p.121.
[44] Stütz, A.E. *Iminosugars as glycosidase inhibitors,* Wiley-VHC, Weinheim, **1999**; Wrodnigg, T.M.; Stütz, A.E.; Withers, S.G. *Tetrahedron Lett.*, **1997**, *38*, 5463-5466.

of the Maillard reaction, melanoidins, brown condensation and polymerization products are formed (Figure 10).[45]

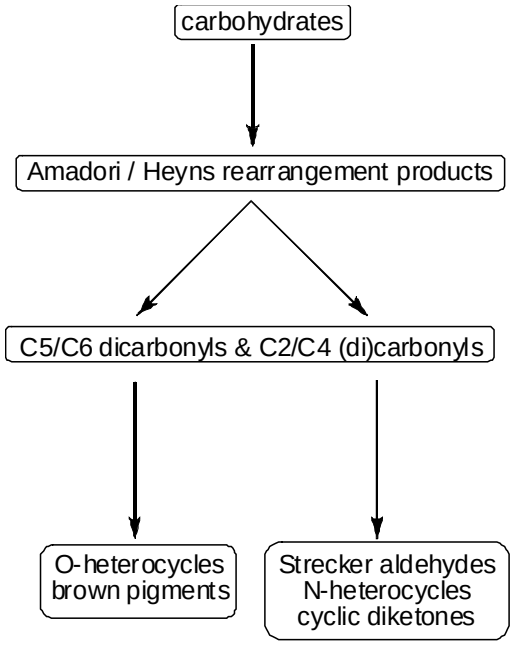
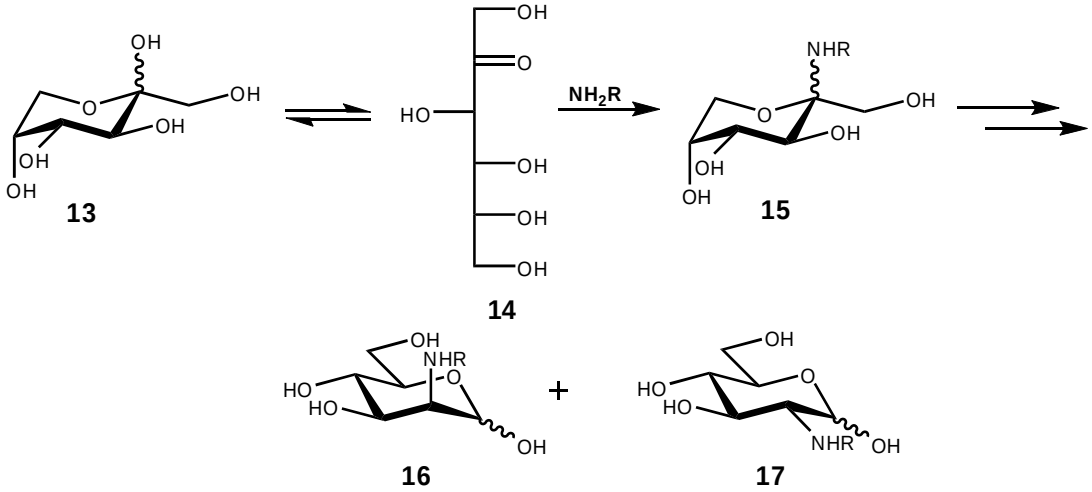


Figure 10: Major Maillard reaction pathway to the formation of heterocycles and brown pigments.[46]

However, the Maillard reaction is not the only drawback of the Amadori rearrangement. Additionally, the glycosyl amine precursor might not enter the equilibrium at all and potentially undergoes hydrolysis back to the starting material which results in mixtures of a lot of compounds that afford quite sophisticated separation techniques. Moreover, depending on the basicity of the amine, Amadori rearrangement and Lobry de Bruyn – Alberda van Eckenstein rearrangement[42] compete for the aldehyde.

A reaction that is strongly related to both, the Amadori rearrangement and the Lobry de Bruyn – Alberda van Ekenstein rearrangement, is the Heyns rearrangement (Scheme 6).[47] In this case, the reaction of a ketose **13** with a suitable amine gives the corresponding 2-amino aldoses **16** and **17** in an epimeric mixture. Via the Heyns rearrangement, ketoses also enter the Maillard reaction cascade and finally end up as (predominantly meat-) flavour products very much like Amadori products.[46]

[45] Namiki, M. *Adv. Food Res.*, **1988**, *32*, 115-184.
[46] De Roos, K.B. In *Flavour Precursors*, Teranishi, R.; Takeoka, G.R.; Güntert,, M. Ed. ACS Symposium Series 490, **1992**, 201-216.
[47] Heyns, K.; Meinecke, K.-H., *Chem. Ber.*, **1953**, *86*, 1453-1462.



Scheme 6: Heyns rearrangement.

1.4. N-Acetyl-D-glucosamine

Amino sugars are highly distributed in nature and play significant roles in vital processes with D-glucosamine being the most abundant one which in most cases exists in *N*-acetylated form β-glycosidically linked to proteins or peptides. Moreover, it is quite frequently incorporated in structural polysaccharides such as chitin (Figure 1) or murein (Figure 11) which constitutes the basic component of the bacterial cell wall.

Another GlcNAc containing polysaccharide which is a topic of intensive research is hyaluronic acid (Figure 11) also called hyaluronan. This oligosaccharide represents the major structural element in the extracellular matrix and is responsible for the viscoelastic properties of cartilages and synovial fluids. Additionally, it is involved in protein interactions where it influences natural processes such as angiogenesis, cell motility, adhesion, wound healing and cancer.[48]

[48] DeAngelis, P.L. *J. Biol. Chem*., **1999**, *274*, 26557-26562;
Kogan, G.; Šoltés, L.; Stern, R.; Gemeiner, P. *Biotechnol. Lett*., **2007**, *29*, 17-25.

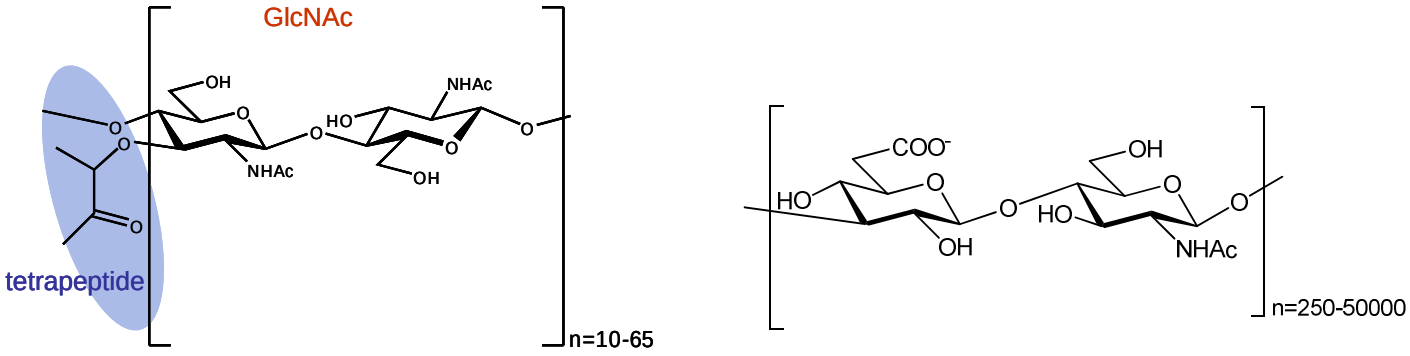


Figure 11: Murein and hyaluronic acid, hyaluronan.

Furthermore, GlcNAc is associated with cancer constituting two common epitopes (Figure 12) on the surface of tumor-associated antigens. These antigens can be detected immunologically by their antibodies and thus play an important role in diagnosis of malignant cells.[49]

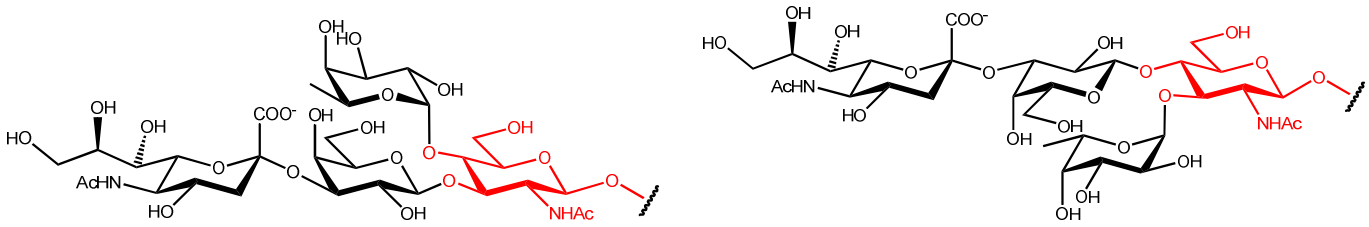


Figure 12: Sialyl-Lewis A (sLe^a) and Sialyl-Lewis X (sLe^x).

Moreover, *O*-glycosylation with GlcNAc moieties turned out to be of high importance in dynamic posttranslational modifications of proteins. Glycosylation in general is locally restricted to the lumen or extracellular regions and therefore is not suspected to directly effect signal transduction. However, GlcNAc residues can be found in nucleocytoplasmatic proteins where they seem to participate in signalling. In many proteins this modification with *O*-GlcNAc has been characterized including transcription factors, cytoskeletal proteins, nuclear pore proteins, oncogeneproducts and tumor suppressors.[50] *O*-GlcNAc is also associated with brain diseases such as Parkinson as the position of the OGT (*O*-GlcNAc-transferase) locus in humans maps to the same region of the X

[49] Izawa, M.; Kumamoto, K.; Mitsuoka, C.; Kanamori, A.; Ohmori, K.; Ishida, H.; Nakamura, S.; Kurata-Miura, K.; Sasaki, K.; Nishi, T.; Kannagi, R. *Cancer Res.*, **2000**, *60*, 1410-1416; Patel, K.D.; Cuvelier, S.L.; Wiehler, S. *Semin. Immunol.*, **2002**, *14*, 73-81; Ugorski, M.; Laskowska, A. *Acta Biochim. Pol.*, **2002**, *49*, 303-311.

[50] Comer, F.I.; Hart, G.W. *J. Biol. Chem.*, **2000**, *275*, 29179-29182; Wells, L.; Vosseller, K.; Hart, G.W. *Science*, **2001**, *291*, 2376-2378; Whelan, S.A.; Hart, G.W. *Circ. Res.*, **2003**, 1047-1058.

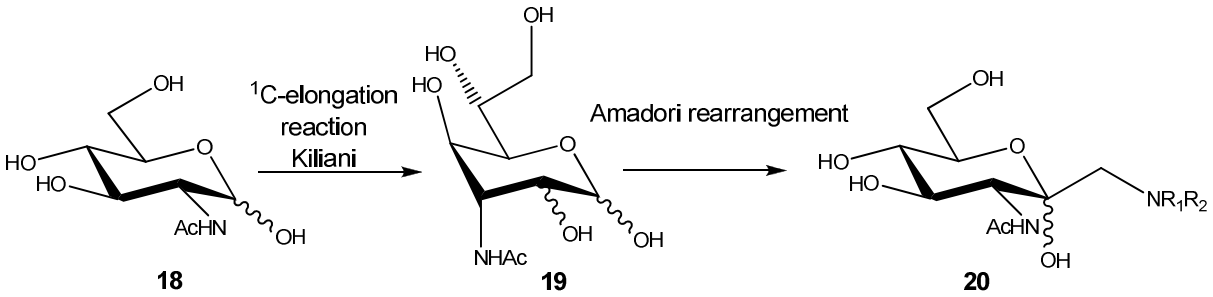
chromosome as the dystonia-parkinsonism syndrome locus.[51] On some proteins, the *O*-glycosylation with GlcNAc is reciprocal to phosphorylation, a phenomenon called "yin-yang" hypothesis. The protein Tau (microtubule-associated-protein) is a famous representative of this class. Tau of patients suffering from Alzheimer's disease is hyperphosphorylated whereas it is multiply *O*-glycosylated with GlcNAc residues in healthy brains.[52]

[51] Shafi, R.; Iyer, S.P.N.; Ellies, L.G.; O'Donnell, N.; Marek, K.W.; Chui, D.; Hart, G.W.; Marth, J.D. *Proc. Natl. Accad. Sci. U.S.A.*, **2000**, *97*, 5735-5739;
Stathakis, D.G.; Lee,D.; Bryant, P.J. *Genomics*, **1998**, *49*, 310-313.

