

**Tools to Unravel Secrets of Glycobiology:
Synthesis of Glycocluster Peptides
and Functional Oligosaccharides**



Dissertation

Zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

submitted in English by
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Kiel 2008

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Defence date: 08-07-2008

Der experimentelle Teil der vorliegenden Arbeit wurde in der Zeit von Dezember 2003 bis Mai 2008 am Institut für Organische Chemie der Christian-Albrechts-Universität zu Kiel durchgeführt.

Frau Prof. Dr. Th. K. Lindhorst danke ich für die Überlassung des Themas, die zahlreichen Diskussionen und ihr stetes Interesse am Verlauf der Arbeit.

Declaration

The research work embodied in this thesis has been carried out under the supervision Prof. Dr. Th. K. Lindhorst, and no other than the mentioned sources and support have been used to accomplish this thesis. This work is original and has not been submitted in part or full, for any degree or diploma to this or any other university.

Harun A. Shaikh

Dedicated to my beloved parents

Index of Abbreviations

Abbreviations

| | |
|------------------------------------|--|
| Ac | Acetyl |
| Ac ₂ O | Acetic anhydride |
| Asp | Aspartic acid |
| BF ₃ ·Et ₂ O | Boron trifluoride diethyl etherate |
| Bn | Benzyl |
| BSA | Bovine serum albumin |
| Bz | Benzoyl |
| Cbz | Carboxybenzyl |
| CCA | α-cyano-4-hydroxycinnamic acid |
| CD | Circular dichroism |
| calcd. | Calculated |
| COSY | Correlated Spectroscopy |
| CRD | Carbohydrate Recognition Domain |
| CSA | Camphor sulphonic acid |
| d | Day |
| d (NMR) | Doublet |
| DBU | 1,8-Diazabicyclo[5.4.0]undec-7-ene |
| DCM | Dichloromethane |
| DHB | 2,5-Dihydroxybenzoic acid |
| DIC | Diisopropylcarbodiimide |
| DIPEA | <i>N</i> -Ethyl- <i>N</i> -diisopropylamine |
| DMF | <i>N,N</i> -Dimethylformamid |
| DMSO | Dimethylsulfoxid |
| ELISA | Enzyme-Linked ImmunoSorbent Assay |
| EtOH | Ethanol |
| Et ₂ O | Diethyl ether |
| Eq. | Equivalent |
| Fmoc | Fluorenylmethoxycarbonyl |
| Gly | Glycine |
| GPC | Gel permission chromatography |
| GPI | Glycosylphosphatidylinositol |
| GFP | Green fluorescent protein |
| h | Hour |
| HATU | <i>O</i> -(7-Azabenzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate |
| HBTU | <i>O</i> -(Benzotriazol-1-yl)- <i>N,N,N',N'</i> -tetramethyluronium hexafluorophosphate |

| | |
|-------------------|---|
| HCl | Hydrochloric acid |
| HCOOH | Formic acid |
| HOAt | 1-Hydroxy-7-azabenzotriazole |
| HOBt | 1-Hydroxybenzotriazole |
| HPLC | High performance liquid chromatography |
| HRESI | High resolution electrospray ionisation |
| HSQC | Heteronuclear Single Quantum Coherence |
| HMBC | Heteronuclear Multiple Bond Correlation |
| m (NMR) | Multiplet |
| MALDI | Matrix Assisted Laser Desorption Ionisation |
| Man | Mannose |
| m _c | Multitplate centred |
| MeOH | Methanol |
| min | Minute |
| MPLC | Medium pressure liquid chromatography |
| MS | Mass Spectroscopy |
| NIS | N-Iodo succinimide |
| NMR | Nuclear Magnetic Resonance |
| NOESY | Nuclear Overhauser Enhancement Spectroscopy |
| PBS | Phosphate buffered saline |
| PBST | Phosphate buffered saline, 0.05 % Tween20© |
| PCC | Pyridinium chloro chromate |
| Pd | Palladium |
| Py | Pyridine |
| q (NMR) | Quartett |
| quant. | Quantitativ |
| RP | Reversed Phase |
| RT | Room Temperature |
| s (NMR) | Singlet |
| Sat. | Saturated |
| Ser | Serine |
| SPPS | Solid Phase Peptide Synthesis |
| t (NMR) | Triplet |
| TBABr | Tetra butyl ammonium bromide |
| ^t Bu | <i>tert</i> -butyl |
| TFA | Trifluoro acetic Acid |
| TfOH | Trifluoromethanesulfonic acid |
| Tf ₂ O | Trifluoromethanesulfonic anhydride |
| THF | Tetrahydrofuran |

| | |
|-------|--|
| TLC | Thin Layer Chromatography |
| TMS | Trimethyl silane |
| ToF | Time of Flight |
| t_R | Retention time |
| TRIS | 2-Amino-2-hydroxymethyl-propane-1,3-diol |
| UV | Ultra violet |

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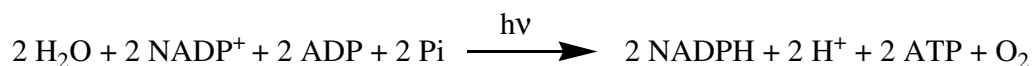
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1. Introduction

1.1 Carbohydrates in nature

Nucleic acids, proteins and carbohydrates are four major classes of organic macromolecules. The carbohydrates are one of the most important ones built up of monosaccharides units as a backbone.^[1] They are produced via photosynthetic pathway in which the energy of light is stored in the form of adenosine triphosphate (ATP) and nicotinic amide adenine dinucleotide phosphate (NADPH). In the later part CO₂ and H₂O are reduced to form carbohydrates and oxygen, a process which is also known as Calvin's cycle.



Starch and glycogen are high molecular weight structures made up of glucose and are important for the plants and animals. Besides their function as an energy source carbohydrates also form a framework in cell walls of animals and plants, e.g. as cellulose and chitin.

The deoxyribose and ribose are main constituents of respective DNA and RNA nucleotides and are responsible for the information transfer in translation and transcription processes. The structural diversity of the carbohydrates can also be viewed in the monomeric form; they make a whole realm of stereochemistry. As they exist in different forms like open or closed chains, in α - or β - form. They exist also in different ring forms as five membered rings known as furanoses and six membered rings as pyranoses. A carbohydrate monomer bears several functionalities either in the form of hydroxyl groups or in the form of amino groups which can be further functionalised to achieve different derivatives. The hydroxyl group at anomeric position can undergo glycosylation reactions to give higher degrees of carbohydrates known as oligo- and polysaccharides. e.g. through these possible variation the structural diversity of the carbohydrates is exceeding the diversity reached by oligonucleotides.^[2]

In the form of oligosaccharides, carbohydrates are important components of the cell membrane. These complex and highly branched macromolecular carbohydrate structures are embedded in the lipid bilayer membrane. This carbohydrate layer on the eukaryotic cell membrane is known as 'glycocalyx'. The thickness of this carbohydrate coating on

cells varies between different cell types and can reach up to 1400 Å.^[3,4] Cells are building their own glycocalyx through converting the monosaccharides, provided from outside of the cell in the endoplasmic reticulum (ER) and the Golgi apparatus, as shown in Figure 1.

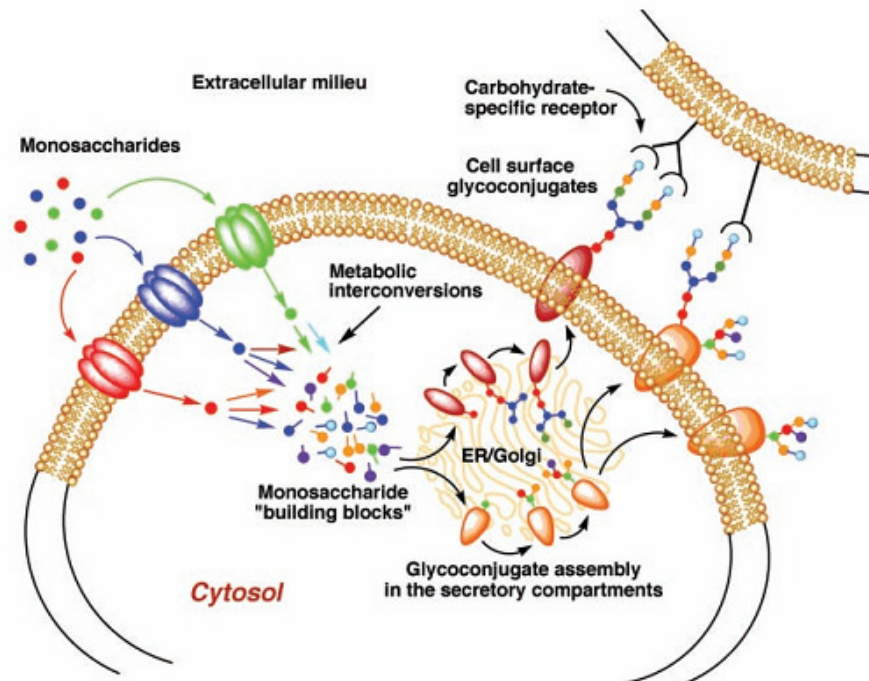


Figure 1: Cartoon, abbreviating the biosynthesis of complex cell surface oligosaccharides, which are important for molecular recognition processes.^[5]

In brief the monosaccharides are first converted into glycosyl donors and then taken to the endoplasmic reticulum (ER) to form glycoproteins, which are further processed in the Golgi apparatus, where they are converted into complex glycoproteins with assistance of glycosyl transferases. Besides glycoprotein synthesis, oligosaccharides are reacted with lipids to form so called glycolipids. Then the newly formed glycoconjugates are transported to the cell wall and are integrated in the lipid bilayer membrane in such a way that the carbohydrate moieties are exposed to the exterior of the cell.^[5]

The glycocalyx is characteristic for each cell type and typical for the development of the cells as well as pathogenic state of the cell. The carbohydrates on the cell surface have unique quality to distinguish diseased and healthy cells and that is why it plays an important role in various biological processes such as cell-cell communications, infection processes and phagocytosis.

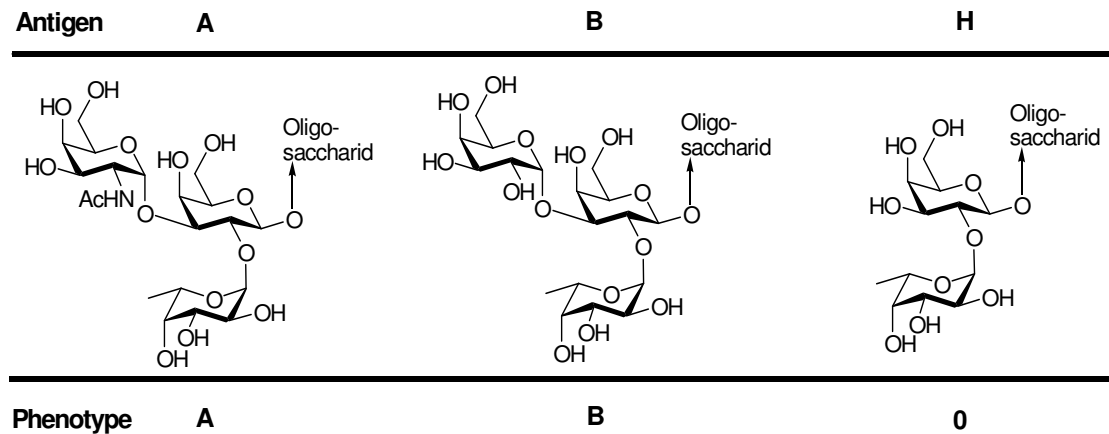


Figure 2: Variation of terminal oligosaccharide constituents of erythrocytes in various blood groups.

A very good example for the biological relevance of the glycocalyx is the glycoconjugates present on the erythrocytes that are determining the blood groups. A very small structural variation in the terminal saccharide units is differentiating the blood groups (Figure 2). The basic structure is L-fucose which is bound to the 2-position of D-galactose. In the case of blood group A the terminal galactose moiety of the erythrocyte glycoprotein is glycosylated to *N*-acetyl-galactosamine whereas in blood group B it is attached to galactose by α -1,3-GalNAc transferase and α -1,3-Gal transferase respectively. Exceptionally, antigen-H is expressed on the cells of all group 0 individuals, where the terminal galactose is not further glycosylated.^[6]

1.2 Carbohydrate-protein interactions

Lectins belong to a class of proteins which is found in most organisms ranging from micro organisms to plants and animals. Lectins, which vary in form, size and arrangement of the recognition units can interact specifically with carbohydrates. Thus, lectins are carbohydrate-specific proteins, but not enzymes or anti-carbohydrate antibodies. The protein-carbohydrate interaction is strongly influenced by hydrogen bonds, often water molecules, or Ca^{2+} ions that have a complexation effect. Another binding effect is caused by van der Waals interactions between the hydrophobic parts of the carbohydrate and the aromatic side chains of the protein.

Lectins bind mono- and oligosaccharides reversibly and with high relatively specificity. Typically, they can be divalent or polyvalent molecules and the binding sites are referred as CRD (Carbohydrate Recognition Domain). They are historically classified into five groups on the basis of their specificity for D-mannose, D-galactose, D-*N*-acetylgalactosamine, D-*N*-acetylglucosamine, L-fucose and D-*N*-acetylneuraminic acid, which are typical constituents of surfaces of eukaryotic cells.^[7] When they interact with the cell e.g. in case of the erythrocytes they not only interact with carbohydrates but also cause crosslinking of these cells resulting in the precipitation called as cell agglutination. (They are also crosslinked with polysaccharides and glycoproteins resulting in their precipitation.)

Nowadays lectins are divided into groups according to their amino acid sequence and 3D-structure. There are three important types of lectins called as C-type, S-type and P-type lectins. Their binding mode is as follows: C-type lectins are Ca^{2+} -dependent lectins that can bind to the large variety of sugars, while the S-type (galectine) binds selectively to the galactose and *N*-acetylgalactosamine, and the P-type binds specifically to mannose. The selectins are crucial members of C-type lectins and are classified according to location. L-selectins are found on lymph nodes, E-selectins on endothelial cells and P-selectins on platelets.

An example of lectin-carbohydrate interaction is the leucocyte recruitment process^[8] (Figure 3). In case of injury, leucocytes are transported to the site of injury in the early stage of immune response. Leucocytes are producing selectins on their surface while endothelial cells are also producing selectins on their surface as immune response. In case of injury, the endothelial cells are activated by cytokines expressing P- and E-selectins on their surface which are recognized by the carbohydrate ligand sialyl-Lewis-X (sLe^x). It is a tetrasaccharide (Figure 4) that binds all three selectins and is present on both the

leucocytes and endothelial layer. The interaction between sLe^x and E- and P-selectin leads to slowing down of leucocytes known as 'rolling' process.

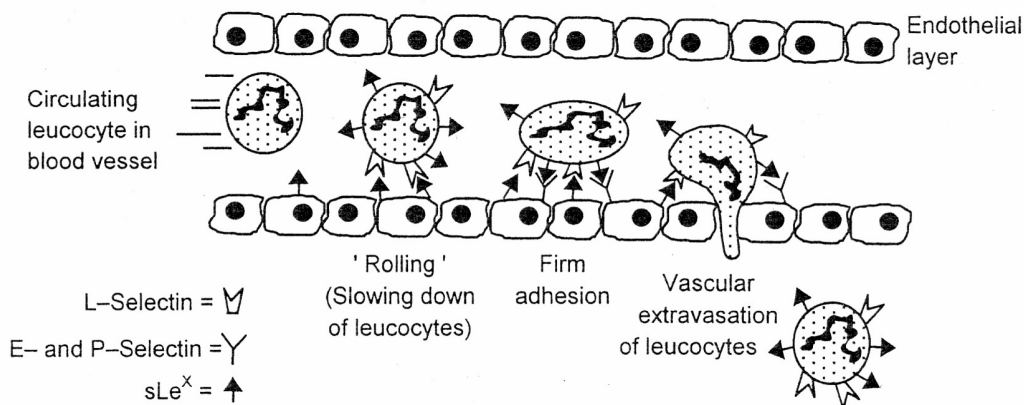


Figure 3: Leucocyte employment at the site of injury.^[15]

In the next step there are strong protein-protein interactions leading to firm adhesion of leucocytes to the endothelial layer and then through vascular extravasation the leucocyte is migrated to the site of injury.

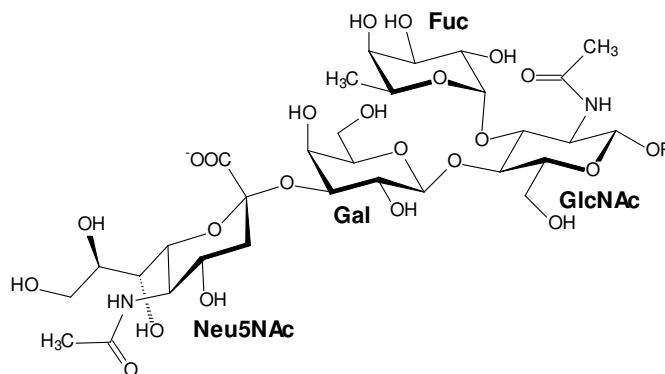


Figure 4: Structure of sLe^x.

Another example of a process, which is mediated by carbohydrate-protein interactions is the fertilisation of egg by sperm which is a key event in the fertilisation process.^[9] The mammalian egg is enclosed by two layers which have to be penetrated by the sperm for fertilisation (Figure 5). The outer layer is made up of cumulus cells and inner layer is made up of cell type-specific glycoproteins ZP1, ZP2 and ZP3 called as zona pellucida. The outer layer of egg is penetrated by the sperm with the help of its hyper-activated

motility and glycosylphosphatidylinositol (GPI)-anchored surface hyaluronidase, called PH-20.

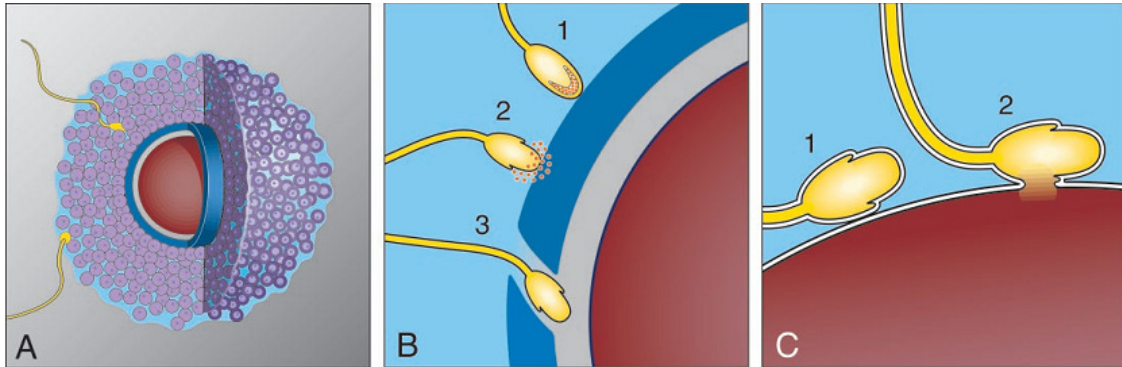


Figure 5: (A) Sperm penetration of the cumulus cells to reach zona pellucida. (B) Sperm 1 binds to zona pellucida, exocytosis of sperm 2 and release of acrosomal content, sperm 3 penetrates the zona pellucida. (C) Sperm 1 binding to plasma membrane of egg and fusion of sperm 2 with egg.^[10]

Then the sperm reaches the zona pellucida and binds to it, which leads to exocytosis, releasing the content of acrosome. In particular the proteins on the sperm bind to the *O*-linked galactoside on the ZP3, which is essential for sperm receptor activity. In absence of these proteins sperm adhesion is not possible.^[10,11]

1.3 Multivalency

The interaction between proteins and carbohydrates is based on non-covalent interactions like H-bonding, van der Waals interactions, electrostatic interactions or the interactions with divalent cations. Multivalency is a natural phenomenon occurring in many biological processes. Multivalent interactions occur between the most diverse biological units, like cells and viruses, cell-cell, cell-molecule and molecule-molecule contacts. The attachment of viruses to the host cell, e. g., is the first event in the infection process. The polyvalent surface of viruses interacts with polyvalent surface of the host cell to give firm adhesion. In later step the cell undergoes endocytosis and releases DNA of virus in cytoplasm.^[12]

In order to understand the phenomenon of multivalency many multivalent ligands have been synthesized and tested. The multivalent ligand can bind to the receptors with high avidity to make them become potent inhibitors. The potency of a multivalent ligand depends on its mode of action (Figure 6). Monovalent ligands bind to single receptor (a) and seldom dimerize whereas the multivalent ligands interact with their receptor in different binding-modes. These are termed as chelate effect (b), subsite binding (c), steric stabilisation (d), statistical rebinding (e) and receptor clustering (f).^[13]

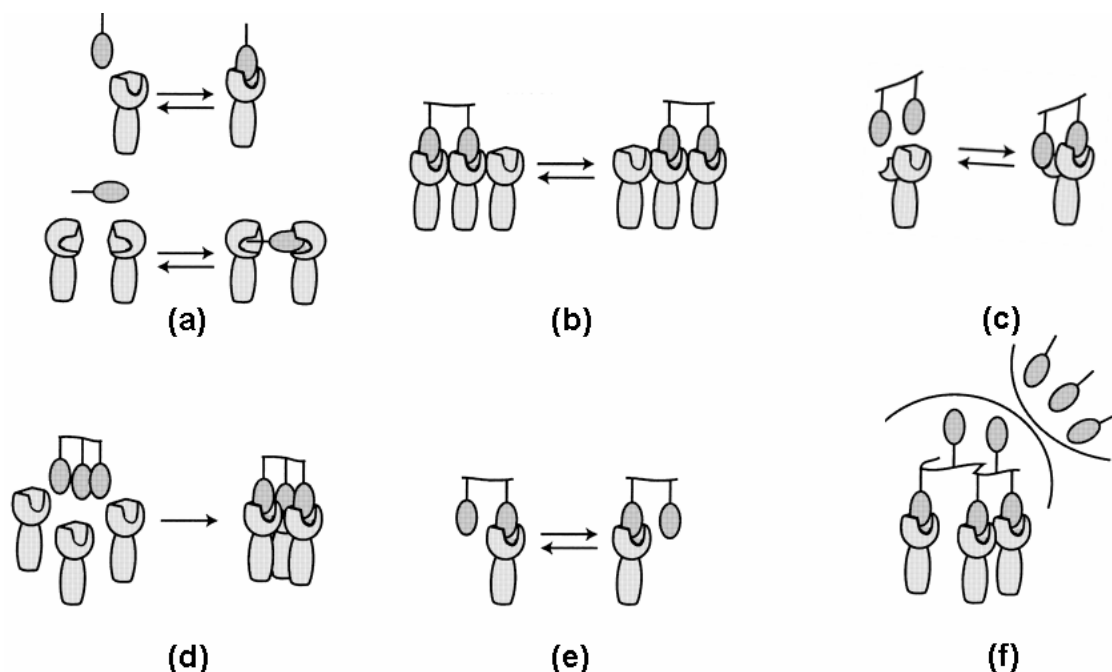


Figure 6: Mechanism of ligand-receptor binding. (a) Monovalent ligand binding; (b) Chelate effect; (c) Subsite binding; (d) Receptor clustering; (e) Statistical effect; (f) Steric stabilization.^[13]

By multivalency a single weak bonding interaction is strengthened through multiple interactions. The monovalent interaction between carbohydrate and protein is in the millimolar to upper micromolar range. In order to have a biological signal, a sufficient stability of the receptor ligand complex is essential.

Due to the multiple interactions between several carbohydrate structures with multiple receptors the strength and specificity of an interaction can be increased. This allows not only large scale surface contact, but also the fine setting of the interaction is possible. The stereochemistry of the carbohydrate and the three-dimensional arrangement of functional groups in a carbohydrate receptor complex are of crucial importance for the detection processes. This allows even the smallest structural change to have a major biological effect.

The strength of the multivalent interaction results from synergistic effect of all monovalent interactions. It can be described through three phenomena, the positive cooperation (synergistic effect), the non-cooperative (additive effect) and the negative cooperation (interfering) interaction. The cluster effect is an effect of positive cooperation, where a linear increase of the receptor ligand interaction leads to an exponential increase in the interaction strength. It was first described in 1978 by Y. C. Lee with the sialoglycoprotein receptor.^[14]

1.4 Glycoconjugates

The abundance of the carbohydrates and glycoconjugates found in nature varies from 1 % to 99 %. The glycoconjugates are composed of approximately twenty different monosaccharides out of which most common ones are L-fucose, D-galactose, D-mannose, D-*N*-acetylneuraminic acid, D-*N*-acetylgalactosamine and D-*N*-acetylglucosamine. Relatively small set of monosaccharide building blocks are used in the biosynthesis of glycoconjugates. The cell surface bears a thick layer of glycoconjugates, a glyco-coat in which additional glycoconjugates are hooked to the glycoproteins by adhesive forces. The glycoproteins are connected to the lipid bilayer through a hydrophobic transmembrane region with the carbohydrate unit normally bound through an *O*- or *N*-glycosidic linkage. These oligosaccharides are known as *O*-glycans or *N*-glycans, respectively.

Another kind of glycoconjugates are the glycolipids where the oligosaccharide unit is attached to the lipid molecules forming the lipid bilayer membrane. They are carrying a hydrophilic and a hydrophobic part making them amphiphilic molecules. The lipid part of the glycolipids often contains either 1,2-di-*O*-diacylglycerol or *N*-acylsphingosine. There is also a third type of glycoconjugates where the carbohydrates are linked to the proteins via ethanolamine phosphate; such linkage is found in glycosylphosphatidylinositols, known as GPI anchors.^[15]

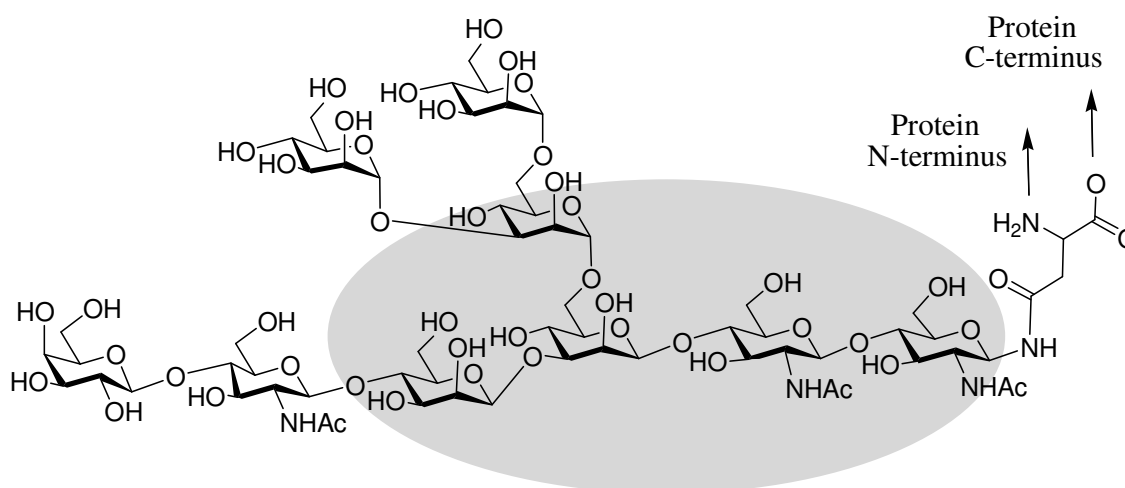


Figure 7: Hybrid type *N*-glycan (core pentasaccharide shaded in gray).

The *N*-glycans are further divided into different classes depending on the amount and type of the carbohydrates attached to the core pentasaccharide, which is common in all *N*-glycoproteins (Figure 7). It is always an asparagine chain of the protein, which is connected to *N*-acetyl glucosamine of the carbohydrate moiety. In the *O*-glycans, the saccharide is connected to a serine or a threonine side chain.

There are far more glycolipids than glycoproteins in the lipid bilayer membrane. The glycolipids are the other kind of glycoconjugates bearing a hydrophilic sugar part and hydrophobic lipid chains. They are mainly known as glycoacylglycerolipids and glycosphingolipids when a hydrophobic part attached to the oligosaccharide contains 1,2-di-*O*-diacylglycerol or *N*-acylsphingosine, respectively (Figure 8). The glycosphingolipids are an important part of eukaryotic cells where they are anchored to the plasma membrane exposing the oligosaccharide part to the exterior part of the cell. The hydrophobic *N*-acylsphingolipid contains a *trans*-configured double bond derived from ceramide.

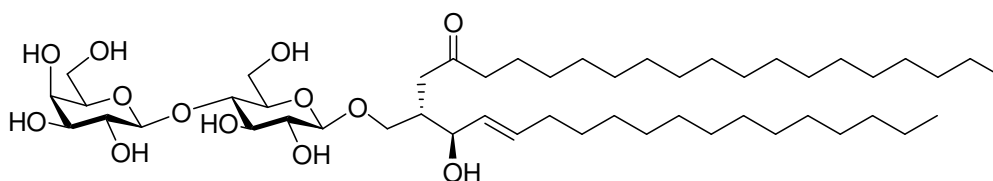


Figure 8: Structure of lactosyl ceramide.

Similar to glycosphingolipids, the lipid moiety could also be attached through a phosphate group in case of sphingophospholipids. However, in the case of glycerophospholipids, the lipid chains are provided by phosphoric acid esters.

1.5 Membrane proteins

It is well known that the biological membranes contain proteins as well as lipids. Eukaryotic plasma membranes typically contain about 50 % of protein. These proteins play an important role in various biological processes like in signal transduction, as ion channels, as sensors and are all glycosylated extracellularly. There are two kinds of proteins; the ones going through the membrane are called transmembrane proteins and can function on both sides of the membrane. They can transport molecules from one side to another whereas the proteins that are associated only with one side of the lipid monolayer can only function on that side of the membrane and are called membrane associated proteins.

A large number of proteins undergo post-translational modifications. Among these glycosylation is the most important one and about 50 % of all proteins are thought to be glycosylated.^[16] The oligosaccharide structures attached to membrane proteins are part of the 'glycocalyx', a complex supramolecular structure on the cell surface.

The transmembrane proteins are amphiphilic in nature having hydrophilic and hydrophobic regions. The hydrophobic region is interacting with the lipid bilayer membrane, whereas, the hydrophilic part is exposed to water. Membrane proteins can be classified into five different types (Figure 9); (A) type-1 membrane proteins; (B) type-2-membrane proteins; (C) multipass transmembrane proteins; (D) lipid chain-anchored membrane proteins and (E) GPI-anchored proteins.^[17]

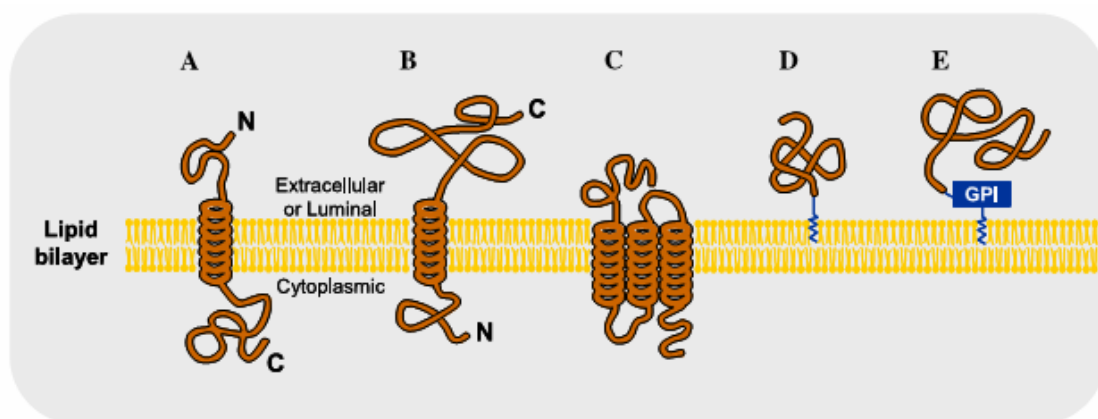


Figure 9: (A) type-1-membrane protein; (B) type-2-membrane protein; (C) multipass membrane protein; (D) lipid chain-anchored membrane protein; (E) GPI-anchored proteins.^[17]

The type-1-membrane protein is a single pass protein which passes through the lipid bilayer membrane and has its C-terminus in cytoplasm whereas the N-terminal end is projected towards the extracellular environment. The type-2-membrane protein is also a single pass protein like type-1-membrane protein but in this case the C-terminus is in the extracellular matrix while the N-terminal end is in cytoplasm. The third type, the multipass membrane protein stands for proteins passing several times through the bilayer membrane. The other two types are lipid chain-anchored proteins and GPI-anchored membrane proteins; they do not pass through the bilayer membrane.