[52] Avila, J. *FEBS Letters*, **2006**, *580*, 2922-2927;
Zachara, N.E.; Hart, G.W. *Chem Rev.*, **2002**, *102*, 431-438.

2. Aims

This diploma thesis is focused on the synthesis of *C*-glycosyl type glycoconjugates with a GlcNAc residue **18** as carbohydrate motif.
As key reaction of the synthetic pathway towards these glycoconjugates, the Amadori rearrangement will be investigated on its applicability to 3-acetamino-3-deoxy-D-gluco-D-*ido*/D-*gulo*-heptose **19**.



Scheme 7: Reaction pathway.

As starting material, the corresponding 3-acetamino-3-deoxy-D-gluco-D-*ido*/D-*gulo*-heptose moiety **19** has to be synthesized. Therefore different C-elongation methods will be studied.

3. Results and discussion

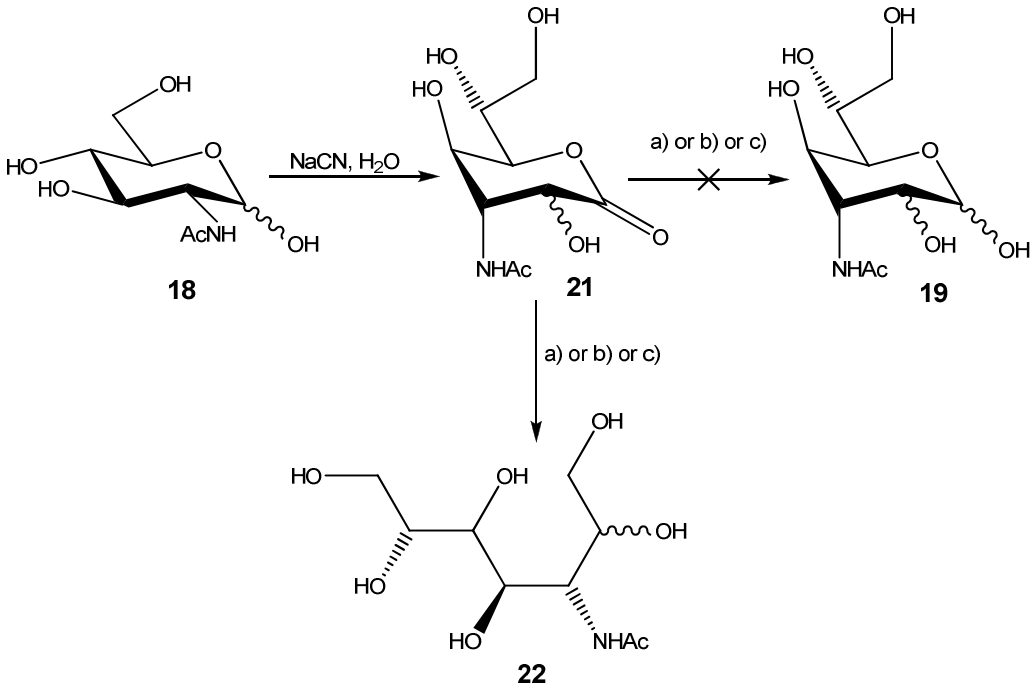
3.1. Chain elongation

The first approach towards the desired heptoses **19** was the Kiliani-Fischer reaction as it is the most common method for the chain elongation of carbohydrates and no use of protecting groups is necessary.[53] The original Kiliani-Fischer synthesis is achieved by addition of hydrogen cyanide to a carbohydrate moiety in aqueous solution, followed by saponification of the obtained nitriles and subsequent acidification.[54] The formed acids can be isolated as lactones if the pH is adjusted accordingly. Consecutive reduction of the lactone with sodium amalgam in weakly acidic medium results in the formation of the respective one carbon elongated aldose.[55]

In order to avoid the quite inconvenient handling of hydrogen cyanide, a modified and very often successfully applied method has been chosen.[56]

Following the literature, an aqueous solution of GlcNAc **18** was cooled to 0°C and sodium cyanide was slowly added. For the subsequent acidic hydrolysis, which substantially affords the corresponding lactones **21**, an accurate pH control is necessary. In this context, either acetic acid[57] or ion exchanger Amberlite IR 120^{+} [58] was used whereas the latter allows the removal of potentially interfering sodium ions at the same time. Since NMR data of the crude products **21** were satisfactory, no further purification was performed. For the subsequent reductive hydrolysis, again, several methods are proposed in literature. Either sodium borohydride[59] (Scheme 8a) or $Pd/BaSO_4$ with H_2[57] (Scheme 8b) was applied as reducing agent. Another reported method for the reduction of lactones uses a borane solution in THF as reductive agent (Scheme 8c).[60]

[53] Richtmyer, Nelson K. *Meth. Carbohydr. Chem.,* **1962**, *1*, 160-167;
Varma, R.; French, D. *Carb. Res.*, **1972**, *25*, 71-79.
[54] Kiliani, H. *Ber.,* **1886**, *19*, 767-772.
[55] Fischer, E., *Ann.*, **1892**, *270*, 64-107.
[56] a) Frampton, V. L.; Foley, L. P.; Smith, L L.; Malone, J. G. *Anal. Chem.*, **1951**, *23*, 1244-1247;
b) Hudson, C. S. *J. Am. Chem. Soc.*, **1951**, *73*, 4498-4499;
Adjé, N.; Vogeleisen F.; Uguen D. *Tetrahedron Lett.*, **1996**, *37*, 5893-5896.
[57] King-Morris, Melinda J.; Bondo, Paul P. *Carb. Res.*, **1988**, *175*, 49-58.
[58] Pratt, James W.; Richtmyer Nelson K. *J. Am. Chem. Soc.*, **1955**, *77*, 6326-6328.
[59] Bock, K.; Lund, I.; Perderson, *C.Carb. Res.*, **1981**, *90*, 7-16.
[60] Bhattacharjee Shyam S.; Schwarcz Joseph A.; Perlin Arthur S. *Carb Res.*, **1975**, *42*, 259-266.



Scheme 8: Different conditions for the reduction of the lactone to the corresponding heptose. a): $NaBH_4$, $EtOH/H_2O$, IR 120^+. b): $Pd/BaSO_4$, H_2, H_2O, Acetic acid. c): BH_3-THF.

Although sodium borohydride is generally not applicable as reducing agent for acyclic esters[61] it is known to reduce lactones.[62] Hence, reduction of such internal esters with sodium borohydride in slightly acidic aqueous conditions should afford the corresponding aldehydes. A range of different pH-values, from strongly acidic to nearly neutral and different temperatures (Table 1) was investigated but in our hands the alditols **22** were the only products that were obtained with reasonable certainty. The addition of sodium borohydride was carefully controlled by TLC which determines the stated amounts. The same result was found for reductions with $Pd/BaSO_4$ and BH_3.

Table 1: Conditions fort he reduction with $NaBH_4$.

Solvent	$NaBH_4$	pH	Temperature
	3eq	2	0°C
	2eq	6-7	0°C
	0.85eq	4	0°C
$EtOH/H_2O$ 1/2	0.7eq	1	0°C
	0.6eq	5	0°C
	0.5eq	4	0°C
	0.5eq	1	-10°C

[61] Chaikin, S. W.; Brown, H. B.; *J. Am. Chem. Soc.*, **1949**, *71*, 122-125.
[62] Wolfrom, M. L.; Wood, H. B. *J. Am. Chem. Soc.*, **1951**, *73*, 2933-2934.

The distinctive polarity of hexose **18** and heptose **19** is very similar. Furthermore, the two pyranoid epimers (α and β anomer each) are not the only possible configurations formed during the reaction. Potentially, also the furanoid epimers can be generated which results in at least eight possible configurations of the desired heptose **19** (Figure 13). Taking into account, that still two anomers of GlcNAc are present together with the always formed heptitols (Figure 14), the reaction mixture can consist of up to 12 different structures. This results in a very demanding detection and separation. Therefore, especially in the case when pH was adjusted to 4, a formation of the desired heptose cannot be totally excluded.

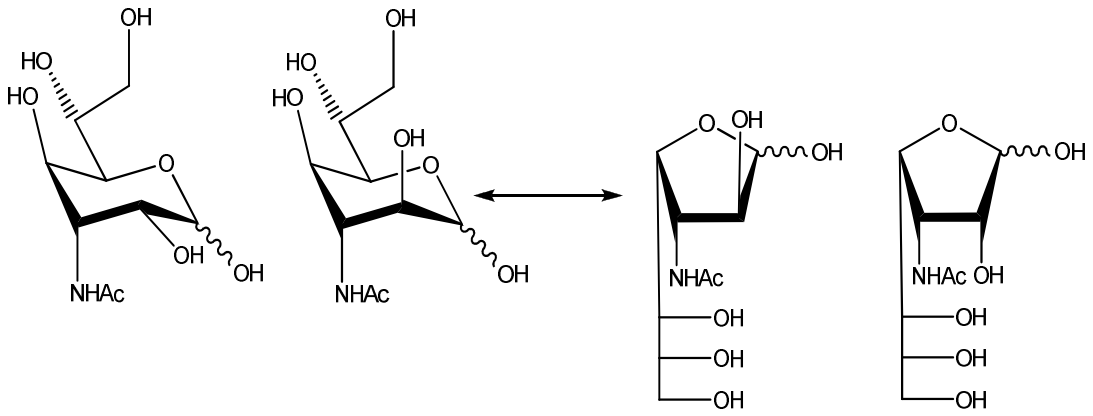


Figure 13: Possible configurations and conformations of heptose **19** (α/β anomer each).

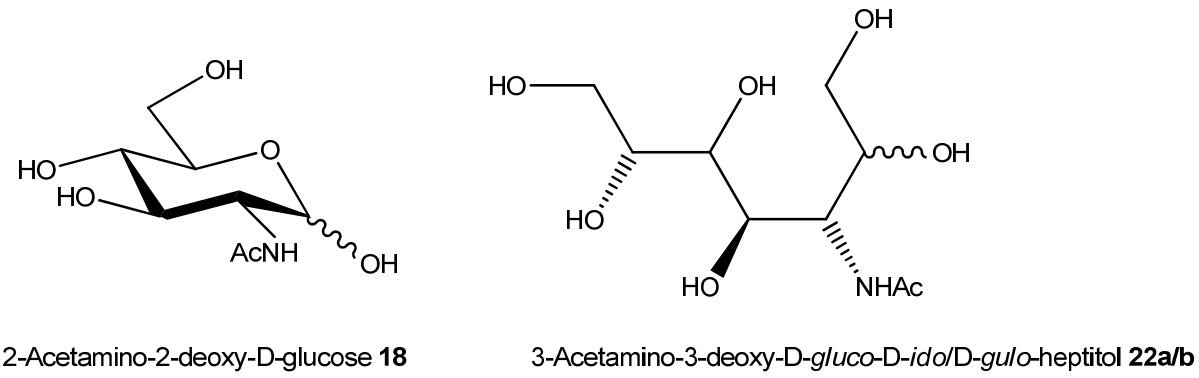
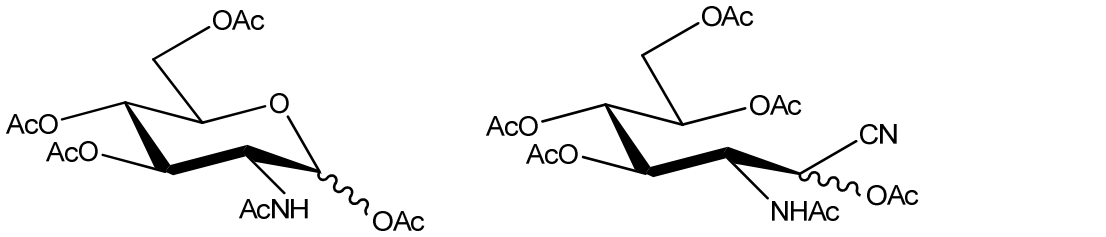


Figure 14: Structures of compounds **18** and **22 a/b**.