2. Concept

The oligosaccharides present on eukaryotic cell surfaces are involved in many important biological processes, e.g. they play a major role in cell-cell signalling. To understand a process like 'signalling', in which information is brought into the cell from outside and vice versa, study of glycoproteins is important. With the help of mimics of the natural structures, such as the herein introduced glycocluster peptides, such studies could be facilitated to enhance the knowledge about the functions of glycoproteins in cell communication

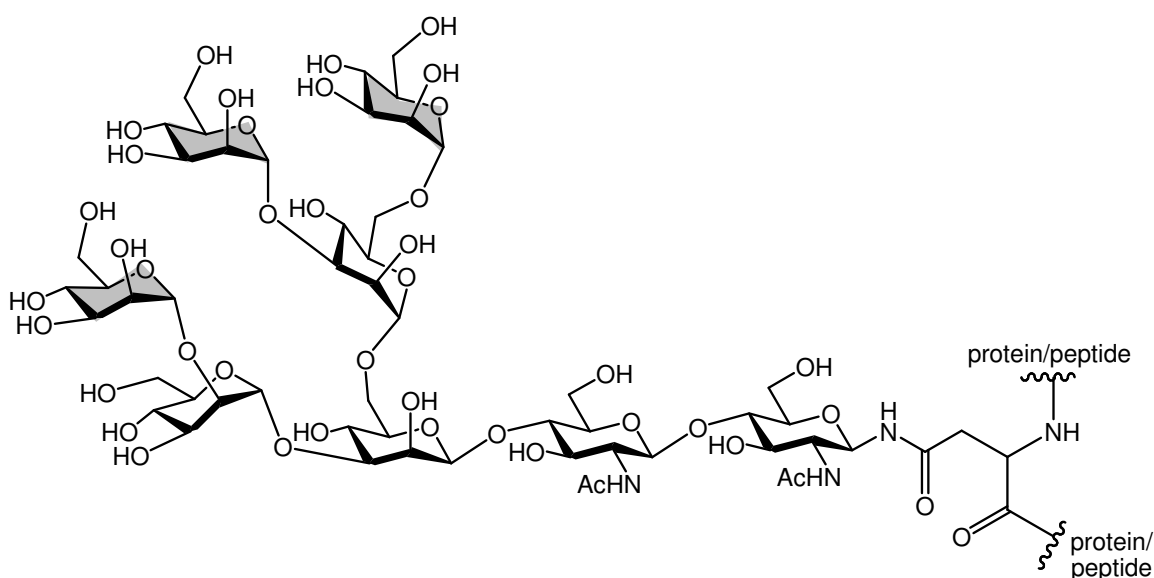


Figure 10: Structure of oligosaccharide moiety found in natural glycoproteins (gray sugars indicate outer layer of the glycoprotein).

As glycoconjugates obtained from nature are limited for research due to the low amounts and microheterogeneity, in this thesis, a feasible method of achieving simple glycoprotein mimics is described where dendrimer chemistry was employed to receive oligosaccharide mimics (Figure 11). The chosen molecular design should allow to test, which carbohydrates are important in cell recognition and what effect occur on the carbohydrate moiety upon interaction with carbohydrate-specific proteins, such as the lectins. The designed glycopeptide mimetics could also help to understand the effect of molecular recognition processes on the supramolecular structure of the peptide and protein part, respectively. The targeted glycocluster peptide could be synthesized in two parts: (a) a glycocluster part mimicking an oligosaccharide (Figure 11 shaded in gray) and (b) the

peptide part which can represent the transmembrane protein part of the natural glycoprotein structures.

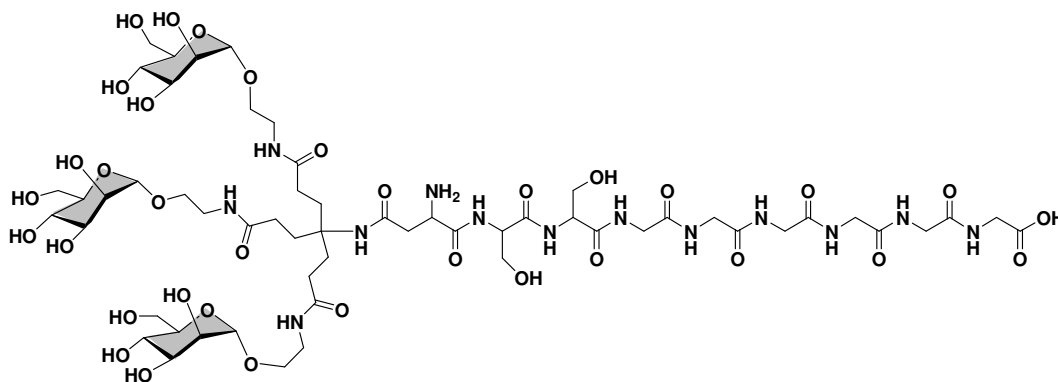


Figure 11: Designed glycocluster peptide.

In another part of this work the intention was to supply a complex oligosaccharide as a multifunctional sugar scaffold. Glycobiological studies require molecules which are close to the natural example structures and which are orthogonally functionalised to allow as many as possible variations for functional studies and further modification of various kinds to encounter glycoconjugate classes such as the glycolipids as well as GPI anchors.

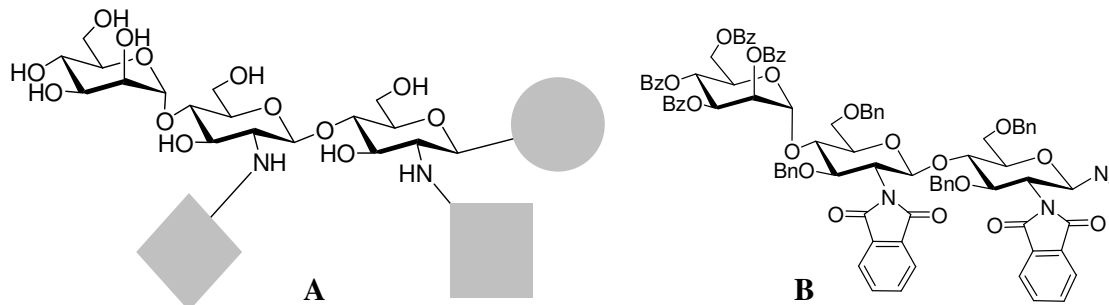


Figure 12: Designed multifunctional oligosaccharide mimic. (A) designed trisaccharide with variable functionality (shaded in gray); (B) targeted structure of trisaccharide.

It was intended to synthesize a trisaccharide unit of the core pentasaccharide structure of *N*-glycans. The trisaccharide could serve as glycoconjugate mimic and can undergo several functional modifications. The trisaccharide carries an azide function at the anomeric centre of the terminal glucosamine residue which could be used for the Staudinger ligation with lipid chains and then the amino function of the glucosamine residues could be set free delivering a GPI anchor mimic. In another approach, the amino functions could also be coupled to lipid chains to have a simple mimic of lipopolysaccharides (Figure 12).

3. Glycocluster peptides as transmembrane protein mimetics

3.1 Introduction

As it was already discussed earlier that the eukaryotic cell is covered with highly branched and complex carbohydrate structures making the glycocalyx. The glycocalyx constituents are attached to the lipid bilayer membrane through different types of aglycon biomolecules, such as from the wide spectrum of glycoconjugates. Thus the glycocalyx 'superstructure' is in contact with the cell through lipids, proteins, proteoglycans or GPI anchors (Figure 13).^[18]

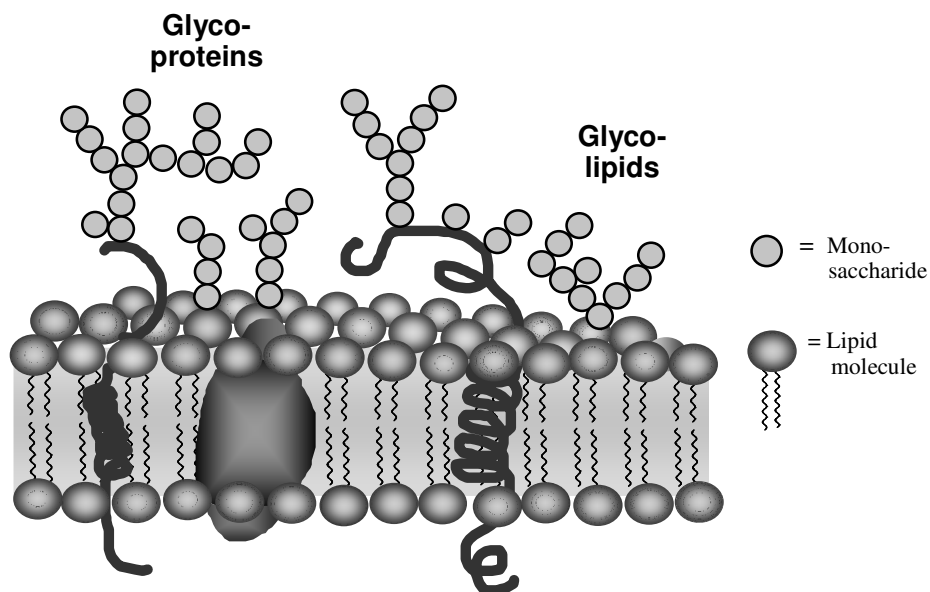


Figure 13: The plasmamembrane of the eukaryotic cell with sugar decoration.^[18]

The glycocalyx plays a vital role in many biological processes. The cellular communication process is one of the important phenomena responsible for infection and also immune response. Information from the extracellular environment is transmitted to the intracellular medium by transmembrane proteins present in the lipid bilayer membrane. All transmembrane proteins are highly glycosylated; thus the first encounter of a cell with foreign bodies takes place at the carbohydrate layer, and then by unknown mechanism information is sent to the cell through transmembrane proteins for example.

Thus it has become very important to investigate the function of the glycocalyx which has not been fully understood yet.

3.2. Motivation

To canvass the biochemistry of the glycocalyx, synthetic glycoconjugates serve as an important tool, because glycoconjugates from natural material can not be isolated in sufficient amount and purity. So there has been a demand of synthetic glycoconjugates as analogs of the natural glycoconjugates. Moreover, the synthesis of such molecules is difficult and time consuming. The incentive of this research was to investigate the biological function of the glycocalyx. The synthesis of natural glycoproteins is tedious and is a mammoth task, as the carbohydrate part of the glycoprotein is very complex and the protein part is composed of a number of amino acids which can be difficult to assemble. Here, glyocluster peptides have been discussed as glycoconjugate mimetics allowing rapid preparation, easy variation of molecular diversity and functional properties (Figure 14).

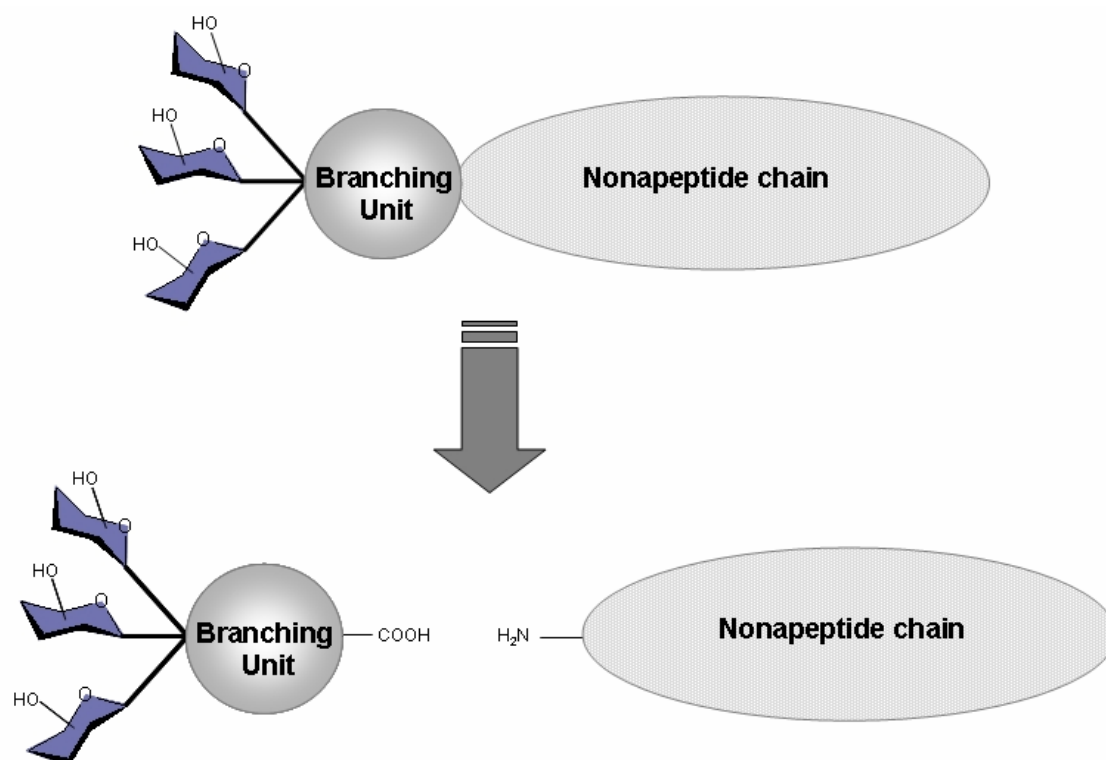


Figure 14: Retrosynthesis of targeted glyocluster peptides.

In glycoclusterpeptides a peptide is attached to an oligosaccharide mimetic. This glycopeptide mimic comprises a glycocluster wedge with a peptide mimicking a transmembrane protein (Figure 15). The glycoclusters were synthesized on the basis of dendrimer chemistry and for the synthesis of the peptide moiety highly flexible peptide synthesis on solid phase was employed. The two building blocks were then connected to each other in a convergent approach (Figure 14).

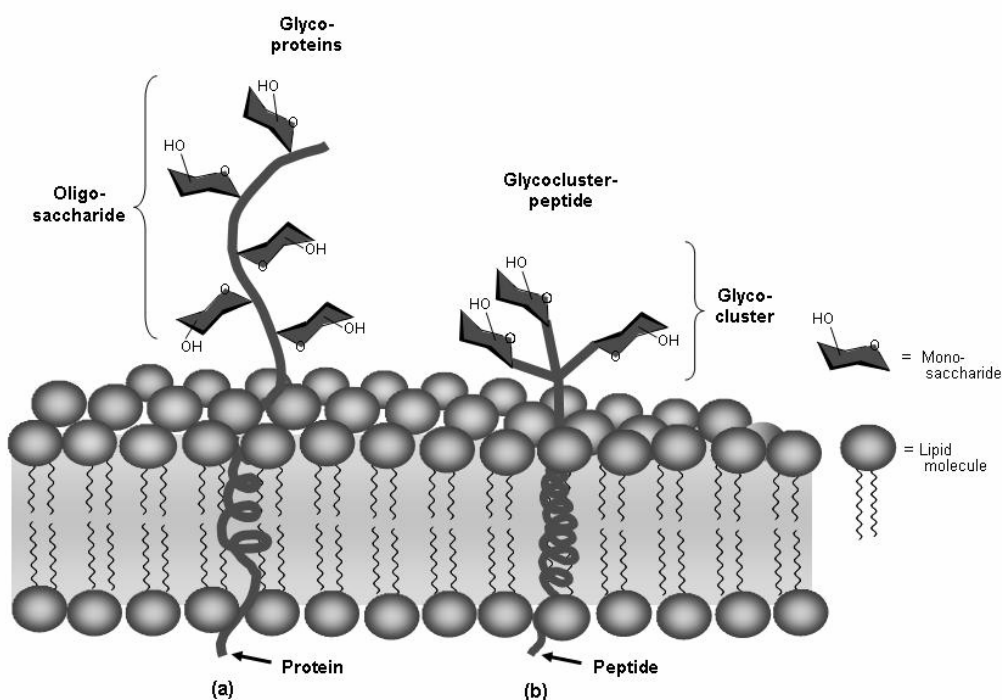


Figure 15: Schematic presentation of glycoprotein mimic: (a) transmembrane protein; (b) glycocluster peptide.

The approach to synthesize glycocluster peptides combines a number of favorable properties. It is easy to vary carbohydrate multiplicity of the branched glycosylated head moiety of the conjugate according to the principles of dendrimer chemistry. It is also possible to vary the peptide part as the synthetic plan is convergent, whereas orthogonal functionalisation of the peptide part enables bio-labelling, which is often a requirement for biological assaying. For synthesis of the designed glycocluster peptide, mannose was chosen because it is a central moiety in glycoproteins of the high-mannose type. The approach was to build a mannosyl glycocluster and attach it to a tailored peptide on solid phase. The design of the molecule was so that the glycocluster part represents the branched glycocalyx structures while the peptide part represents the transmembrane protein. The transmembrane protein is exhibiting different secondary structures like α -

helix and β -sheets. Synthesizing a transmembrane protein is rather difficult and pragmatically a nonapeptide was designed which has aspartic acid, serine and glycine as a constitutional material. In natural glycoproteins, especially in *N*-glycans the carbohydrate moieties are always attached to an asparagine. The transmembrane protein part in the bilayer membrane bears quite unpolar amino acids, ^[19,20] so a hexaglycine building block was chosen which mimics the transmembrane protein part in the lipid bilayer membrane. Thus, the peptide was designed to be of sequence Asp-Ser-Ser-Gly-Gly-Gly-Gly-Gly, i.e. DSSGGGGG. The mannosyl glycocluster representing the glycocalyx would be connected to the aspartic acid end making a simple glycoprotein mimic.

As mentioned above the synthesis of the glycocluster peptide was planned in two parts: mannosyl cluster part and peptide part. The peptide was decided to synthesize in a solid phase synthesis and the cluster mannoside in a solution phase. The cluster mannoside was planned to be attached to the peptide chain on solid phase; to avoid the steric problems to react the focal point to the peptide aspartic acid was first attached to the cluster mannoside.

3.3 Dendrimers

Dendrimers are monodispers macromolecules which are developed through iterative synthesis. The name “dendrimer” is derived from the Greek words *dendron* for tree and *meros* for part. Starting from a multifunctional core molecule the dendrimer is branched out forming several layers which are named as generations.^[21]

Syntheses of dendrimers consist of two or more reaction steps where one reaction cycle is always yielding the next generation.

Those functional groups are the starting point for the next one. In principle a dendrimer can be constructed in two different ways the divergent and the convergent one. The divergent

approach (Figure 16) is starting with a core molecule which is having two or more functional groups. The molecule is reacted with a branching unit that contains several protected functionalities and one group that is reacting with the core molecule. In the next step the protecting groups are deprotected and can be reacted with another branching unit to build up the second generation. The coupling and deprotection steps can be repeated alternating for higher generations.

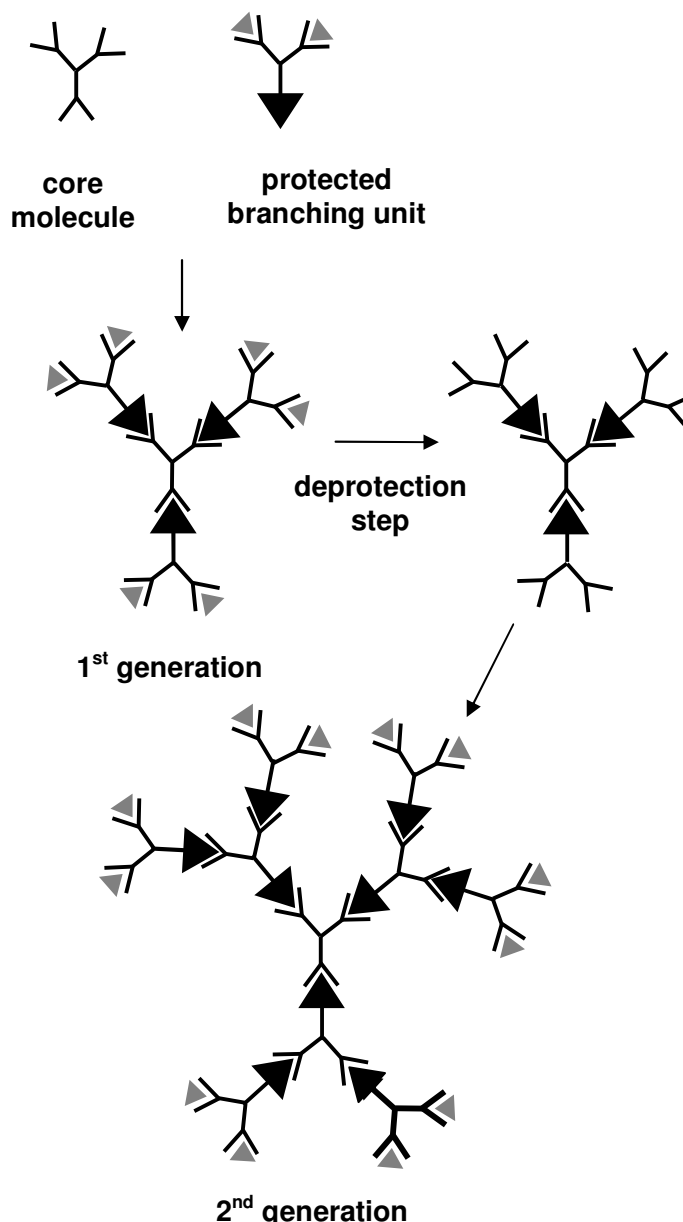


Figure 16: Divergent synthesis of dendrimers.

The coupling and deprotection steps can be repeated alternating for higher generations.

Like this the highly branched dendrimer grows from “within”, while the number of functional groups grows exponentially.

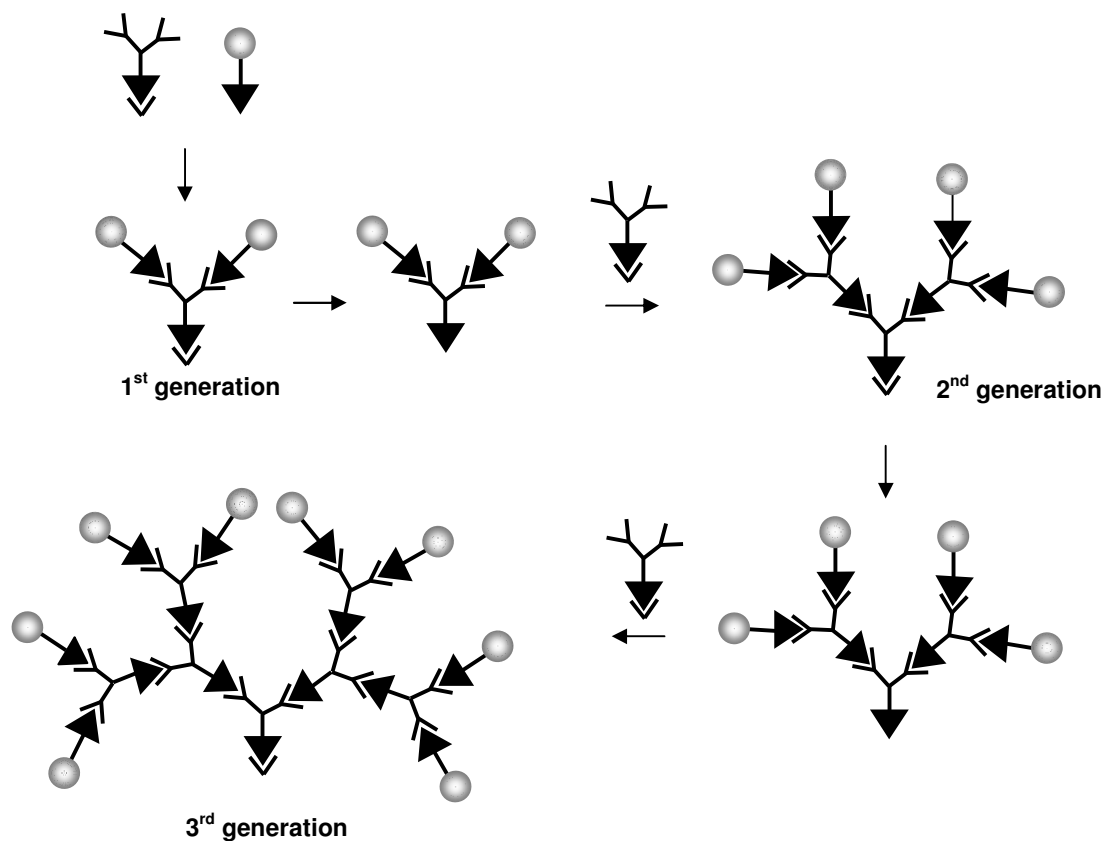


Figure 17: Convergent approach for dendrimer synthesis.

In a convergent synthesis the dendrimer is growing from outside towards inside (Figure 17). In most cases one is using trifunctional building blocks with two free functionalities as branching unit. These get reacted with the later surface molecule and then the remaining functional group is getting deprotected. This can then react with another branching unit forming a dendrimer segment that is called “dendron”. In the last step the dendrons get reacted with a core molecule forming the final dendrimer.

3.4 Solid phase peptide synthesis (SPPS)

Proteins are one of the most important natural products exhibiting vital properties in the living organisms. It has been of much interest to synthesize biologically active peptides whereas solution phase peptide synthesis of such peptides gets much tedious with increasing number of amino acid residues. The introduction of polymer supported peptide synthesis in 1962 made the long chain peptide synthesis a lot easier than before. This advance in peptide chemistry earned Robert Bruce Merrifield, the inventor of this method, a Nobel Prize in chemistry in 1984.

In the simplified method, a first amino acid is covalently attached to the functionalised solid polymer support (also called as resin beads), followed by a deprotection step of the amino group and the addition of the succeeding amino acid.^[22] At the end of the synthesis, the whole peptide is removed from the polymer support. It was important to make a right choice of polymer which would be insoluble in all solvents used for the synthesis having stable physical form in order to permit filtration. Besides this it was also important to have a functional group to which the first protected amino acid could be covalently attached. Merrifield^[23] *et al.* investigated cellulose, polyvinyl alcohol, polymethacrylate and sulfonated polystyrene as polymer support with different attachment modes; they found that a chloromethylated copolymer of styrene (Figure 18) and divinylbenzene gave the best results. The first resin used for the solid phase synthesis was chloromethylated polystyrene in form of 200-400 mesh beads.

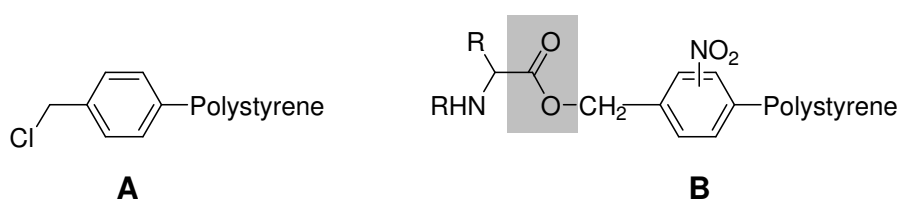


Figure 18: The resin used by Merrifield in the first peptide synthesis on solid phase (A), mode of attachment to the resin via ester bonding (gray) (B).

The peptide linked to the resin by ester bonding, highlighted in gray colour in Figure 18, was cleaved in the end by sodium hydroxide solution to receive the peptide.

Advantages of the SSPS:

- The peptide on the insoluble solid support could be easily filtered
- It could be washed so that the impurities and by-products are washed away
- Solubility problems and purifications are minimised
- Convenient and time saving

3.5 Resin types

After the use of the first polymer resin for peptide synthesis by Merrifield there have been several developments in this field. There are mainly three kinds of polymer materials available for the peptide synthesis with a broad spectrum of linkers allowing varying mode of attachment and cleavage of the peptide from resin.

a) Polystyrene resins

This is one of the most commonly used resins for peptide synthesis. This resin swells to a minimum in dichloromethane and fits to the well size of automated peptide synthesizers that is why it is preferred in automated solid phase synthesis.

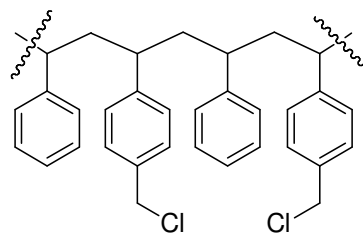


Figure 19: Polystyrene resin.

b) Polyamide resins

This is a polyacrylamide crosslinked resin which was introduced by Sheppard^[24] *et al.* in 1975. It is built up out of dicarboxylic acids and diamines containing either functionalised aliphatic or aromatic systems. Their functional groups are the later connecting unit to the first amino acid. It was shown that polyamide resin was more compatible with the solvents used and gives better results as compared to conventional polystyrene resin.

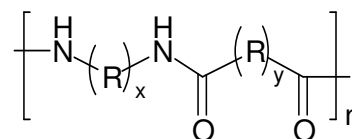


Figure 20: Polyamide resin.

c) PEG-based resins

These are high performance and highly crosslinked amphiphilic resins composed of polyethyleneglycol by crosslinking of its monomers. This resin was introduced by Rapp *et al.* and can be stored in dry form so that it could be easily weighed resulting in minimum losses. This is also used in enzymatic synthesis as it also swells well in water.^[25]

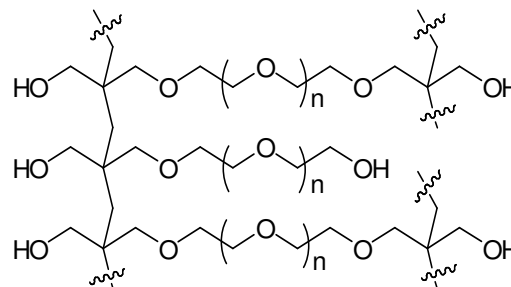


Figure 21: ChemMatrix resin.

3.6 Synthesis of model octapeptide

For synthesis of the peptide on the solid phase conventional Fmoc-strategy was employed in which the Fmoc-protected amino acid is covalently attached to the resin. In a subsequent step the protecting group is removed by the base treatment using piperidine and is coupled with the next amino acid which is also carrying a Fmoc-protection.

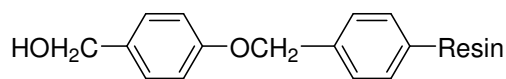


Figure 22: Wang resin.

In order to synthesize the model peptide,

Wang resin (Figure 22) was used as a solid support for the peptide synthesis which was

developed by Su-Sun Wang by modifying Merrifield resin with *p*-benzyloxybenzyl alcohol as functional group.^[26] The first amino acid is esterified to the resin and the peptide is easily cleaved by treatment with TFA while the protecting groups on the side chain remain undisturbed unless they are very acid labile.^[27]

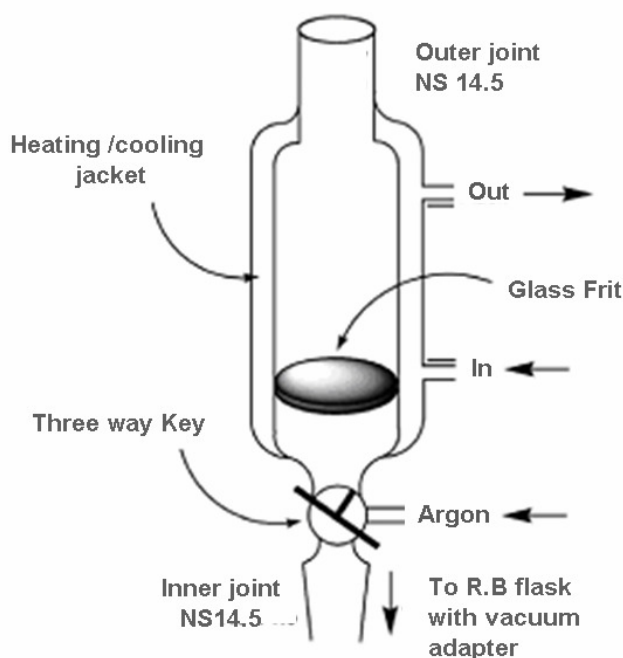


Figure 23: Schematic presentation of glass apparatus used for peptide synthesis.

The peptide synthesis was carried out in a glass apparatus employed by Lindhorst *et al.* (Figure 23).^[28] The apparatus was equipped with a cooling jacket and gas inlet. The apparatus was facilitated by a three

way key to switch between vacuum and nitrogen gas. The resin is placed on the frit in the apparatus

and the activated amino acid is applied to the resin and nitrogen gas was bubbled into it. The completion of coupling reaction was confirmed by 'Kaiser test',^[29] showing no blue colouration of resin beads. In the Kaiser test three different solutions are used (a) ninhydrin solution in ethanol; (b) potassium cyanide solution in pyridine; and (c) phenol solution in ethanol.