Driven by these drawbacks, a procedure proposed by Kuhn and Baschang[63] was examined for the C-elongation reaction. Therefore, compound **18** was dissolved in dry pyridine, triethyl amine and hydrogen cyanide were added and the reaction mixture was stirred until TLC showed full conversion of GlcNAc. For the starting material **18** and the reaction product **23** no difference in R_f-values could be noted as cyanhydrine hydrolyses on the slightly acidic conditions of the TLC surface. Therefore, both compounds were detected as their corresponding peracetylated forms on TLC (Figure 15).



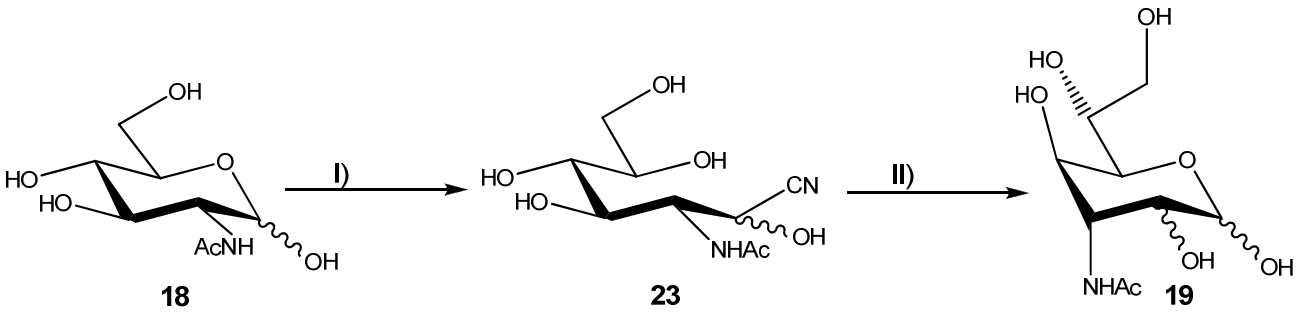
1,3,4,6-Tetra-*O*-acetyl-
2-acetamino-2-deoxy-D-glucose

2,4,5,6,7-Penta-*O*-acetyl-2-acetamino-2-deoxy-
D-*gluco*-D-*ido*/D-*gulo*-heptononitrile

Figure 15: Peracetylated forms of hexose **18** and nitrile **23.**

Subsequent removal of the solvent and acidic reductive hydrolysis with $Pd/BaSO_4$ in water afforded the desired heptoses **19** in almost quantitative yield (Scheme 9). Due to the very low stability of the nitriles **23** and their distinct tendency to undergo hydrolysis back to the starting material, the temperatures during concentration has to be controlled carefully and has to be kept strictly below 30°C and additionally, the isolated material **23** had to be subjected to reduction immediately.

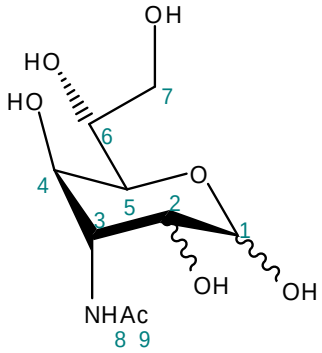
[63] Kuhn, R.; Baschang, G. *Liebigs Ann. Chem*., **1960**, *636*, 164-173.



Scheme 9: Reported reaction pathway: I) pyridine dry, HCN, Et_3N. II) H_2O, acetic acid, $Pd/BaSO_4$, H_2.

Again, the reaction affords up to eight reaction products (as the yield can be considered as quantitative, no GlcNAc is left). Although all characteristic ^{13}C signals are found in the expected region (Table 2), a full interpretation of the NMR spectrum is not possible.

Table 2: ^{13}C NMR signals of heptose **19**.



amid. sarbonyl; C-8	174.4-172.7 ppm
anomer; C-1	94.3- 92.6 ppm
alc. sec.; C-2, C-4, C-5	78.0- 72.2 ppm
alc. sec. excycl.; C-6	69.4- 66.7 ppm
alc. prim.; C-7	65.0- 64.3 ppm
C-3	56.7- 54.2 ppm
CH_3; C-9	23.4- 22.7 ppm

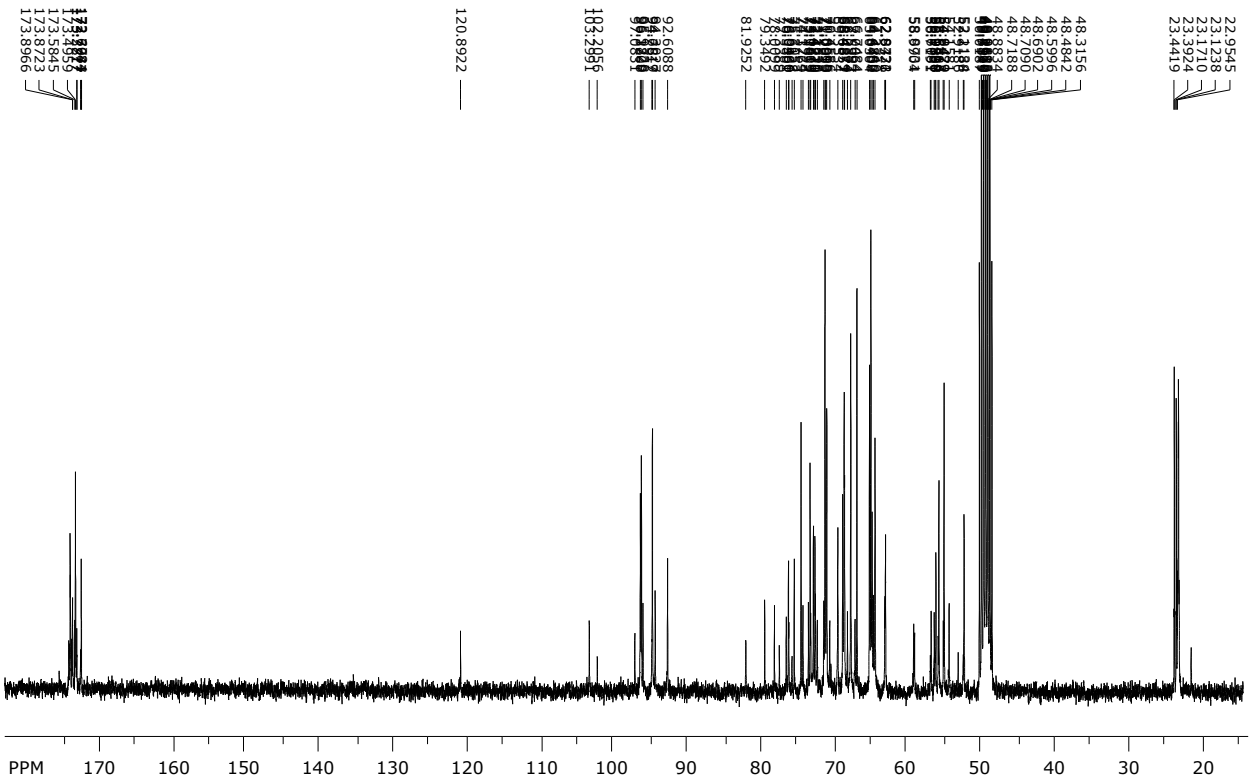
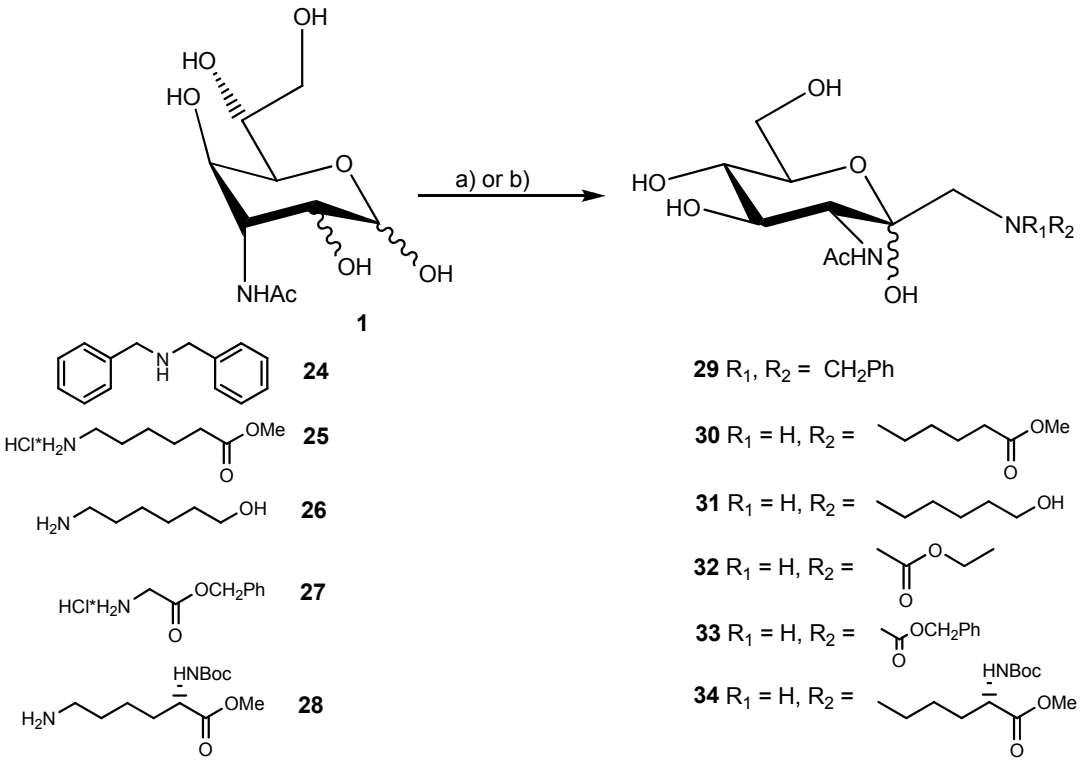


Figure 16: ^{13}C-NMR spectrum of heptose **19.**

3.2. Amadori Rearrangement

Previous works with commercially available D-*glycero*-D-*gulo*-heptose as starting material for the Amadori rearrangement[64] showed very promising results, therefore, the same reaction conditions were applied to heptoses **19**. This reaction pattern should allow easy rearrangement to *C*-glycosyl type carbohydrate amino acid conjugates with the GlcNAc motif in the sugar part (Scheme 10).

[64] Wrodnigg , Tanja M.; Kartusch, C.; Illaszewicz, C *Carb. Res.*, **2008**, *343*, 2057-2066.



Scheme 10: Reaction conditions for the Amadori rearrangement: a): EtOH, acetic acid, approx. 50°C, **24** or **26** or **28**. b): EtOH, Et_3N, approx. 50°C, **25** or **27**.

The reaction conditions were primarily tested with dibenzyl amine, as it was expected that this amine gives the best yields.[64] Furthermore, the two benzyl groups allow for tracking of the reaction by UV on TLC and the purification of product **29** should be less demanding because of the decreased polarity of the rearrangement product. Thereupon, 6-aminohexanol **(26)**, methyl 6-aminohexanoate hydrochloride **(25)**, glycine benzyl ester hydrochloride **(27)** as well as Boc-Lys-OMe **28** as protected amino acid derivative were subjected to the Amadori rearrangement as amino component.

For the aliphatic amines and Boc-Lys-OMe, acetic acid was added in varying amounts (Table 3) to an ethanolic solution of the respective heptose until a pH of ~3 was reached and the reaction mixture was heated slightly while stirring.

Table 3: Amounts of acetic acid added to the reaction mixture.

Amine	Acetic acid
Boc-Lys-OMe	1.0eq
Dibenzyl amine	1.8eq
6-Aminohexanol	3.8eq

In case of products **30**, **32** and **33**, the corresponding amine hydrochlorides were stirred with one equivalent of triethyl amine for 30 minutes prior to the addition of heptose **19**. This assures the liberation of the free amine from its hydrochloride and no further addition of acid is necessary.

Since the all-equatorial orientation of the hydroxyl groups as well as of the quite bulky amine side chains induces increased stability, the $^4C_1$ D-*gluco* conformation of the α-anomer was the expected product. In accordance with these expectations, the α-anomers of all title compounds were the exclusively observed products.

In order to avoid the possible degradation reactions of the Maillard reaction cascade, the reactions had to be stopped with a notable amount of starting material still present. Furthermore, the distinct polarity and basicity of the unprotected reaction products leads to a very challenging purification by silica gel chromatography. Up to 30% of ammonia had to be added to the eluent to avoid adsorption of the compounds on the silica gel. These so far unsolved problems of the purification of Amadori rearrangement products cause the relatively low yields in all examples. Interestingly, the benzyl group of compound **27** seems to be quite unstable in basic medium. Obviously, a transesterification with the solvent catalyzed by triethyl amine had taken place and both, the benzyl ester **33** as well as the corresponding ethyl ester **32,** were isolated.

The mechanism for the Amadori rearrangement when applied on heptose **19** is shown in Figure 17.

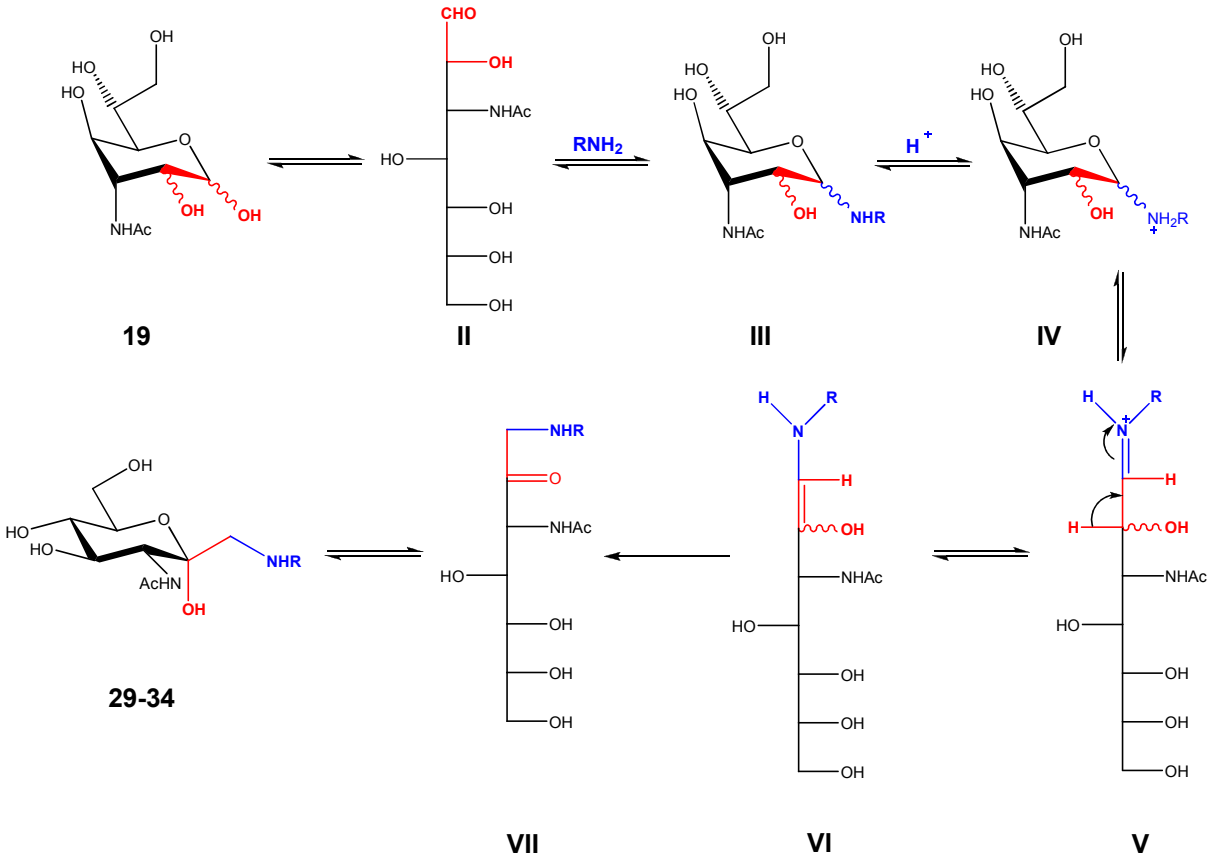


Figure 17: Mechanism of the Amadori rearrangement.

4. Conclusion and outlook

In this diploma thesis a suitable method for the synthesis of *C*-glycosyl type glycoconjugates composed of different amines and GlcNAc as the sugar moiety was successfully developed. Additionally, an appropriate strategy for the chain elongation towards heptose **19** could be found. Furthermore, the applicability of the Amadori rearrangement on heptose **19** could be confirmed.

The next step would be to optimize the yields of the Amadori rearrangement reaction by optimization of the reaction conditions and investigation of alternative purification methods such as HPLC or reversed phase column chromatography.

Additionally, other amino acids as well as smaller peptides as amino compounds should be applied on heptose **19** and other heptoses such as those derived from *N*-acetylgalactosamine which is of biological interest almost as much as GlcNAc. Such *C*-glycosyl type glycoconjugates can serve as building blocks for glycosylation reactions and monomers for peptide synthesis.

5. Experimental section

5.1. General methods and materials

NMR spectra were recorded on a Bruker Ultrashield 300 MHz (^{1}H 75.53 MHz and ^{13}C 300.36 MHz). Chemical shifts are listed in ppm employing residual, non-deuterated solvent as a standard. All spectra were measured at room temperature and data reported as follows:

Table 4: Abbreviations used for reporting NMR signals.

s	singlet
d	doublet
t	triplet
q	quadruplet
bs	broad singlet
dd	doublet of doublets
ddd	doublet of doublet of doublets
m	multiplet

Optical rotations were measured on a Perkin Elmer 341 polarimeter at the wavelength of 589 nm and a path length of 10 cm at 20°C. Analytical TLC was performed on precoated aluminium plates Silica Gel 60 F254 (E. Merck), detected with UV light (254 nm), 10% vanillin/sulphuric acid as well as ceric ammonium molybdate (100 g ammonium molybdate/8 g ceric sulphate in 1 L 10%H_2SO_4) and subsequent heating. Preparative TLC was performed on precoated glass plates Silica Gel 60, 0.5 mm (E. Merck). For column chromatography Silica Gel 60 (230-400 mesh, E. Merck) was used.
Unless otherwise stated, all chemicals were purchased commercially and used without further purification.

5.1.1. HCN-formation[65]

All equipment involved in the formation or reaction of HCN was placed in a special well-ventilated hood. The required amount of HCN was always freshly prepared. Therefore, a saturated solution of NaCN was added drop wise to an aqueous solution of H_2SO_4 (60%) at 80°C and the formed HCN transported

[65] Avi, M.; Gaisberger, R.; Feichtenhofer, S.; Griengl, H. *Tetrahedron*, **2009**, *65*, 5418-5426.

through a $CaCl_2$ drying column with a N_2 stream. Subsequently, the HCN was collected in a cooling trap at -12°C. All waste solutions and equipment were treated with aqueous NaOCl (10%) and subsequently neutralised with aqueous H_2SO_4.

5.1.2. Peracetylation

The respective sugar was dissolved in dry pyridine, a catalytic amount DMAP was added and the solution cooled to 0°C. Three equivalents of acetic anhydride per hydroxyl group were added drop wise and the reaction mixture allowed to come to room temperature and stirred for 12 hours. The reaction was quenched by addition of MeOH and worked up by subsequent dissolution with CH_2Cl_2, extraction with aqueous $NaHCO_3$, aqueous HCl and evaporation of the organic fractions. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure.

5.2. Experimental procedures

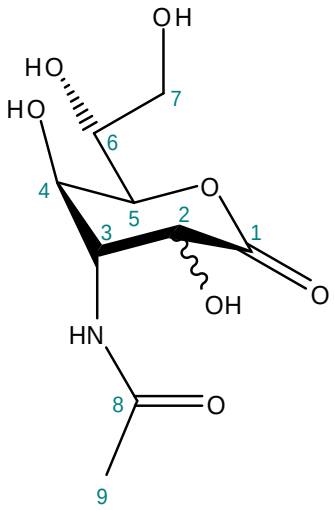
5.2.1. 3-Acetamino-3-deoxy-D-*gluco*-D-*ido*/D-*gulo*-heptonolactone **(21)**

Method A: Compound **18** (3 g, 13.56 mmol) was dissolved in water (20 ml) at 0°C, NaCN (1.20 g, 24.40 mmol, 1.8 eq) was added and the solution was stirred at 0°C for 12 hours. After addition of another equivalent of NaCN (0.66 g, 13.56 mmol) at 0°C, the reaction mixture was allowed to come to room temperature, stirred for 24 hours and then heated under reflux for 12 hours. The pH was then adjusted to 4 with ion exchanger Amberlite IR 120^+ (washed with H_2O). The resin was filtered off and the filtrate concentrated *in vacuo* to give a yellow oil.

Method B: Compound **18** (3 g, 13.56 mmol) was dissolved in water (20 ml) at 0°C, NaCN (1.20 g, 24.40 mmol, 1.8 eq) was added and the solution stored in the fridge for 4 days. After heating under reflux for 12 hours, the initial pH of 14 was adjusted to 6 via acetic acid. Solvent removal *in vacuo* afforded a yellowish oil which was subjected to column chromatography.

Method C: Compound **18** (3 g, 13.56 mmol) was dissolved in water (20 ml) at 0°C, NaCN (1.20 g, 24.40 mmol, 1.8 eq) was added and the solution stored in the fridge for 4 days. The pH was adjusted to neutral with acetic acid. $Pd/BaSO_4$

was added and the reaction mixture stirred for 4 hours under H_2-atmosphere. The catalyst was removed by filtration, rotary evaporation and further cleaning by silica gel chromatography afforded the title compound.

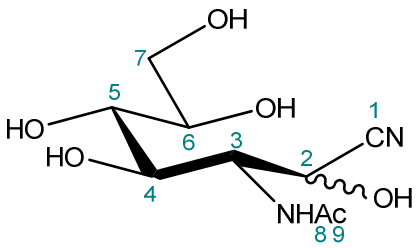


TLC- S: $CHCl_3$/MeOH /H_2O 1/2/0.25
MW: 249.22 ($C_9H_{15}NO_7$)
C- S: $CHCl_3$/MeOH/H_2O 1/1/0.25- 1/2/0.25- 1/4/0.25- 0/1/0
Yield: 45%

NMR- data: ^{13}C (D_2O): δ=177.6 (C-1); 174.1 (C-8); 72.6 (C-6); 72.2 (C-5); 71.3 (C-2); 68.2 (C-4); 62.5 (C-7); 54.1 (C-3); 22.2 (C-8). **^{1}H:** δ= 4.12 (dd, 1H, $J_{3,4}$= 4.5 Hz, H-3); 4.06-4.01 (m, 1H, H-6); 3.94 (dd, 1H, $J_{4,5}$=3.6 Hz, H-4); 3.74-3.41 (m, 4H, H-2, H-5, H-7, H-7'); 1.9 (s, 3H, H-9).

5.2.2. 3-Acetamino-3-deoxy-D-*gluco*-D-*ido*/D-*gulo*-heptononitrile **(23)**

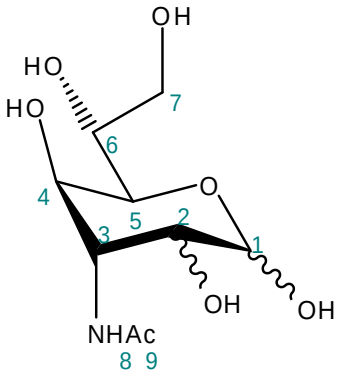
GlcNAc **18** (3 g, 13.56 mmol) was dissolved in dry pyridine (45 ml, 0.56 mol). To the slightly yellow suspension consecutively triethyl amine (0.15 ml, 1.08 mmol, 12.6 eq) and hydrogen cyanide (4.5 ml, 0.115 mol, 8.48 eq) were added. After 4 days quantitative conversion was observed through TLC of the peracetylated reaction mixture. The solvent was removed under reduced pressure. Crystallization started during first or second codistillation with ethanol and was finished after 2 hours in the fridge. The white crystals were washed with a 1:1 mixture of EE/diethylether. Compound **23** was used in the next step without further characterization.