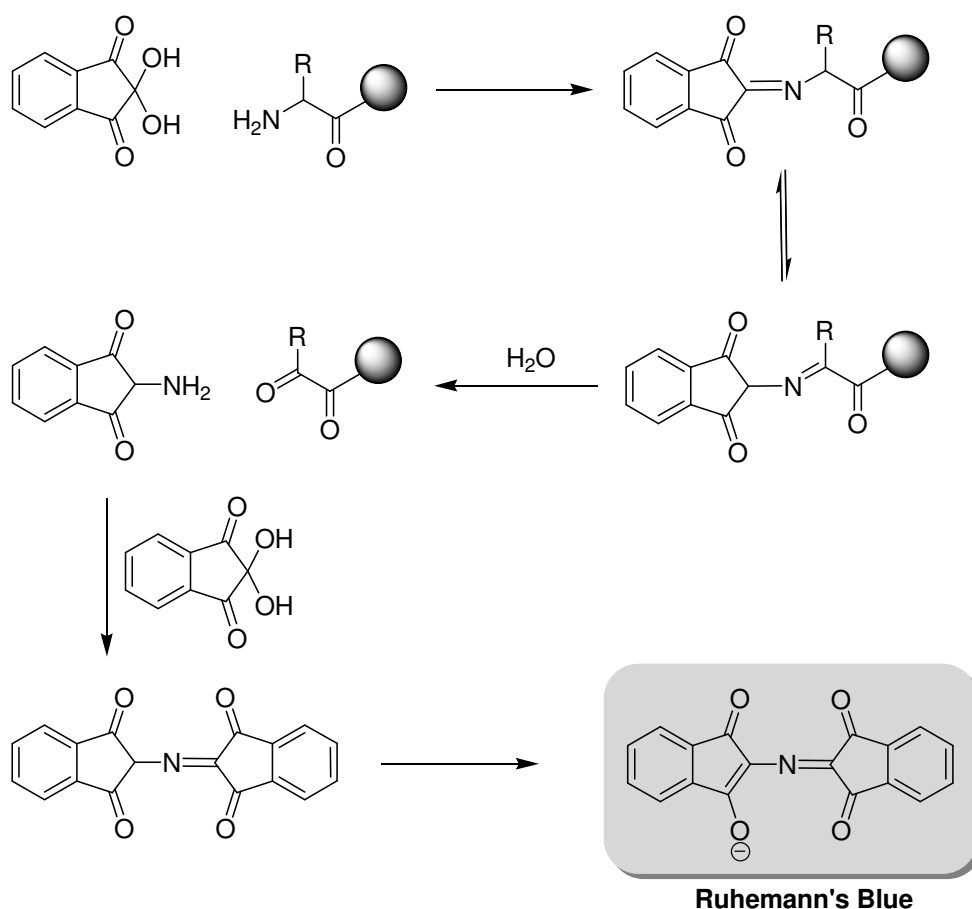


Figure 24: Mechanism of the Kaiser test.

The reaction between ninhydrin added to resin forms an intermediate which further reacts with ninhydrin to give a dimeric structure and the enolate form of this structure is known as 'Ruhemann's Blue'; showing blue coloured beads (Figure 24).^[30] The Kaiser test is applied both qualitatively and quantitatively.^[31]

3.7 Designing solid phase synthesis

After retrosynthesis of the model peptide, the molecule was disconnected in five building blocks. Initially, the first glycine (a) unit could be attached to the resin, followed by coupling with diglycine (b) and triglycine (c) units to get the desired hexaglycine building block (Figure 25). The reason why it was necessary to go through three different couplings procedures instead of attaching a hexaglycine in one step was to avoid the

synthetic difficulties to attach a longer peptide chain to the resin. Another reason for choosing longer a route was to invalidate the possibility of rudimentary deprotection of Fmoc-group used as protecting group of *N*-terminal of the amino acid.

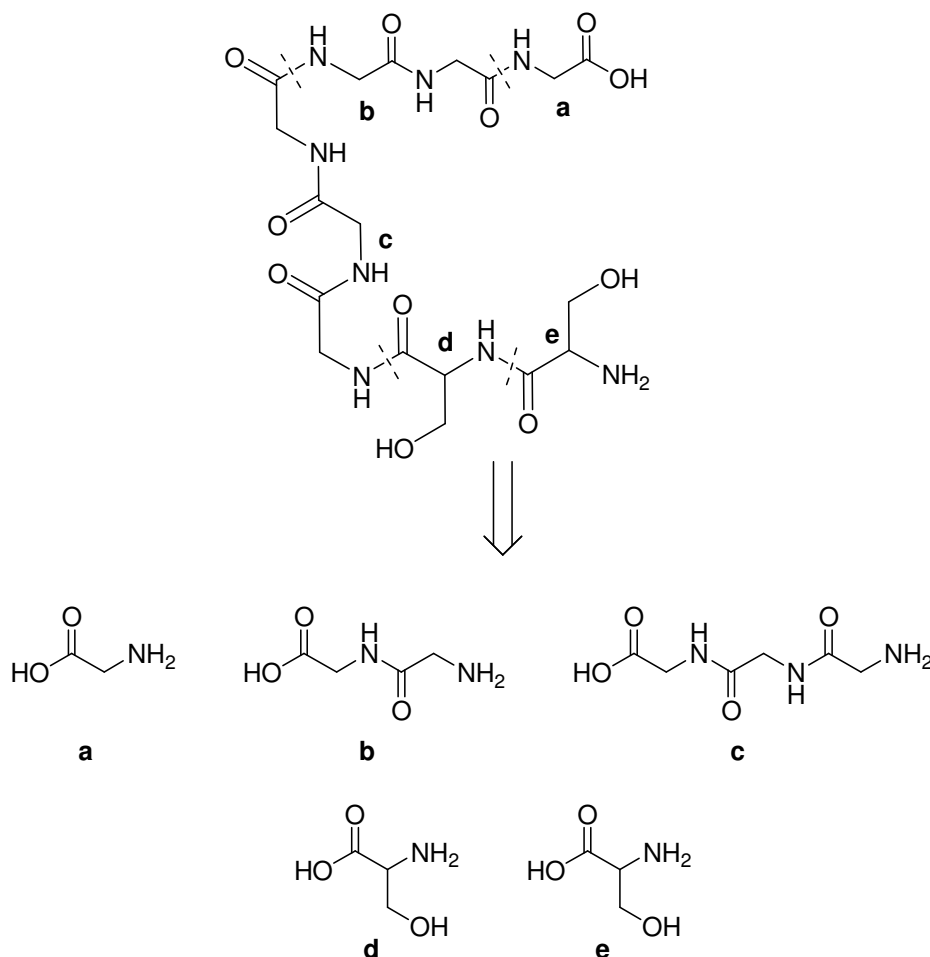
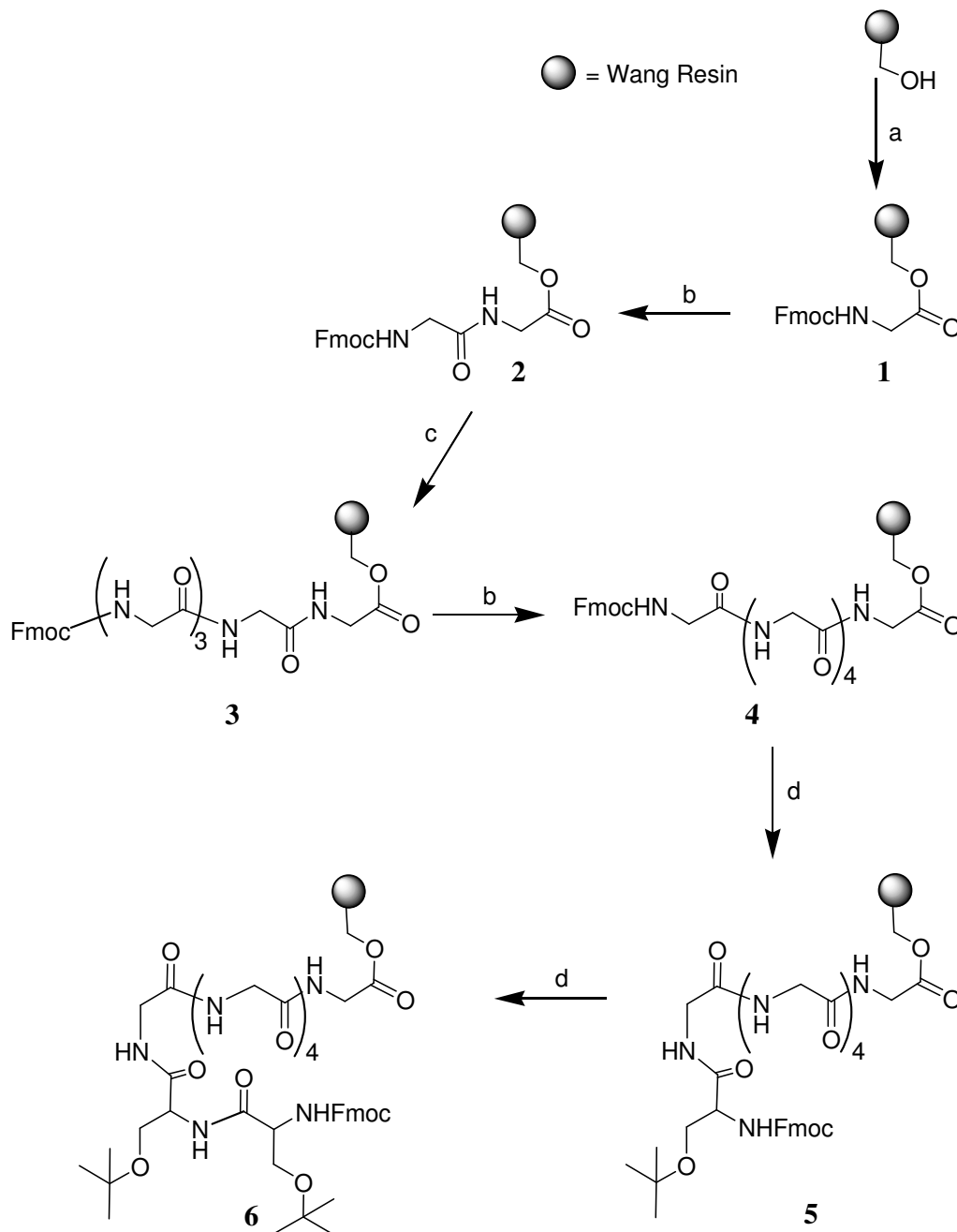


Figure 25: Designing synthesis of peptide on solid phase.

For the synthesis of the model octapeptide, first the glycine derivative was attached to the resin using *N,N'*-diisopropylcarbodiimide (DIC), 1-hydroxybenzotriazole (HOBT) and *N,N'*-diisopropylethylamine (DIPEA) as coupling reagents through ester bond to Wang resin. The unreacted surface of the resin was then blocked by treatment with acetic anhydride and pyridine to get the resin bound glycine **1** (Scheme 1). The resin was then dried to a constant weight to determine the exact load of Fmoc-Gly-OH on the resin.

The resin was then treated with piperidine solution in order to cleave the Fmoc-group. The resin was subsequently washed with DMF and treated with 25 % piperidine solution

in DMF to remove the Fmoc protection. The procedure was repeated after each coupling reaction. However, the coupling sequence could not be carried out as initially planned due to the poor solubility of Fmoc-Gly-Gly-OH.



Scheme 1: Synthesis of model octapeptide **6** on Wang resin. a) (i) Fmoc-Gly-OH, DIC, HOBT, DIPEA, DMF, (ii) Ac₂O, pyridine; b) (i) 20 % piperidine in DMF, (ii) Fmoc-Gly-OH, HBTU, HOBT, DIPEA; c) (i) 20 % piperidine in DMF, (ii) Fmoc-Gly-Gly-OH, HBTU, HOBT, DIPEA; d) (i) 20 % piperidine in DMF, (ii) Fmoc-Ser-O^tBu-OH, HBTU, HOBT, DIPEA.

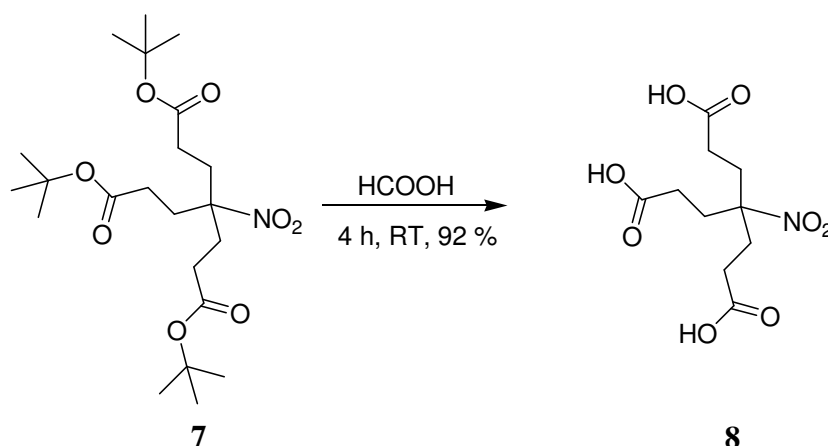
The synthesis was slightly modified and instead of coupling Fmoc-Gly-Gly-OH, the resin was treated with another Fmoc-Gly-OH moiety to get **2** by using HBTU, HOBt and DIPEA as coupling reagents. HOBt was used as an additive to the coupling reagent to avoid probable racemization.^[32,33] To judge the completion of coupling reaction 'Kaiser test' was performed, the completion was marked by the absence of blue beads. In subsequent procedure of Fmoc-deprotection and coupling with Fmoc-Gly-Gly-Gly-OH, Fmoc-Gly-OH gave the hexaglycine **4**. Then followed coupling with Fmoc-Ser-O^tBu for twice finally gave the octapeptide **6**.

After synthesis of the peptide **6**, a small portion of resin was subjected to peptide cleavage to calculate the exact load of peptide. The peptide load was found to be 0.46 mmol/g of the total amount of resin. The NMR spectra and mass spectrometry confirmed the formation the title peptide **6**.

3.8 Glycocluster amino acids

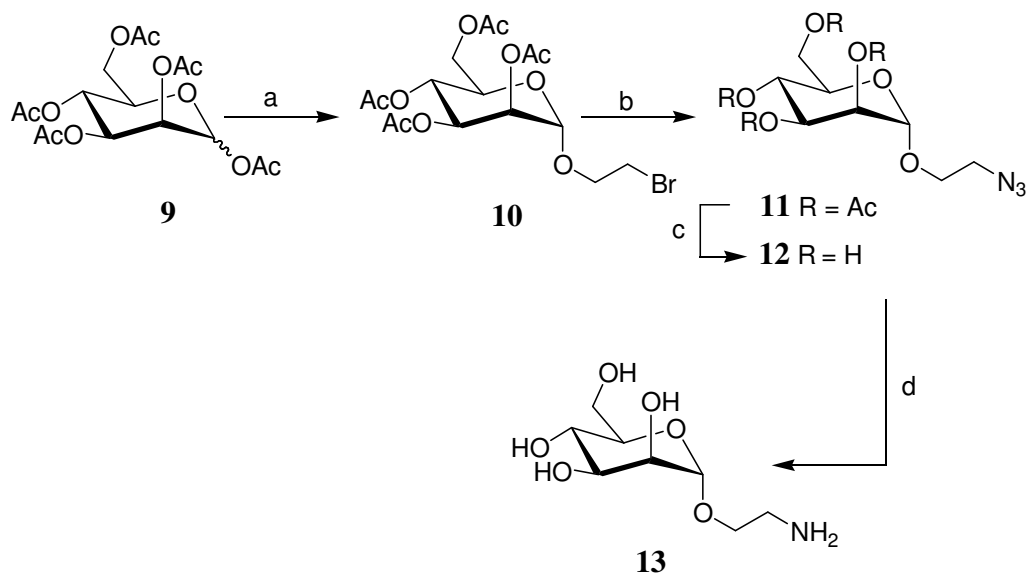
In order to synthesize the target glycocluster peptides, the octapeptide **6** was synthesized on Wang resin. This octapeptide then had to be coupled to different glycocluster amino acids. These glycocluster amino acids were synthesized using different trivalent dendritic wedges. The mannose derivatives were attached to the wedge either through peptide coupling or via glycosylation reaction. The mannosyl residues could be attached to the wedge through spacers with various lengths.