MW: 248.23 ($C_9H_{16}N_2O_6$)
Crude yield: 100 %
TLC- S: EE/C 4/1 (for peracetylated compound)

5.2.3. 3-Acetamino-3-deoxy-D-*gluco*-D-*ido*/D-*gulo*-heptose **(19)**

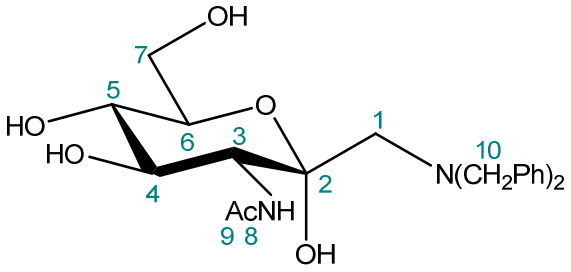
To a solution of compound **23** (3.5 g, 14.1 mmol) in water (39 ml) acetic acid (1.23 ml, 21.5 mmol, 1.52 eq) and Pd/$BaSO_4$ (2.1 g) were added and the reaction mixture stirred under H_2-atmosphere for 72 hours. After TLC of the peracetylated reaction mixture showed acceptable conversion of the starting material, the pH was adjusted to 3 with Amberlite IR 120^+ (washed with H_2O) and the reaction mixture stirred for 30 min. The solvent was evaporated and twofold codistillation from water (20 ml each) afforded white crystals which were subjected to the next steps without further purification.



MW: 251.23 ($C_9H_{17}NO_7$)
Crude yield: 80 %
TLC- S: EE/C 4/1 (for peracetylated compound)

5.2.4. 1-(*N,N*-Dibenzyl)amino-2-acetamino-1,2-dideoxy-α-D-*gluco*-hept-2-ulopyranose **(29)**

Compound **19** (300 mg, 1.19 mmol) was dissolved in EtOH (6 ml), DBA (0.46 ml, 2.38 mmol, 2 eq) and acetic acid (0.12 ml, 2.14 mmol, 1.8 eq) were added to give a slightly orange solution. The mixture was stirred at 40°C for 3 hours. When TLC showed almost quantitative conversion of the starting material, the reaction mixture was reduced under diminished pressure and the afforded oil subjected to chromatography.

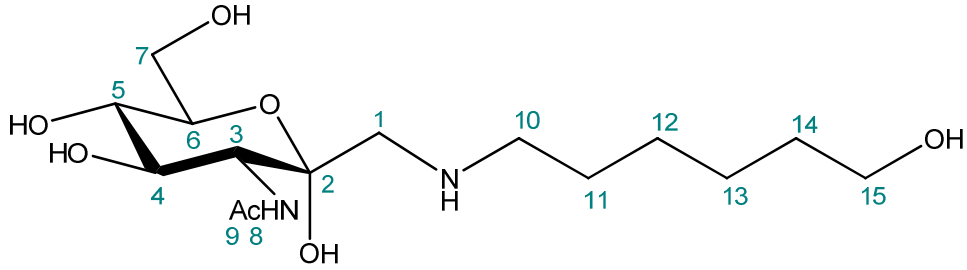


TLC- S:	$CHCl_3$/MeOH /NH_3 3/1/1%
C- S:	$CHCl_3$/MeOH /NH_3 8/1/1%
MW:	430.49 ($C_{23}H_{30}N_2O_6$)
$[\alpha]_D^{20}$:	+62.41 (c 0.9 MeOH)
Yield:	41 %

NMR- data: ^{13}C (MeOD): δ= 173.4 (C-8); 140.1, 130.4; 129.6, 128.5 (6C, Ph); 98.3 (C-2); 74.4, 73.8 (2C, C-4, C-5); 72.3 (C-6); 63.1 (C-7); 60.5 (2C, C-10, C-10'); 57.5 (C-1); 56.8 (C-3); 23.0 (C-9). **^{1}H:** δ = 7.23-7.07 (m, 10H, H-Ph); 3.98 (d, 2H, $J_{10,10}$=13.2 Hz, H-10, H-10'); 3.71-3.43 (m, 5H, H-3, H-4, H-5, H-7, H-7'); 3.31-3.25 (m, 1H, H-6); 3.26 (d, 2H, $J_{10,10}$=13.0 Hz, H-10, H-10'); 1.47 (s, 3H, H-9).

5.2.5. 1-(*N*-6-Hydroxyhexyl)-amino-2-acetamino-1,2-dideoxy-α-D-*gluco*-hept-2-ulopyranose **(31)**

Compound **19** (300 mg, 1.19 mmol) was dissolved in EtOH (6 ml) and subsequently 6-aminohexanol **(26)** (0.22 g, 1.90 mmol, 1.6 eq) and acetic acid (0.26 ml, 4.54 mmol, 3.8 eq) were added to give a white suspension. The reaction mixture was stirred at 60°C for 24 hours and consecutive removal of the solvent in vacuo afforded a brown oil which was purified via column chromatography.

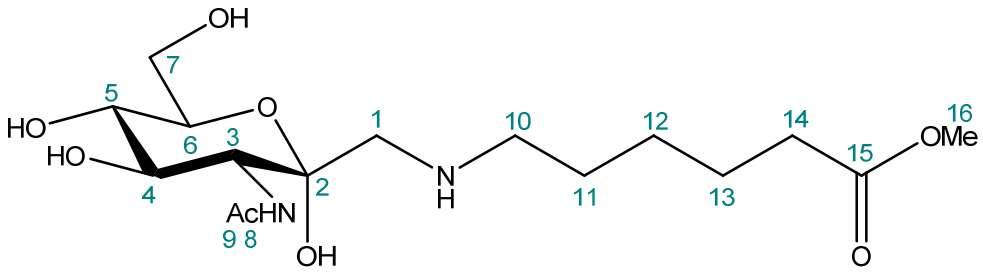


TLC- S:	$CHCl_3$/MeOH /NH_3 2/1/30%
C- S:	$CHCl_3$/MeOH /NH_3 5/1/30%
MW:	350.41 ($C_{15}H_{30}N_2O_7$)
$[\alpha]_D^{20}$:	+18.87 (c 3.1 MeOH)
Yield:	48%

NMR- data: ^{13}C (MeOD): δ= 174.3 (C-8); 98.1 (C-2); 74.2, 73.4 (2C, C-4, C-5); 71.8 (C-6); 63.0 (C-15); 62.6 (C-7); 56.1, 56.0 (2C, C-1, C-3); 50.9 (C-10); 33.6 (C-14); 30.0 (C-11); 28.0, 26.8 (2C, C-12, C-13); 22.8 (C-9). **^{1}H:** δ= 3.84-3.72 (m, 5H, H-3, H-4, H-5, H-7, H-7'); 3.58 (t, 2H, H-15); 3.47 (ddd, 1H, H-6); 2.79 (d, 1H, $J_{1,1'}$=12.3 Hz, H-1); 2.67 (t, 2H, H-10); 2.61 (d, 1H, H-1'); 2.05 (s, 3H, H-9); 1.64-1.51 (m, 4H, H-11, H-14); 1.45-1.36 (m, 4H, H-12, H-13).

5.2.6. 1-(*N*-6-Methylcarboxyhexyl)-amino-2-acetamino-1,2-dideoxy-α-D-*gluco*-hept-2-ulopyranose **(30)**

Methyl 6-aminohexanoate hydrochloride **(25)** (0.35 g, 1.9 mmol, 1.6 eq) was dissolved in EtOH (6 ml), Et_3N (0.26 ml, 1.9 mmol, 1.6 eq) was added and the reaction mixture stirred at room temperature for 30 min. After addition of compound **19** (300 mg, 1.19 mmol) the solution was kept at 40°C for 96 hours. The solvent was evaporated to afford a dark brown oil which was purified with chromatography.



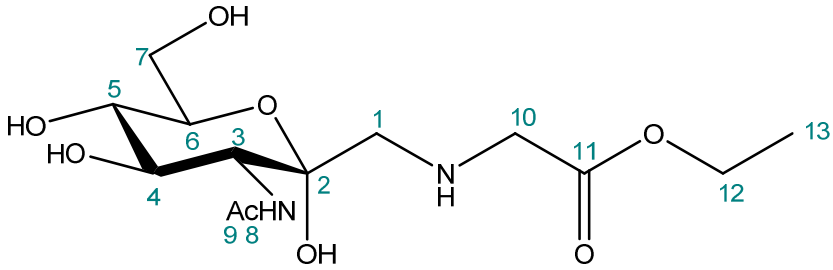
TLC- S: $CHCl_3$/MeOH /NH_3 3/1/1%
C- S: $CHCl_3$/MeOH /NH_3 5/1/1%
MW: 378.42 ($C_{16}H_{30}N_2O_8$)
$[\alpha]_D^{20}$: +5.70 (c 0.9 MeOH)
Yield: 25 %

NMR- data: ^{13}C (MeOD): δ= 175.7, 175.5 (2C, C-8, C-15); 96.7 (C-2); 74.5, 72.1 (2C, C-4, C-5); 71.4 (C-6); 61.7 (C-7); 56.1 (C-3); 54.1 (C-1); 52.2 (C-16); 49.5 (C-10); 34.5 (C-14); 27.1 (C-12); 26.8 (C-11); 25.4 (C-13); 22.6 (C-9). **^{1}H:** δ= 3.92-3.71 (m, 5H, H-3, H-4, H-5, H-7, H-7'); 3.69 (s, 3H, H-16); 3.49 (ddd, 1H, $J_{5,6=6,7}$=9.0 Hz, $J_{6,7'}$=0.6 Hz, H-6); 3.15-2.93 (m, 4H, H-1, H-1', H-10, H-10'); 2.41 (t, 2H, H-14); 2.10 (s, 3H, H-9); 1.86-1.64 (m, 4H, H-11, H-13); 1.54-1.40 (m, 2H, H-12).

5.2.7. 1-(*N*-2-Ethylcarboxyethyl)[a)]/(*N*-2-Benzylcarboxyethyl)[b)]-amino-2-acetamino-1,2-didesoxy-α-D-*gluco*-hept-2-ulopyranose [a)]**(32)**, [b)]**(33)**

Glycine benzyl ester hydrochloride (0.36 g, 1.79 mmol, 1.5 eq) was dissolved in EtOH (6 ml), Et_3N (0.25 ml, 1.79 mmol, 1.5 eq) was added and the mixture stirred at room temperature for 30 min. Compound **19** (300 mg, 1.19 mmol) was added and stirred at 40°C for 96 hours. After another 5 hours of heating under reflux, the solvent was removed under reduced pressure. Column chromatography afforded a mixture of compound **32** and **33**.

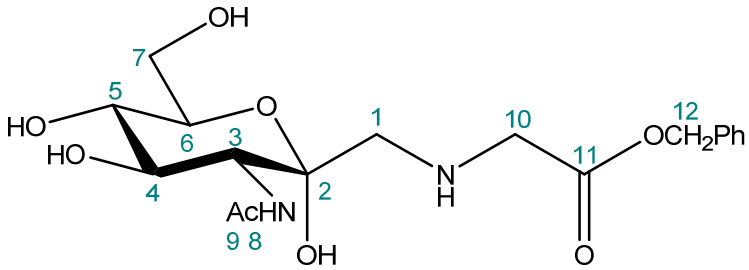
32:



TLC- S:	$CHCl_3$/MeOH /NH_3 3/1/1%
C- S:	$CHCl_3$/MeOH /NH_3 5/1/10%
MW:	336.34 ($C_{13}H_{24}N_2O_8$)
$[\alpha]_D^{20}$:	+25.12 (c 2.0 MeOH)
Yield:	8%

NMR- data: 32 : ^{13}C (MeOD): δ= 174.1, 173.7 (2C, C-8, C-11); 98.8 (C-2); 74.3, 73.9 (2C, C-4, C-6); 72.0 (C-5); 62.8 (C-7); 61.9 (C-12); 55.8 (C-3); 55.6 (C-1); 51.4 (C-10); 22.7 (C-9); 14.6 (C-13). **^{1}H:** δ= 4.21 (q, 2H, $J_{12,13}$= 7.13 Hz, H-12); 3.87- 3.69 (m, 5H, H-3, H-4, H-5, H-7, H-7'); 3.51-3.31 (m, 3H, H-6, H-10); 2.83 (d, 1H, $J_{1,1'}$= 12.5 Hz, H-1); 2.58 (d, 1H, H-1'); 2.04 (s, 3H, H-9); 1.29 (t, 3H, H-13).