The trivalent wedge **7** introduced by Newkome has been used in the synthesis of different dendrimers.^[34] For the synthesis of the first glycocluster amino acid derivative we chose the trivalent wedge **8** as core molecule. This trivalent wedge was synthesized by reacting *tert*-butyl acrylate and nitromethane in a Michael-type addition reaction using Triton B as a base. Then the resulting nitro triester **7** was treated with formic acid to give corresponding nitro triacid **8** (Scheme 2).



Scheme 2: Synthesis of nitro triacid **8**.

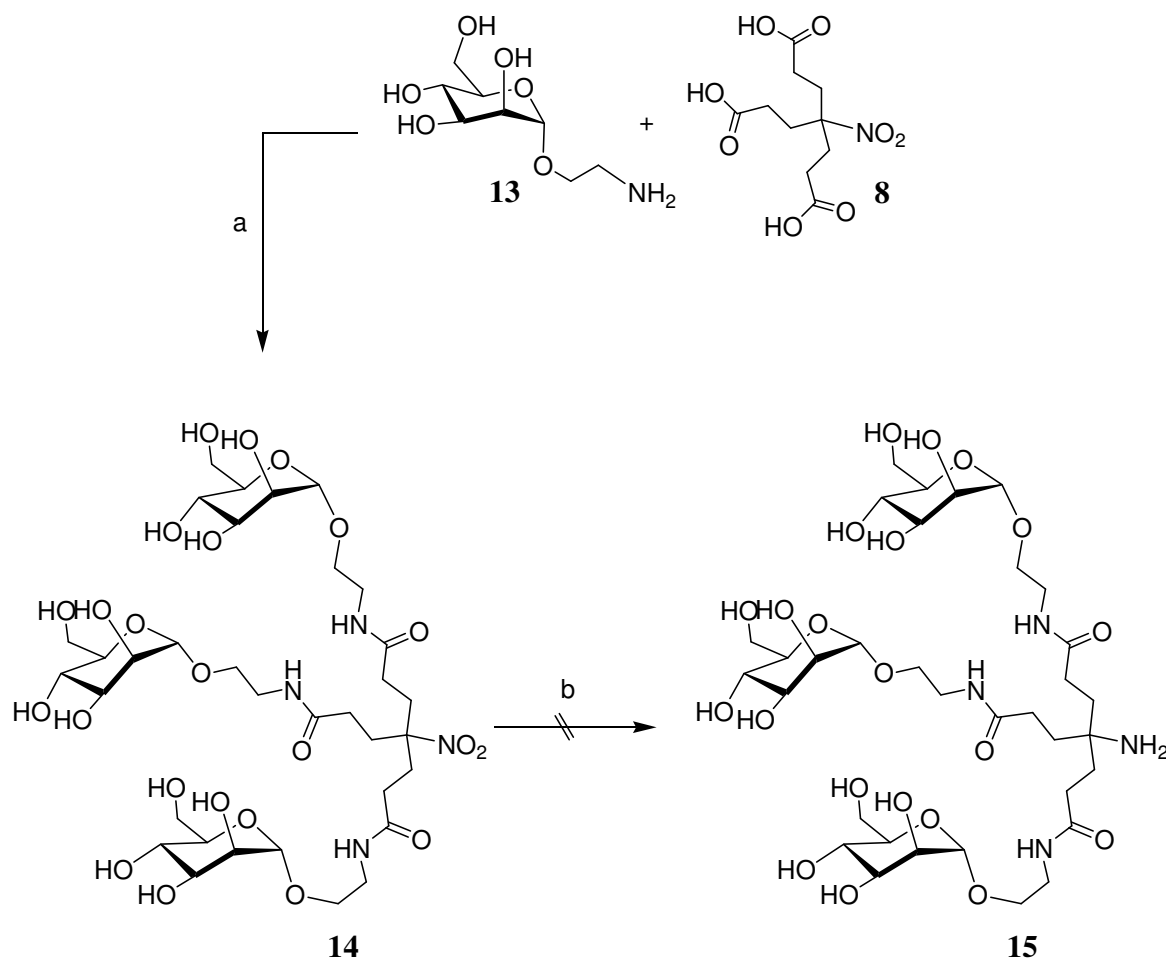
On the other hand a mannose derivative was synthesized which could be coupled to the triacid **8**. In the first reaction step, mannose pentaacetate **9** was glycosylated using BF_3 -etherate catalysis with 2-bromoethanol to give the glycoside **10**^[35] in good yields. Further the bromoethyl mannoside **10** was reacted with sodium azide resulting in compound **11** after $\text{S}_{\text{N}}2$ -type reaction. Later after deprotection of acetyl groups according to the Zemlén^[36] procedure compound **12** was achieved in very good yields. The deprotected azidoethyl mannoside **12** was catalytically reduced using palladium to give the corresponding amine **13** (Scheme 3).



Scheme 3: Synthesis of aminoethyl mannoside **13**. (a) $\text{HOCH}_2\text{CH}_2\text{Br}$, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CH_2Cl_2 , RT, 16 h, 53 %; (b) NaN_3 , TBABr, DMF, 60 °C, 16 h, quant.; (c) CH_3ONa , CH_3OH , 4 h, 86 %; (d) Pd/C, H_2 , CH_3OH , RT, 16 h, quant.

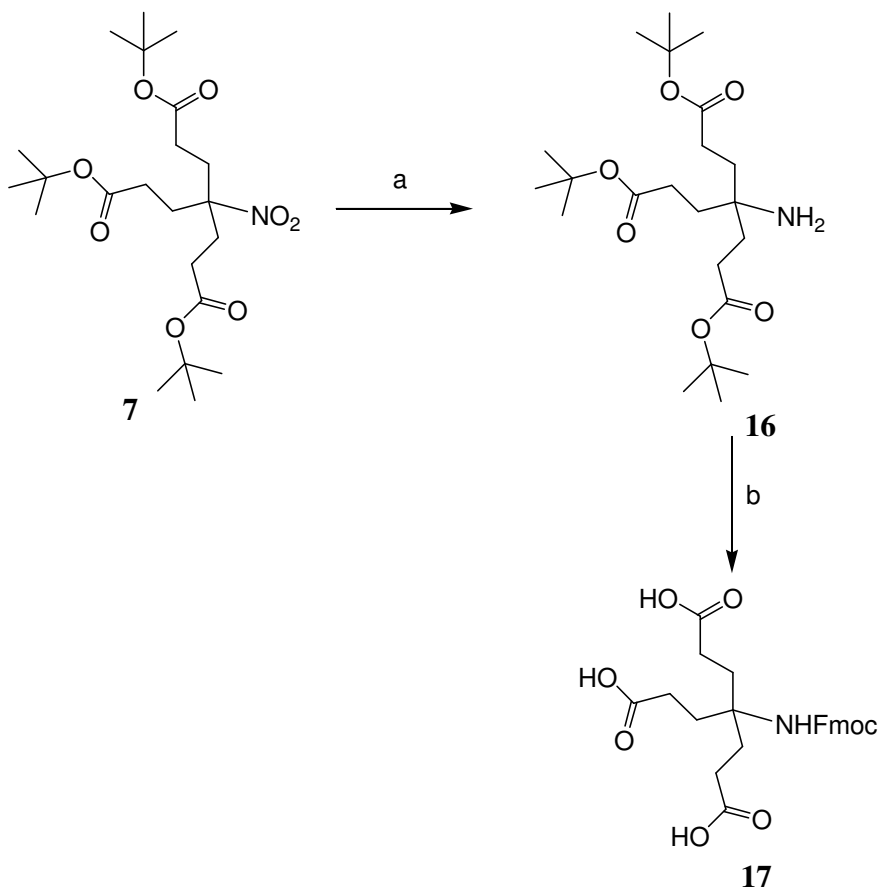
In the next step, the mannoside derivative **13** was reacted with triacid wedge **8**^[37] via peptide coupling reaction using HATU as a coupling reagent to give **14** in moderate yield. The coupling reagent HATU is an uronium salt derived from 1-hydroxy-7-azabenzotriazole which has been reported to speed up the reaction with less loss of chiral integrity.^[38] After the successful coupling reaction the mannosyl tricluster **14** was isolated (Scheme 4).

However, the reduction of the nitro group at the focal point of tricluster **14** to the corresponding amine **15** was not successful. It has also been known from the literature that the reduction of nitro group is difficult when the carbohydrates are attached to the trivalent wedge. Steric hindrance at the focal point could be a reason behind the difficulty in reduction of the nitro group.^[39]



Scheme 4: Attempted synthesis of glycocluster **15**. (a) HATU, DIPEA, DMF, 0 °C → RT, 12 h, 51 %; (b) H₂, Pd/C, ammonium formate 3 d.

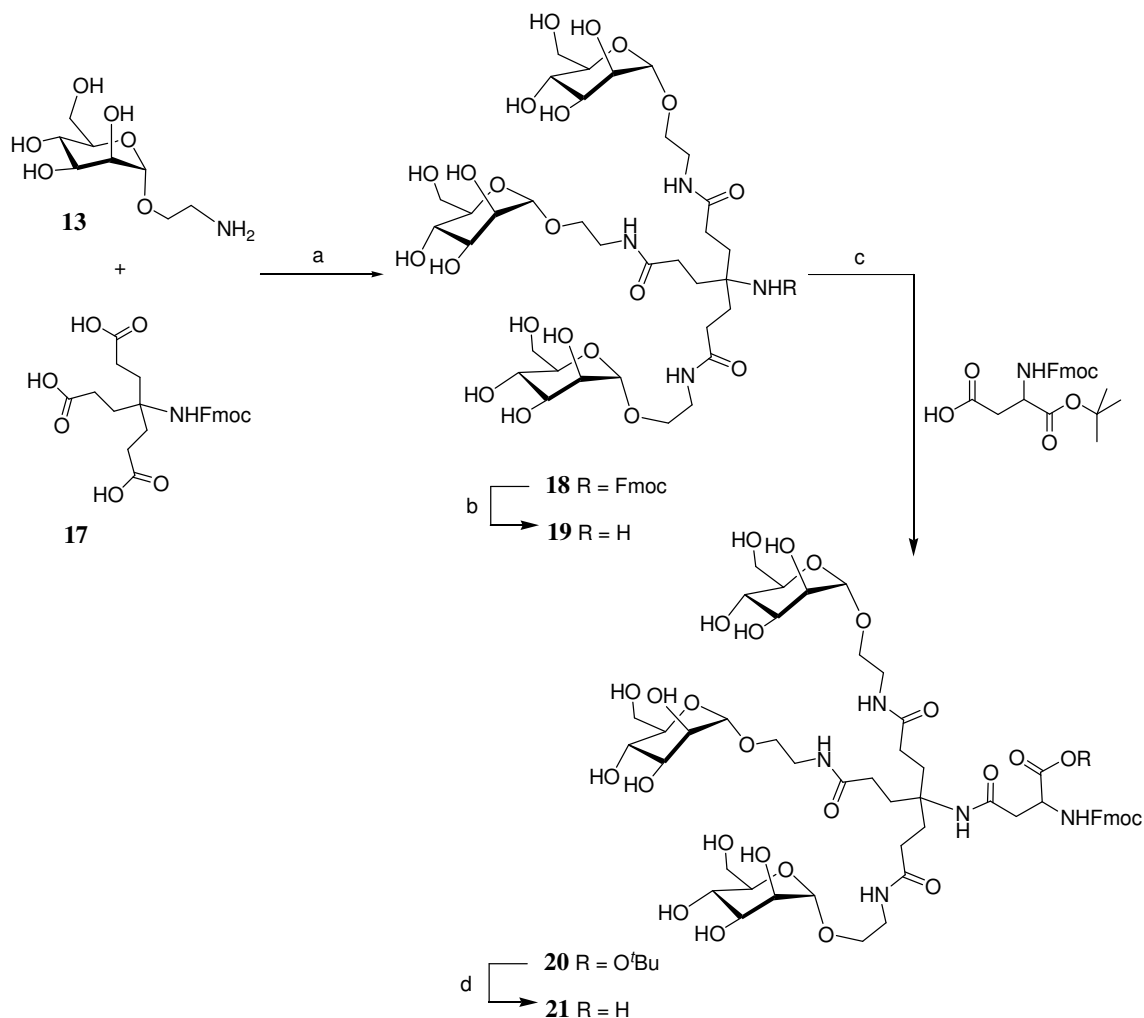
As the preliminary synthetic route could not be carried forward, it was decided to modify the synthetic strategy. Following a new route, compound **7** was first reduced to **16** (Scheme 5) using Raney nickel as reported in the literature.^[40] However, the procedure described by Newkome for the reduction of nitro group requires application of high pressure and autoclave employment. A modified reduction procedure described by Apko *et al.*^[41] requires no high pressure and change of solvent. The reaction solvent ethanol was replaced by heptane. After reduction deprotection of *tert*-butyl groups was envisaged. The reduction of **7** was carried out at atmospheric pressure to get amino triester **16** in quantitative yield. The amine was then protected as Fmoc-group followed by conversion of *tert*-butyl ester to triacid **17** in moderate yield (Scheme 5).



Scheme 5: Synthesis of Fmoc-protected triacid **17**. (a) Raney-Ni, H_2 , heptane, 2 d, quant.; (b) (i) Fmoc-Cl, DIPEA, CH_2Cl_2 , RT, 1 h; (ii) TFA, CH_2Cl_2 , 4 h, 60 %.

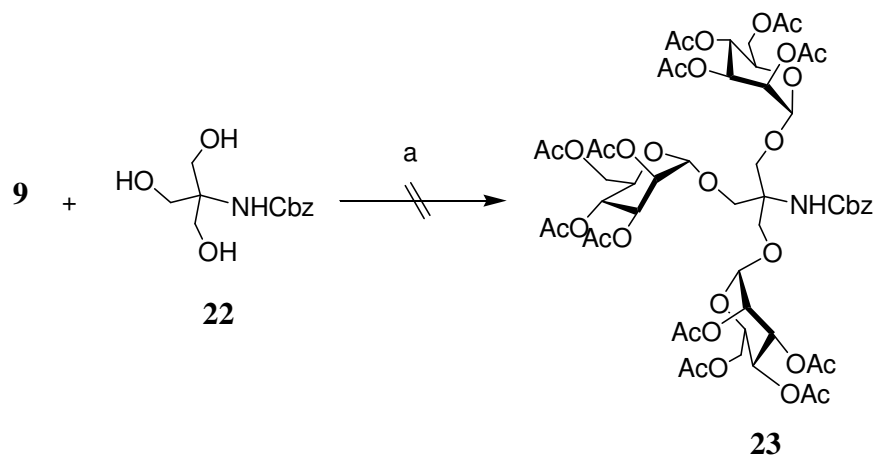
The Fmoc-protected triacid **17** was then reacted with mannosyl derivative **13** using the same reaction conditions as in the case of **14** following the procedure reported by Kleinert^[42] *et al.* The reaction worked well and gave the tricluster mannoside **18** in moderate yield. In the next step, the Fmoc group was deprotected using morpholine in dimethylformamide as the solvent. The cluster mannoside **19** was used in the next step without any further purification, as the by-products were harmless in the next reaction step. Then, the substrate was subjected to the peptide coupling reaction with the orthogonally protected aspartic acid derivative, Fmoc-Asp- O^tBu in presence of HATU and DIPEA. After the reaction, a complex mixture of crude product was isolated, which went through various purification processes. The crude compound was first applied to gel permeation chromatography with Sephadex LH-20 in methanol followed by MPLC purification on reverse phase material. In the last step twice purified substrate was

subjected to HPLC purification to give pure glycocluster amino acid derivative **20** in poor yield. The compound **20** was then treated with water-TFA mixture to give glycocluster amino acid derivative **21** in excellent yield (Scheme 6).



Scheme 6: Synthesis of glycocluster amino acid **21**. (a) HATU, DIPEA, DMF, 0 °C → RT, 56 %; (b) morpholine, DMF, 2 h, RT; (c) HATU, DIPEA, DMF, 0 °C → RT, 4 h, 36 %; (d) TFA/H₂O (7:3), 1 h, 95 %.