33:

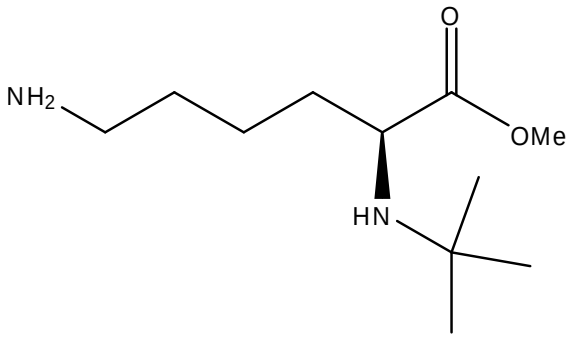


TLC- S:	$CHCl_3$/MeOH/NH_3 3/1/1%
C- S:	$CHCl_3$/MeOH/NH_3 5/1/10%
MW:	398.41 ($C_{18}H_{26}N_2O_8$)
$[\alpha]_D^{20}$:	+23.79 (c 0.8 MeOH)
Yield:	11%

33: ^{13}C (MeOD): δ= 174.1, 173.4 (2C, C-11, C-8); 137.4, 129.6, 128.0 (12C, C-Ph); 98.4 (C-2); 74.1, 73.9 (2C, C-4, C-5); 72.0 (C-6); 67.7 (C-12); 62.8 (C-7); 55.9 (C-3); 55.5 (C-1); 51.6 (C-10); 22.5 (C-9). **^{1}H:** δ= 7.32- 7.10 (5H, H-Ph); 5.08 (bs, 2H, H12); 3.76- 3.55 (m, 5H, H-3, H-4, H-5, H-7, H-7'); 3.46-3.24 (m, 3H, H-6, H-10); 2.72 (d, 1H, $J_{1,1'}$=12.5 Hz, H-1); 2.48 (d, 1H, H-1'); 1.90 (s, 3H, H-9).

5.2.8. Boc-Lys-OMe **(35)**

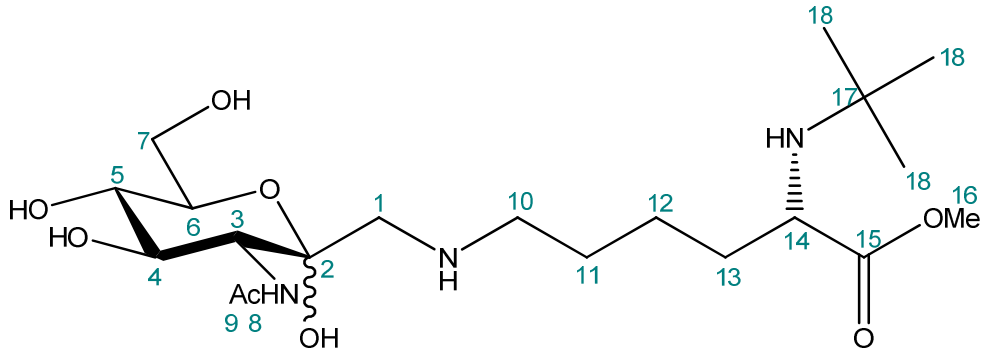
Boc-Lys(Z)-OMe (510 mg, 1.29 mmol) was dissolved in MeOH abs. (10 ml) and drugged with a small amount of $Pd(OH)_2$ on charcoal. After a 30 min-reaction under H_2-atmosphere, the catalyst was removed by filtration and the solvent removed under diminished pressure. The product was reacted on without further purification.



TLC- S: $CHCl_3$/MeOH /NH_3 3/1/1%
MW: 294.35 ($C_{15}H_{22}N_2O_4$)
Yield: 95%

5.2.9. 1-(*N*-5*S*-Benzyloxycarbonylamino-6-methylcarboxypentyl)-amino-2-acetamino-1,2-dideoxy-α-D-*gluco*-hept-2-ulopyranose **(34)**

Compound **35** (0.36 g, 1.22 mmol, 1.03 eq) was dissolved in EtOH (6 ml), acetic acid (0.07 ml, 1.22 mmol, 1.03 eq) and heptose **19** (300 mg, 1.19 mmol, 1 eq) were added and the reaction mixture stirred at 50°C for 36 hours. The solvent was removed under diminished pressure to afford the crude product which was subjected to chromatography.



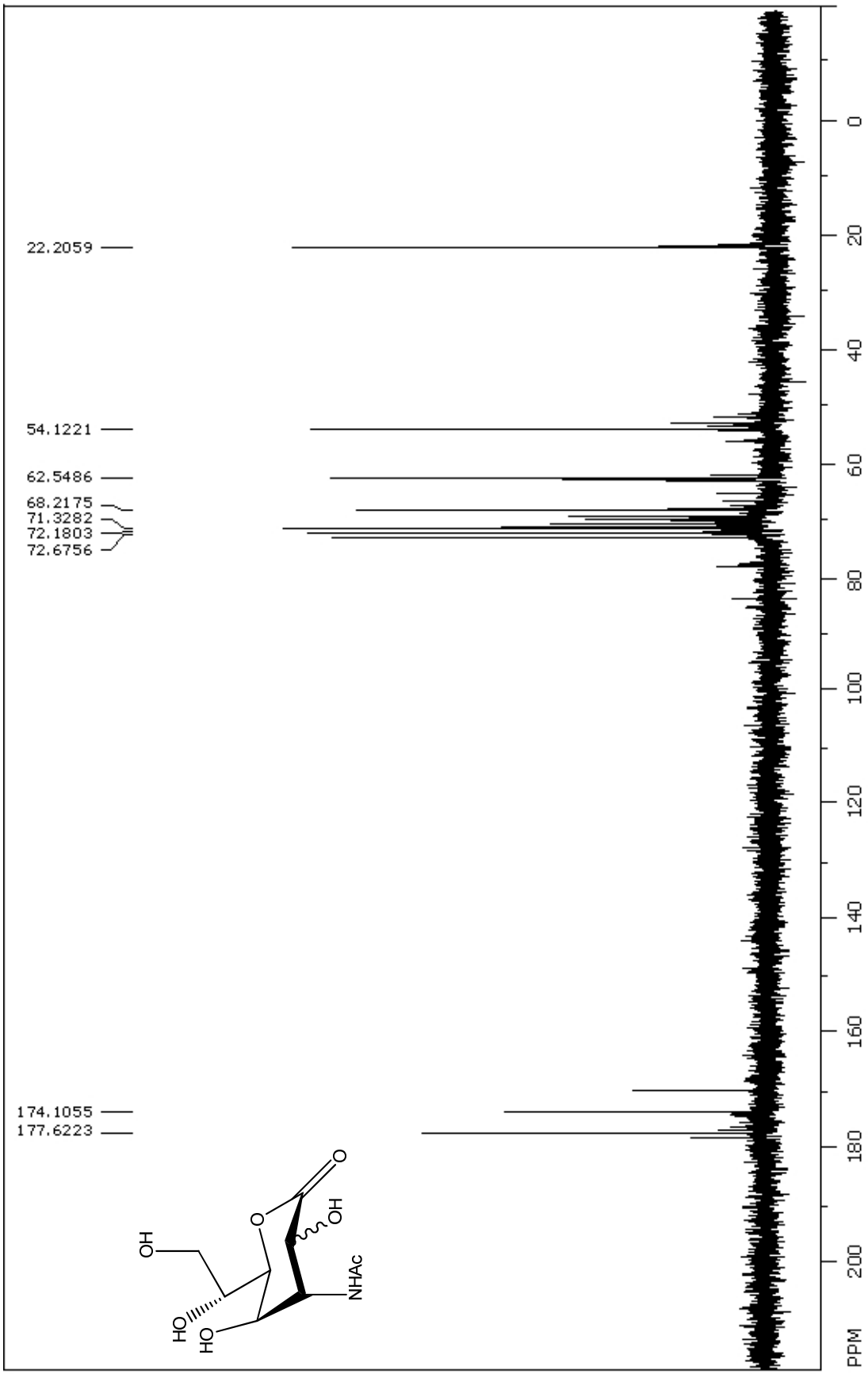
TLC- S:	$CHCl_3$/MeOH /NH_3 3/1/1%
C- S:	$CHCl_3$/MeOH /NH_3 7/1/10%
MW:	449.54 ($C_{20}H_{39}N_3O_8$)
$[\alpha]_D^{20}$:	+11.79 (c 4.0 MeOH)
Yield:	26%

NMR- data: ^{13}C (MeOD): δ= 175.1, 174.1 (2C, C-8, C-15); 98.3 (C-2); 80.6 (C-13); 74.1, 73.7 (2C, C-4, C-5); 71.8 (C-6); 62.6 (C-7); 56.3 (C-1); 55.9 (C-3); 55.1 (C-14); 52.7 (C-17); 50.7 (C-10); 32.5 (C-13); 29.9 (C-11); 28.8 (3C, C-18); 24.5 (C-12); 22.7 (C-9). **^{1}H:** δ= 4.15-4.07 (m, 1H, H-14); 3.88-3.71 (m, 5H, H-3, H-4, H-5, H-7, H-7'); 3.52-3.43 (m, 1H, H-6); 2.75 (d, 1H, $J_{1,1'}$= 12.2 Hz, H-1); 2.62 (t, 2H, H-10); 2.53 (d, 1H, H-1'); 2.04 (s, 3H, H-9); 1.86-1.37 (m, 6H, H-11, H-12, H-13); 1.47 (s, 9H, H-18).