In order to vary the conformational features of the glycocluster head groups for the synthesis of different glycocluster amino acid derivatives, next a glycocluster amino acid with a shorter spacer than in **21** was featured. In this case tris(hydroxymethyl)aminomethane (TRIS) was chosen as the trivalent scaffold which can easily be obtained in its Cbz-protected form **22**^[43] (Scheme 7). Frequently, environmentally hazardous mercury salts such as Hg(CN)₂ were employed as promoters to effect complete *O*-glycosylation of TRIS.^[44]

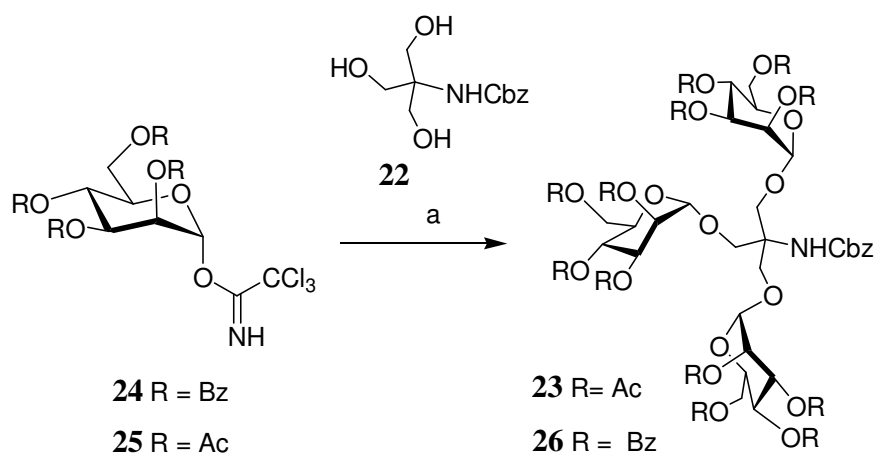


Scheme 7: Attempted synthesis of mannosyl cluster **23**. (a) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CH_2Cl_2 , $0\text{ }^\circ\text{C} \rightarrow \text{RT}$, 16 h.

Mannosylation of TRIS, delivering **26**, has been reported^[45,46] employing protected mannosyl bromide and silver salts according to a Königs-Knorr protocol.^[47] Here, BF_3 -etherate catalysis was employed. In the first glycosylation attempt to achieve glycosylation of the triol **22**, mannose pentacetate **9** was employed as glycosyl donor using BF_3 -etherate as a promoter (Scheme 7). However, this glycosylation reaction did not work and made it necessary to employ a more reactive glycosyl donor.

Then benzoyl-protected mannosyl trichloroacetimidate **24**^[48] was used and this reaction allowed the synthesis of the protected trivalent cluster mannoside **26** (Scheme 8). The mannosylation reaction with *O*-acetyl-protected mannosyl trichloroacetimidate **25** was also successful under similar reaction conditions to give the acetyl-protected cluster mannoside **23**. However, the *O*-benzoyl-protected mannosyl trichloroacetimidate **24** gave 66 % yield, whereas its *O*-acetyl protected counterpart **25** gave 56 % yield.

The synthesis of cluster mannosides **23** and **26** employing BF_3 -etherate as a promoter was successful without the use of heavy metal salts and employing glycosyl trichloroacetimidates as glycosyl donors.^[49] In addition, no orthoester formation (Figure 26) was observed in the mannosylation reaction of **23** and **26**, which is often problematic in multi-mannosylation reactions.^[50]



Scheme 8: Synthesis of glycocluster **23** and **26**. (a) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{RT}$, overnight, 56 % for **23**, 66 % for **26**.

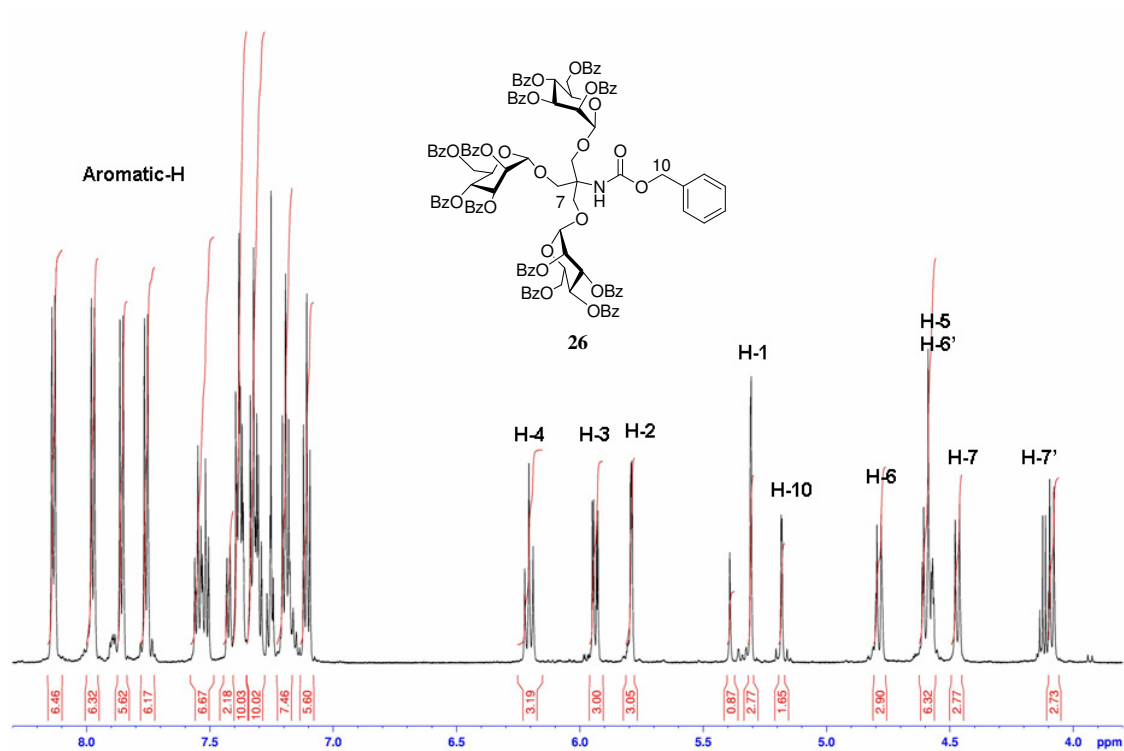
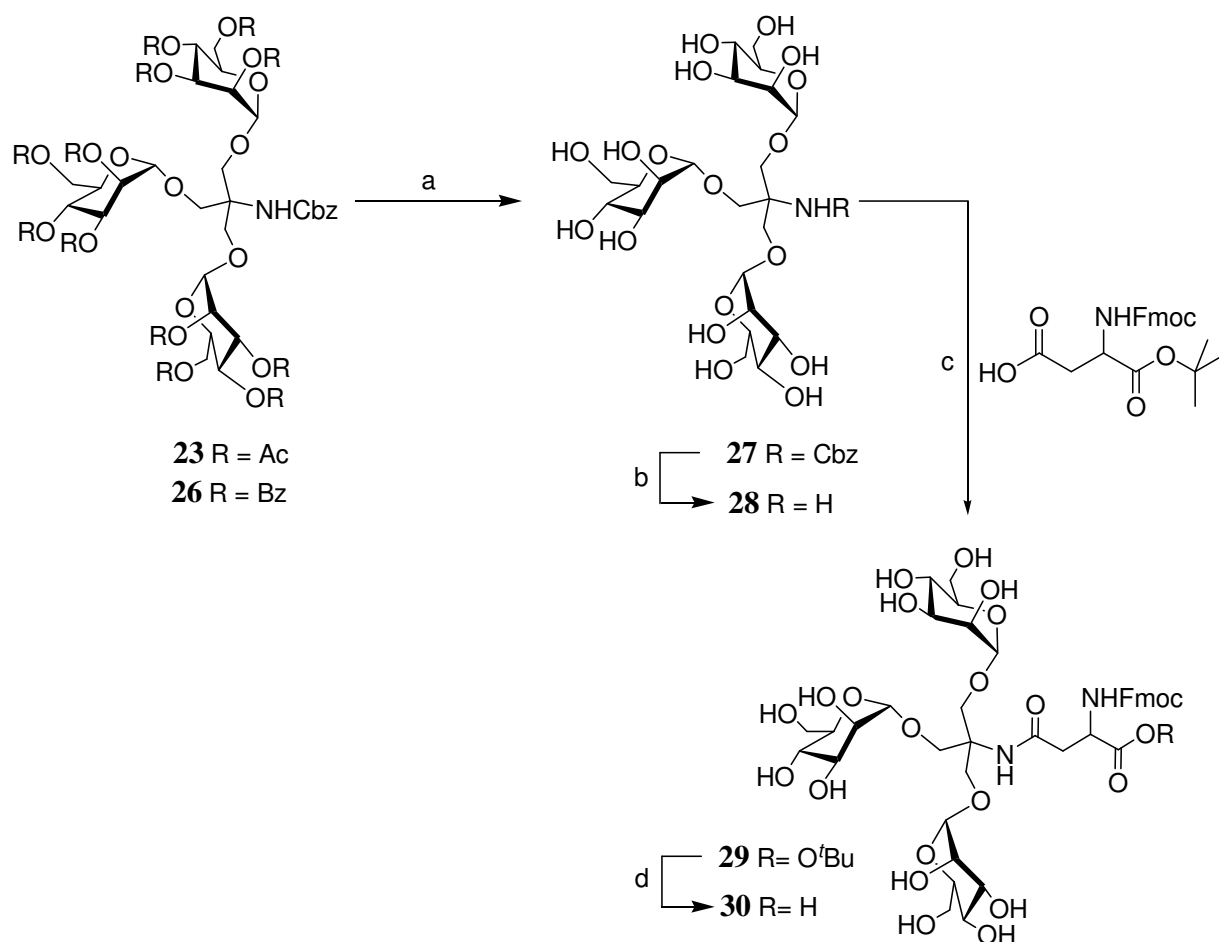


Figure 26: ^1H NMR spectrum of cluster mannoside **26** showing single set of protons.

The ^1H NMR spectrum (Figure 26) confirmed the structure of cluster mannoside **26**. In the NMR shown above, only one set of protons was found for the three mannose residues. The mannose moieties were connected to the quarternary carbon over a methylene group which was well displayed in spectrum. The spectrum clearly indicated no orthoester formation.



Scheme 9: Synthesis of glycocluster amino acid **30**. (a) MeONa, MeOH, RT, 4 h, 96 % from **26**, 88 % from **23**; (b) Pd/C, H₂, MeOH, RT, 12 h, 95 %; (c) HATU, DIPEA, DMF, 0 °C → RT, 4 h, 37 %; (d) TFA/H₂O (7:3), RT, 1.5 h, 94 %.

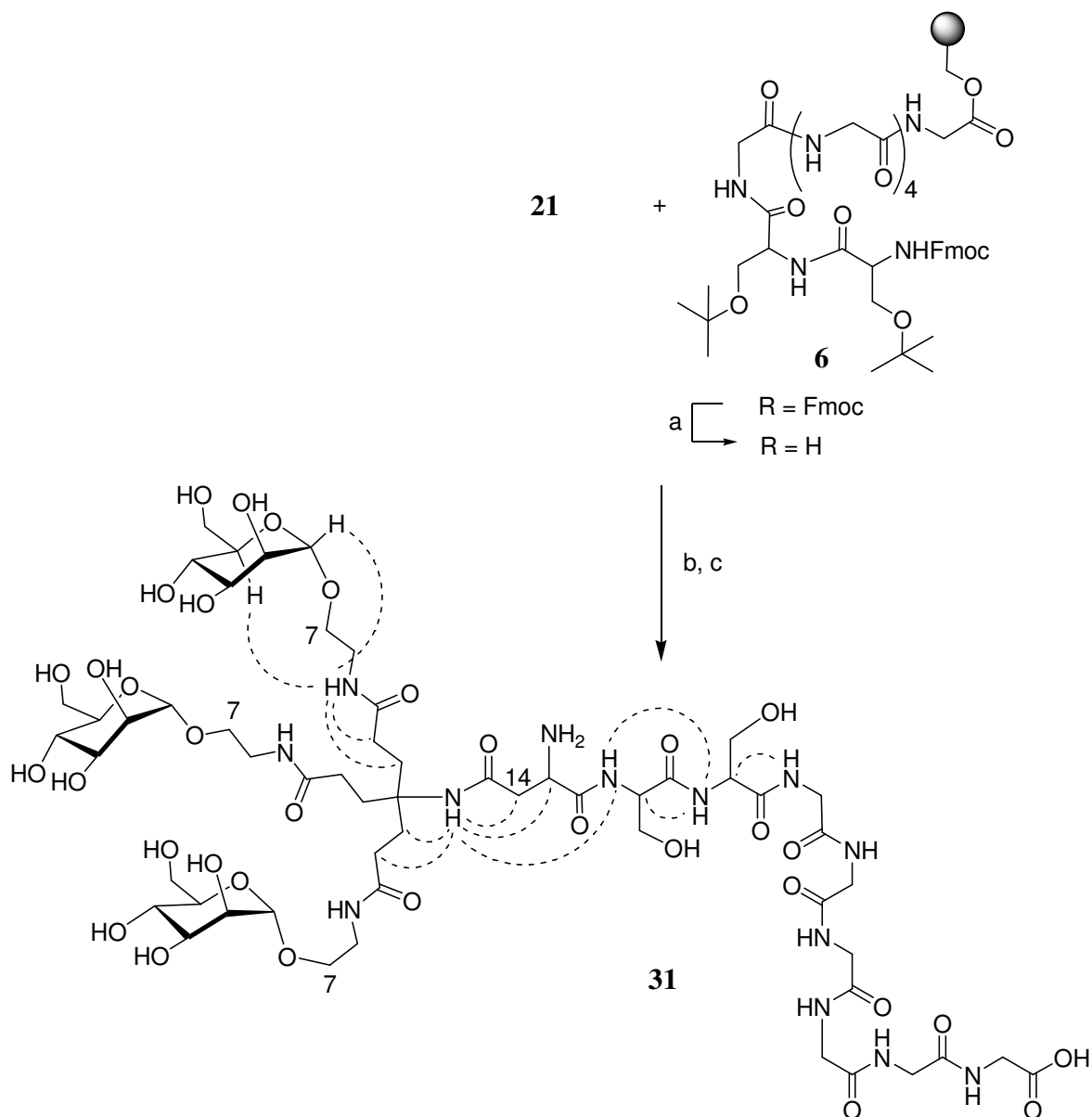
Deprotection of *O*-benzoyl and *O*-acetyl of the fully protected glycocluster **23** and **26** was accomplished according to Zemlén's sodium methanolate procedure to give rise to pure **27**. In case of **26** to **27** purification by size exclusion chromatography on LH-20 gel was required to remove the methyl benzoate by-product formed in the deprotection step. Then, the Cbz-protected amino group of **27** was deprotected via Pd-catalyzed

hydrogenation to give amine **28** in nearly quantitative yield (Scheme 9). Similar to the synthesis of glycocluster amino acid **21**, the amino-functionalized cluster mannoside **28** was functionalized as glycocluster amino acid employing an orthogonally protected aspartic acid derivative.

HATU-mediated peptide coupling to the side chain carboxylic group afforded glycocluster **29** in good yield. Purification of compound **29** was quite tedious and involved gel chromatography to remove low molecular mass impurities and subsequent HPLC employing a water-acetonitrile gradient. This lengthy purification protocol might account for the moderate yield in which the protected glycocluster amino acid **29** was finally obtained after lyophilisation. Lastly, treatment of **29** with a TFA-H₂O mixture (7:3) gave the corresponding carboxylic acid **30** in excellent yield.