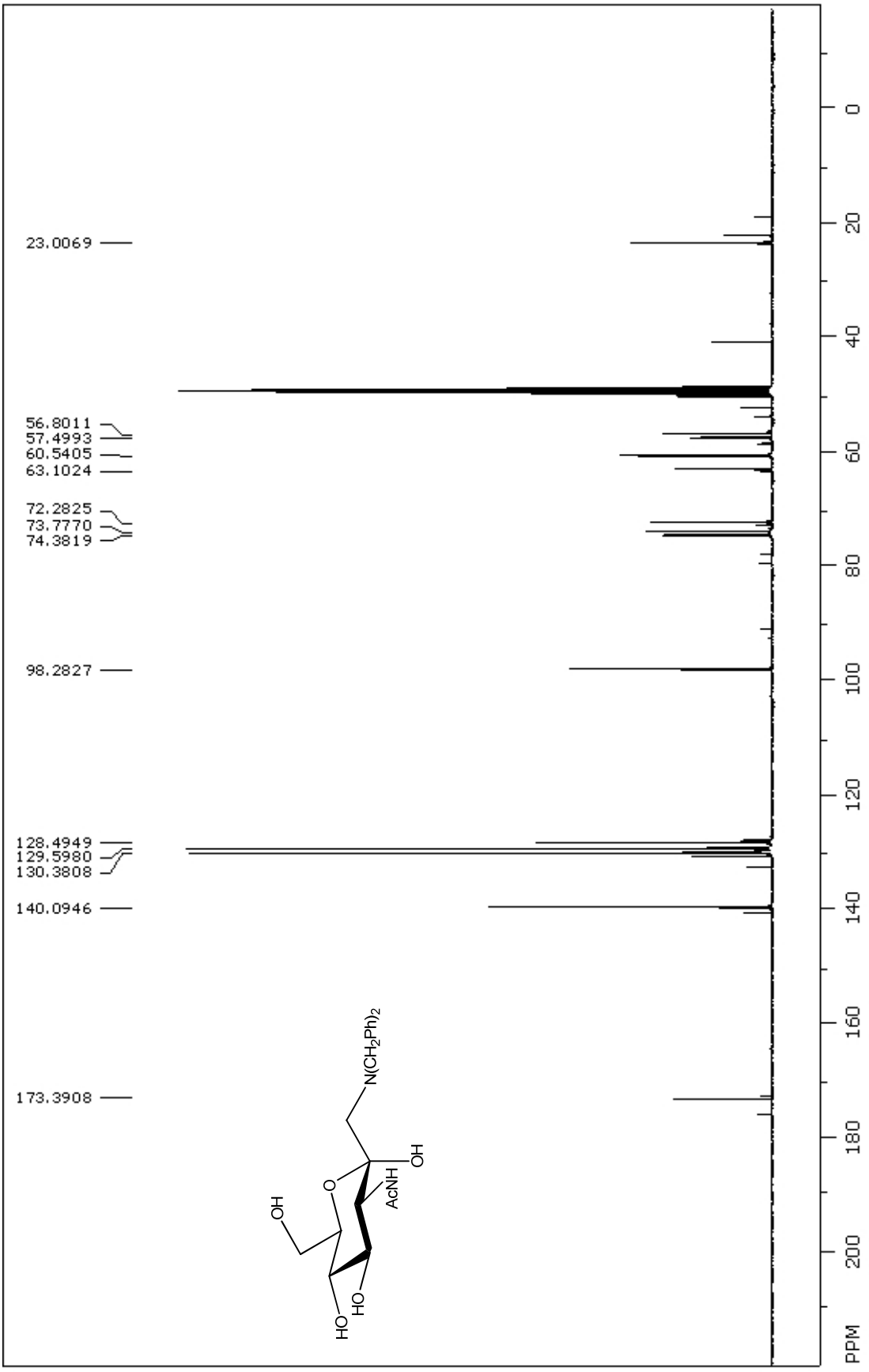
6. Appendix

^{13}C NMR Spectra of final products

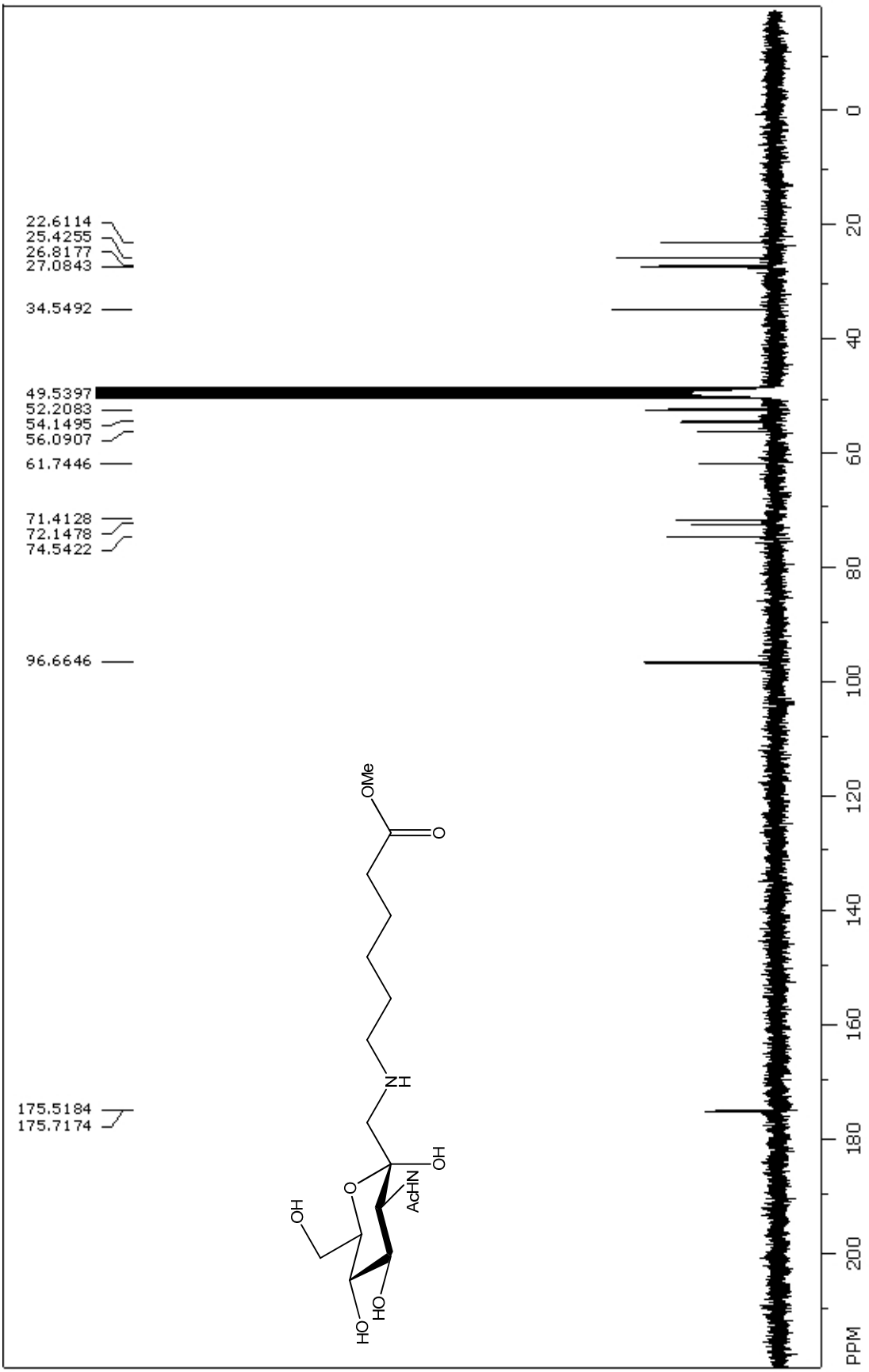
3-Acetamino-3-deoxy-D-*gluco*-D-*ido*/D-*gulo*-heptonolactone **(21)**



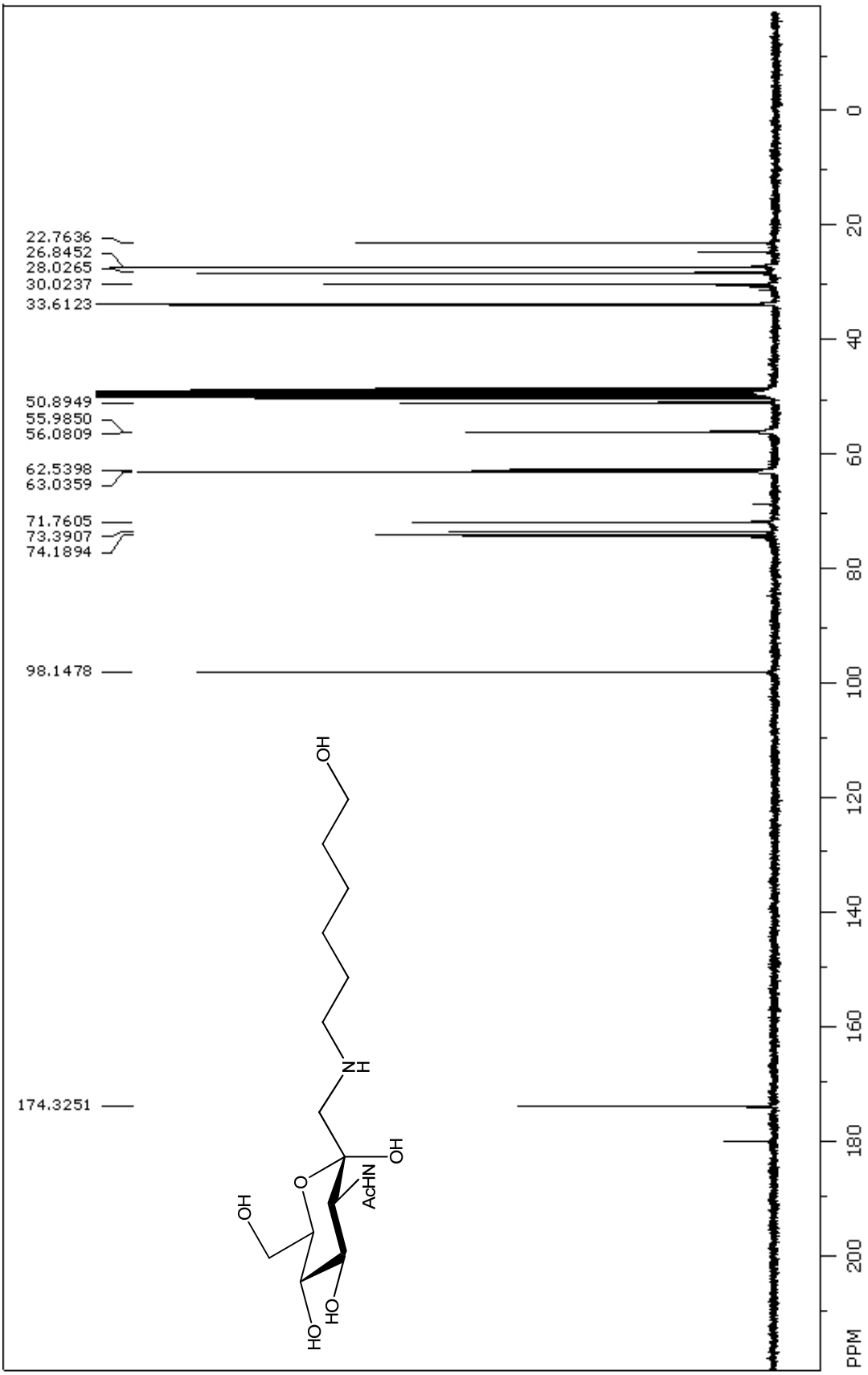
1-(*N,N*-Dibenzyl)amino-2-acetamino-1,2-dideoxy-α-D-*gluco*-hept-2-ulopyranose **(29)**



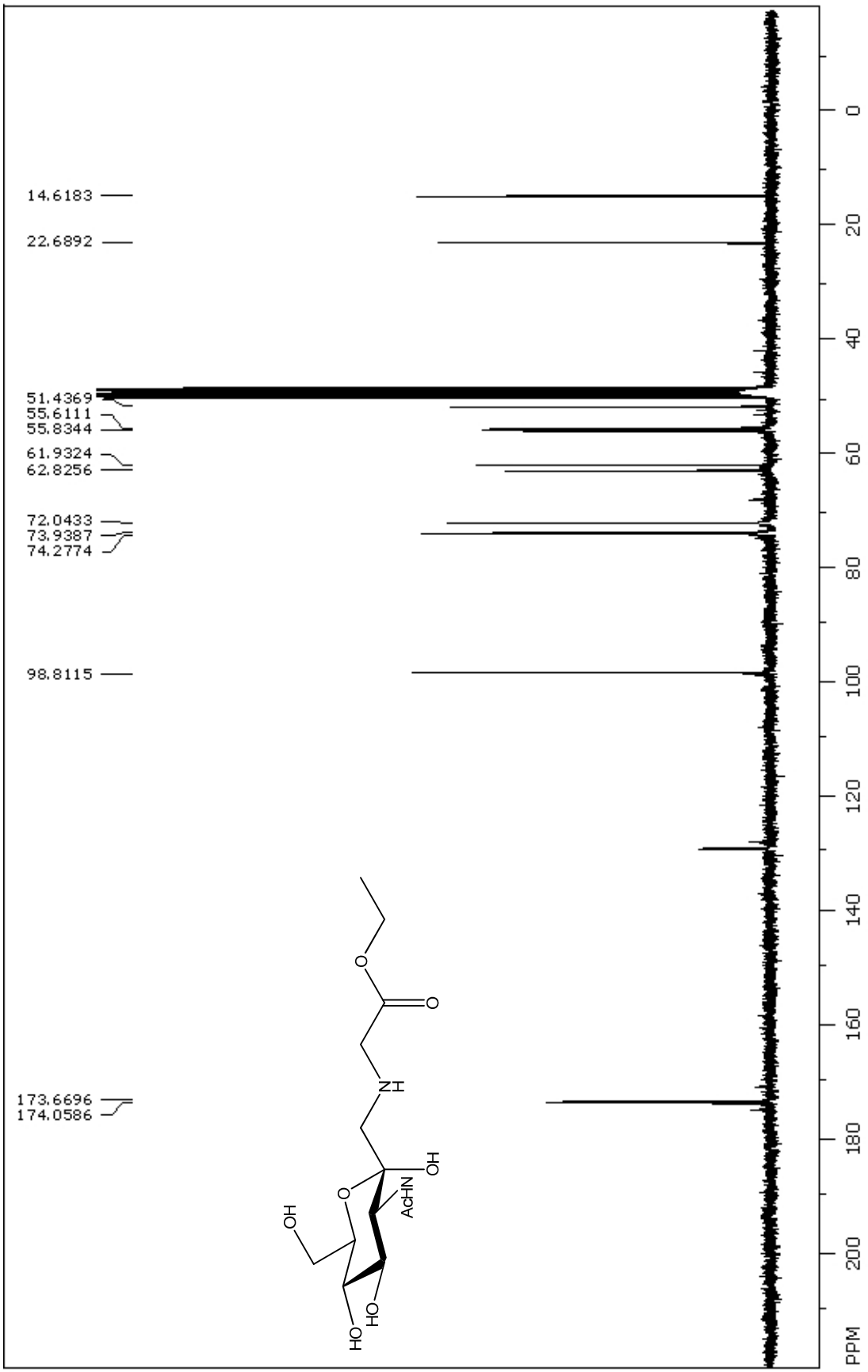
1-(*N*-6-Methylcarboxyhexyl)-amino-2-acetamino-1,2-dideoxy-α-D-*gluco*-hept-2-ulopyranose **(30)**



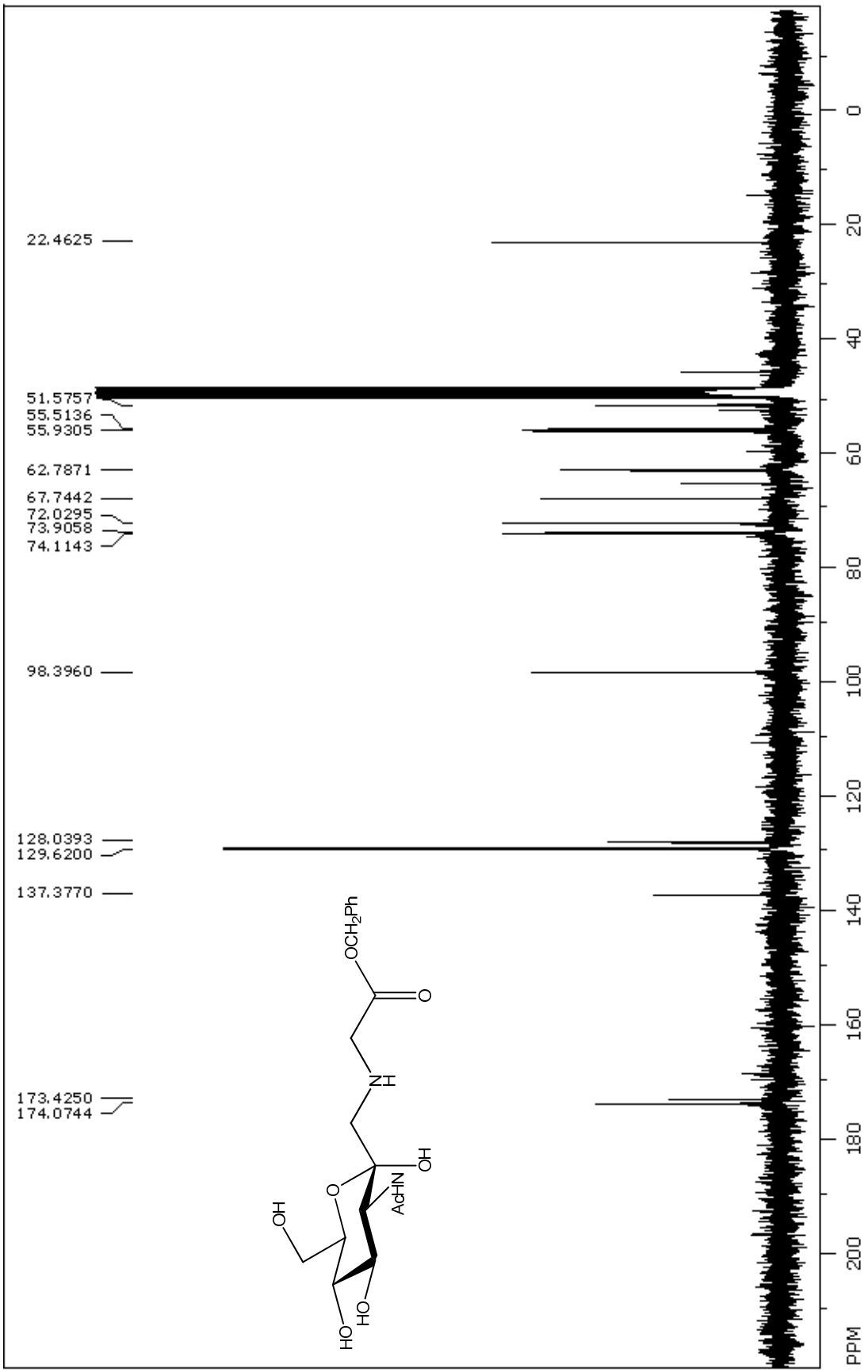
1-(*N*-6-Hydroxyhexyl)-amino-2-acetamino-1,2-dideoxy-α-D-*gluco*-hept-2-ulopyranose **(31)**



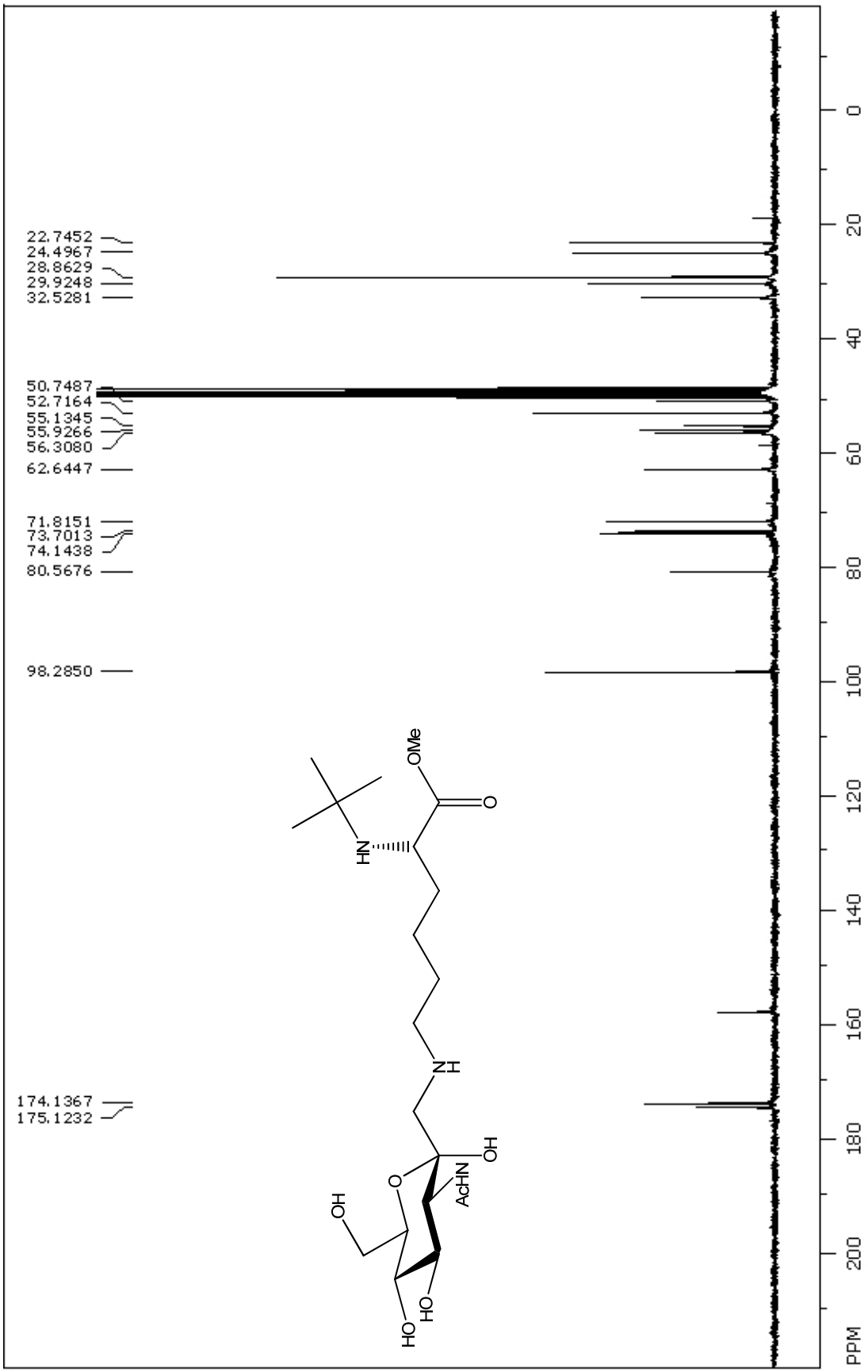
1-(*N*-2-Ethylcarboxyethyl)-amino-2-acetamino-1,2-didesoxy-α-D-*gluco*-hept-2-ulopyranose **(32)**



1-(*N*-2-Benzylcarboxyethyl)-amino-2-acetamino-1,2-didesoxy-α-D-*gluco*-hept-2-ulopyranose **(33)**



1-(*N*-5S-Benzyloxycarbonylamino-6-methylcarboxypentyl)-amino-2-acetamino-1,2-dideoxy-α-D-*gluco*-hept-2-ulopyranose **(34)**



7. Curriculum vitae

Gerit Maria Pototschnig
Born October 9, 1985 in Judenburg, Austria

Education

1992- 1996	Elementary school: Volksschule Moederbrugg
1996- 2004	Grammar school: BG/BRG Judenburg
Jul 2004	University Entrance Certficate, passed with distinction
Oct 2004 – present	Undergraduate studies, Technical Chemistry, Graz University of Technology
Oct 2007 – present	Undergraduate studies, Italian, Karl Franzens University Graz
Mar 2009 – present	Diploma thesis: ***"GlcNAc analogues by Amadori Rearrangement of 3-acetamino-3-deoxyheptoses"***

Internship

Sept 2006 – Oct 2006	Graz University of Technology, Institute of Organic Chemistry. Advisor: Assoc. Prof. Dipl.-Ing. Dr. *techn*. Tanja M. Wrodnigg
Jul 2007 – Aug 2007	Graz University of Technology, Institute of Organic Chemistry. Advisor Assoc. Prof. Dipl.-Ing. Dr. *techn*. Tanja M. Wrodnigg

8. Publications

8.1. Articles in peer-reviewed journals

1. G. Pototschnig, C. Morales De Csáky, J.R. Montenegro Burke, G. Schitter, A.E. Stütz, C.A. Tarling, J. Wicki, S.G. Withers, T.M. Wrodnigg, Biotin-Iminoalditol Conjugates: Molecular Probes for Glycoside Hydrolases, *in preparation.*

2. F. Adanitsch, G. Pototschnig, T.M. Wrodnigg, Synthesis of *C*-glycosyl Type Glycoconjugates of importanat Carbohydrates by the Amadori Rearrangement*, in preparation for the Journal of Carbohydrate Chemistry.*

8.2. Posters

1. "Synthesis of *N*-Acetylglucosamine Containing Glycoconjugates via the Amadori Rearrangement"
   G. Pototschnig, K. Gallas, T.M. Wrodnigg
   15th European Carbohydrate Symposium, Vienna, Austria, July 19 - 24, ***2009.***

2. "The Amadori Rearrangement as powerful method for the Synthesis fo *N*-Acetylglucosamine containing Glycoconjugates"
   G. Pototschnig, K. Gallas, T.M. Wrodnigg
   13. Österreichische Chemietage, Vienna, Austria, August 24 – 27, ***2009****.*

3. "Synthesis of D-*manno* and D-*galacto* Glycoconjugates via the Amadori Rearrangement"
   K. Gallas, G. Pototschnig, T.M. Wrodnigg
   15th European Carbohydrate Symposium, Vienna, Austria, July 19 - 24, ***2009.***

4. "The Amadori Rearrangement: Synthesis of D-*manno* and D-*galacto* Glycoconjugates"
   K. Gallas, G. Pototschnig, T.M. Wrodnigg
   13. Österreichische Chemietage, Vienna, Austria, August 24 – 27, ***2009****.*

8.3. Oral presentations

1. "*Synthese neuartiger lipophiler Iminoaldite mit interessantem chaperoning-Profil*"
   G. Schitter, E. Scheucher, M. Thonhofer, T.M. Wrodnigg, G. Pototschnig, A. Steiner
   12. Österreichischer Kohlenhydrat-Workshop, Universität für Bodenkultur, Vienna, Austria, February 2, ***2008.***

2. *"GlcNAc analogues by Amadori Rearrangement of 3-acetamino-3-deoxyheptoses"*
   G. Pototschnig, T.M. Wrodnigg
   14. Österreichischer Kohlenhydrat-Workshop, Universität für Bodenkultur, Vienna, Austria, February 11, ***2010****.*