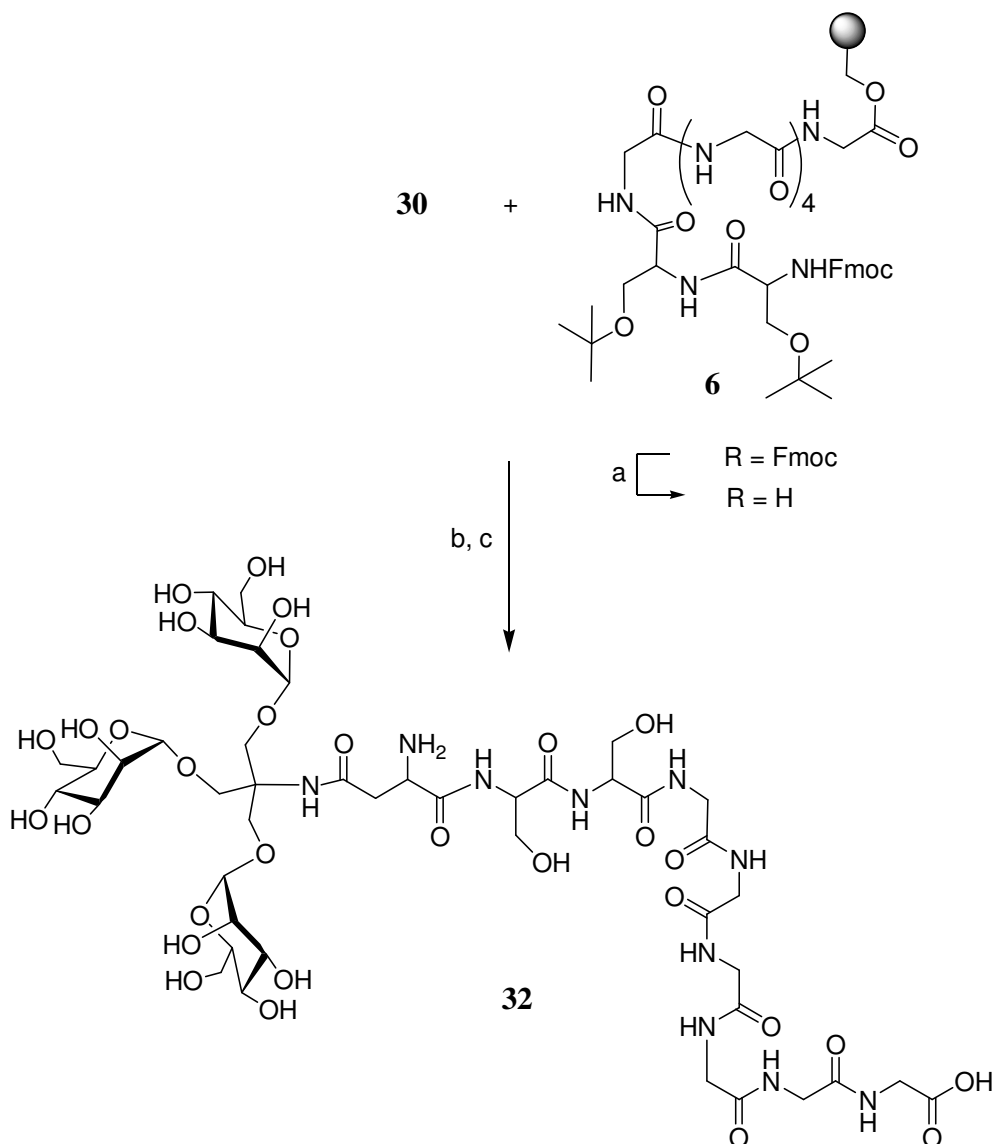
3.9 Glycocluster peptides

After preparing glycocluster amino acid **21** and **30** bearing different spacer length, the target glycocluster peptides were prepared on solid phase. The model peptide **6** was then treated with 25 % solution of piperidine in DMF to remove Fmoc-group at the *N*-terminus.



Scheme 10: Synthesis of glycocluster peptide **31**. (a) 20 % piperidine in DMF; (b) HATU, DIPEA, DMF, 24 h; (c) TFA/CH₂Cl₂ (1:1), 93 %. (NOEs observed are depicted by dotted lines).

After repetitive washing with DMF, glycocluster amino acids **21** and **30** were activated by HATU and DIPEA and applied to the resin in two different steps. Unlike in other coupling steps, the equivalence of acid used was less because of highly valued substances. Because of larger size of the molecules the coupling steps were carried out over longer time. After 'Kaiser test' confirmation, the resin was treated with TFA and water to release the corresponding peptides **31** from **21** (Scheme 10) and **32** from **30** (Scheme 11).



Scheme 11: Synthesis of glycocluster peptides **32**. (a) 20% piperidine in DMF; (b) HATU, DIPEA, DMF, 24 h; (c) TFA/CH₂Cl₂ (1:1), 82%.

Both glycocluster peptides **31** and **32** underwent several NMR experiments. In the ^1H NMR spectra of both **31** and **32**, α -protons of all six glycine residues overlapped at 3.76 ppm. The amide protons of both the peptides were reasonably well dispersed, and resonated between 7-8 ppm. They were sequentially assigned by comparison of COSY and NOESY spectra. In the latter, sequential $d_{\alpha\text{N}}(i,i+1)$ ^[51] NOEs were observed between the first and second serine residues, and between the second serine and the following first glycine. Additionally, the high quality NOE data set of **31** provided further expected NOEs between “spacer NH” to the spatially proximal protons, which facilitated the complete characterization of the spacer structure. The NOEs are highlighted with dotted lines in Scheme 10.

An analysis of the chemical shifts of the structurally sensitive $\text{H}\alpha$ -protons of all amino acids indicates that these shifts are nearly identical to random coil chemical shifts,^[52] which is suggestive of a lack of defined secondary structure. Notably though, a sequential d_{NN} -NOE is observed between the aspartic acid γ -carboxamide proton (HN at the focal point) and the first serine HN in **31**. This is unexpected due to the separation between this aspartic acid γ -carboxamide and the serine, and may indicate a conformational preference in this compound. However, the interpretation of this NOE requires more detailed NMR-spectroscopic studies, which are also warranted in light of putative effects of the spacer length on the cluster structure. In particular, in the somewhat more crowded compound **32**, the peptide conformation and the relative orientation of the carbohydrate moieties may be affected by steric hindrance.^[53]

Prof. Dr. Sönnichsen is thankfully acknowledged for his help in carrying out NMR experiments and interpretation of the data.

4. Glycocluster synthesis via Staudinger reaction

4.1 Introduction

The reaction introduced by Hermann Staudinger between phosphines and azides to form an aza-ylide is called the “Staudinger reaction” and is widely used in organic and biological chemistry. In the presence of water the aza-ylide intermediate is hydrolysed to form a primary amine and phosphine oxide.^[54] The azide and the phosphine react with each other quickly at room temperature and produce the corresponding amine in good yields (Figure 27).

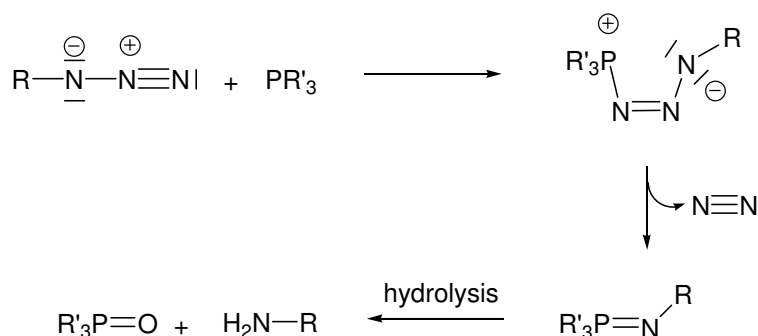


Figure 27: Staudinger reaction.

Recently, it has been shown that a modified Staudinger reaction could be used for ligation.^[55] Instead of producing a primary amine by reacting an azide and the phosphine, an acid is added to produce an amide (Figure 28), a method which is relevant in biological chemistry. In this work a somewhat modified Staudinger reaction was performed to build glycocluster molecules, thus saving time and reaction steps.

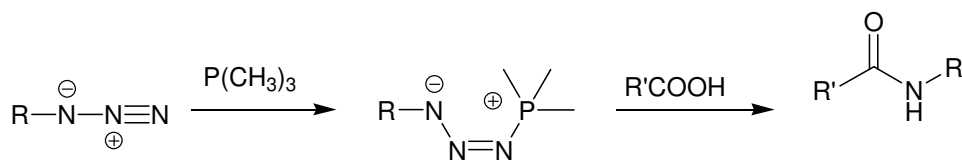
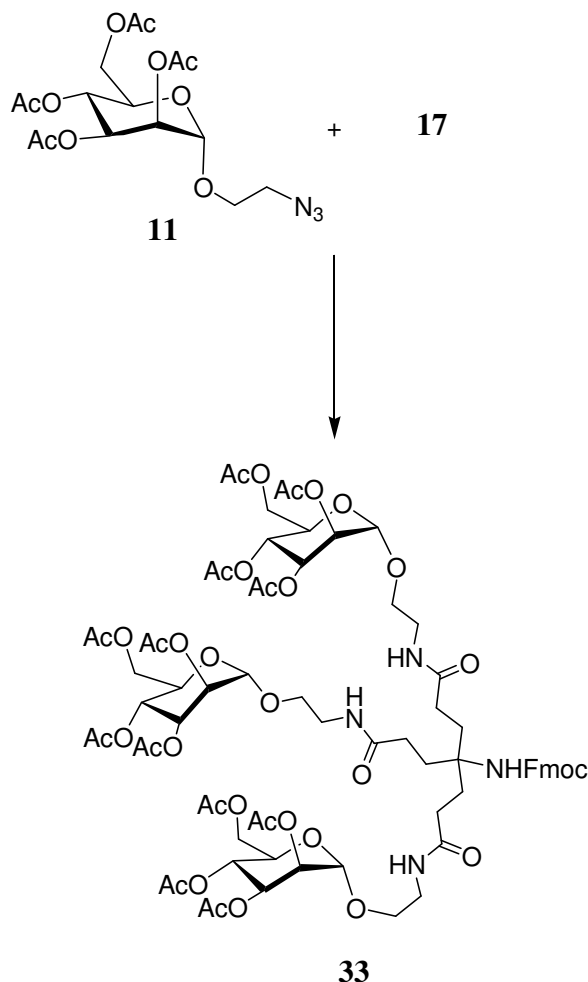


Figure 28: Modified Staudinger reaction.

4.2 Synthesis

The modified Staudinger reaction, carried out between a glycosyl azide and amino acid can be used in glycoscience.^[56,57] Here, this procedure has been adapted for glycocluster synthesis, where Newkome's modified trivalent acid **17**, already mentioned above was used. In the first attempt to synthesize a tricluster, compound **17** was reacted with azidoethyl mannoside **11**. For activation of the carboxylic acid **17** a combination of DIC and HOBT was used, whereas instead of triphenyl phosphine which is quite often used, less hindered trimethyl phosphine was employed. When the reaction was performed at 0 °C, there was no product formation. However, when the reaction was performed at -70 °C the desired glycocluster **33** was obtained in moderate yield (Scheme 12). In addition, formation of defected structure carrying only two mannose moieties was



Scheme 12: Synthesis of glycocluster **33**. (a) DIC, HOBT, P(CH₃)₃, THF, -70 °C, 12 h, 52 %.

observed.

The reaction mechanism of the Staudinger reaction can justify the work under subzero temperature. The intermediate **I** is formed in the reaction between phosphine and azide. The carboxylic acid reacts with the DIC to form activated ester which further reacts with HOBt, the suppressant used against racemization to form ester **II** which reacts with **I** to give an amide bond releasing nitrogen and phosphine oxide (Figure 29).

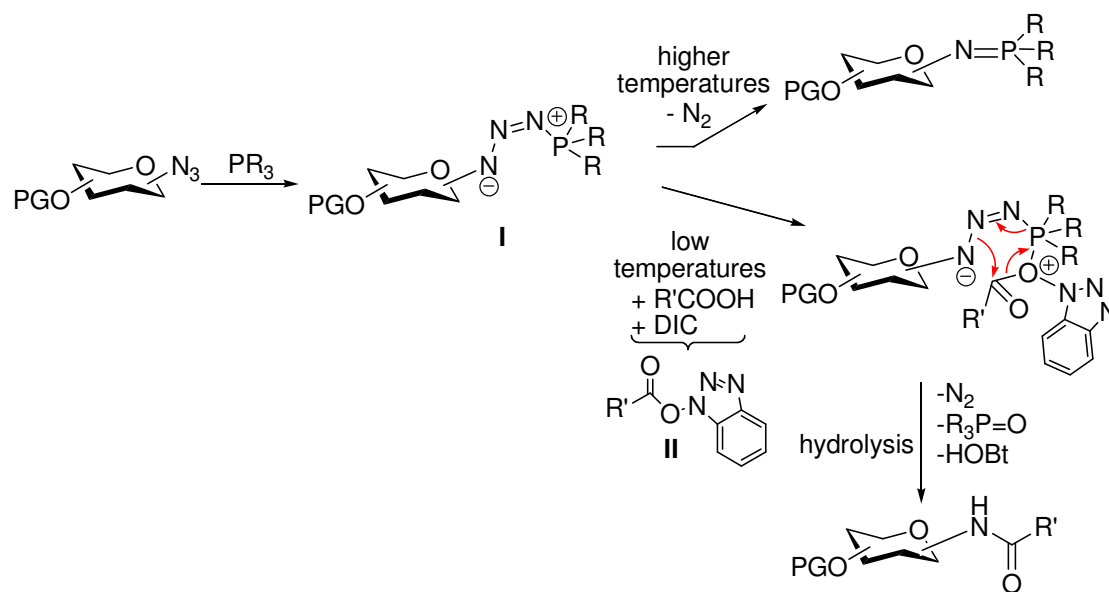
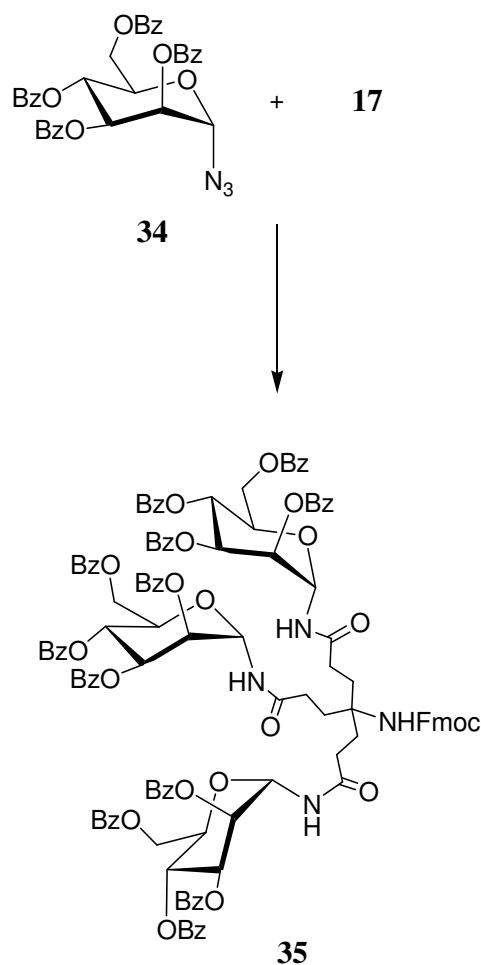


Figure 29: Mechanism of modified Staudinger reaction.

The reaction goes probably through a six membered transition state, favouring the product. When the reaction is carried out at higher temperature, the adduct **I** forms a four-membered ring intermediate, releasing nitrogen to form an iminophosphorane which can not be reacted further in order to form an amide.

Under analogous conditions, the molecular wedge **17** was reacted with *O*-benzoyl-protected mannosyl azide **34**, yielding the trivalent glycocluster **35** as the only product. Interestingly, the ¹H NMR spectrum showed that the product was contaminated with the traces of trimethyl phosphine oxide formed as a by-product of the reaction which was not possible to remove by silica gel chromatography. The difference between molecular masses of product **35** and by-product trimethyl phosphine oxide allowed purification by a size exclusion chromatography to remove parts of the trimethyl phosphine oxide.



Scheme 13: Synthesis of glycocluster **35**. (a) DIC, HOBT, $P(CH_3)_3$, THF, $-70\text{ }^\circ\text{C}$, 16 h, 34 %.

In conclusion, the glycoclusters **33** and **35** were successfully synthesized via modified Staudinger reaction. In this simple method of coupling carboxylic acid and an azide in presence of phosphine is interesting, also large products can be obtained, saving reaction steps and time. This reaction can further be utilized for the synthesis of glycocluster peptides, even more as $O \rightarrow N$ acetyl group migration can be avoided which is often a problem in carbohydrate chemistry.

5. Synthesis of oligosaccharide mimetics

5.1 Introduction

In *N*-glycoproteins, complex oligosaccharides, the *N*-glycans, are connected to a protein chain via an *N*-glycosidic bond. All glycoconjugates of this kind share a peptide-linked pentasaccharide unit which is commonly known as ‘core pentasaccharide’ and is consisting of two GlcNAc moieties and three mannose moieties. They are connected to each other in following way, Man-(α 1,6)[Man-(α 1,3)]Man-(β 1,4)-GlcNAc-(β 1,4)GlcNAc whereas, the terminal GlcNAc is linked to an aspartic acid side chain in every case.

The core pentasaccharide is further elongated by other branched oligosaccharides, leading to three different classes of *N*-glycans, those of the high mannose type (**A**), carrying predominantly mannose residues, those of the complex type (**B**), with [3-Gal-(β -1,4)-GlcNAc- β] as a repeating unit, varying from 2 to 50 copies; and *N*-glycans of the hybrid type (**C**), containing both of the other types (Figure 30).^[58]

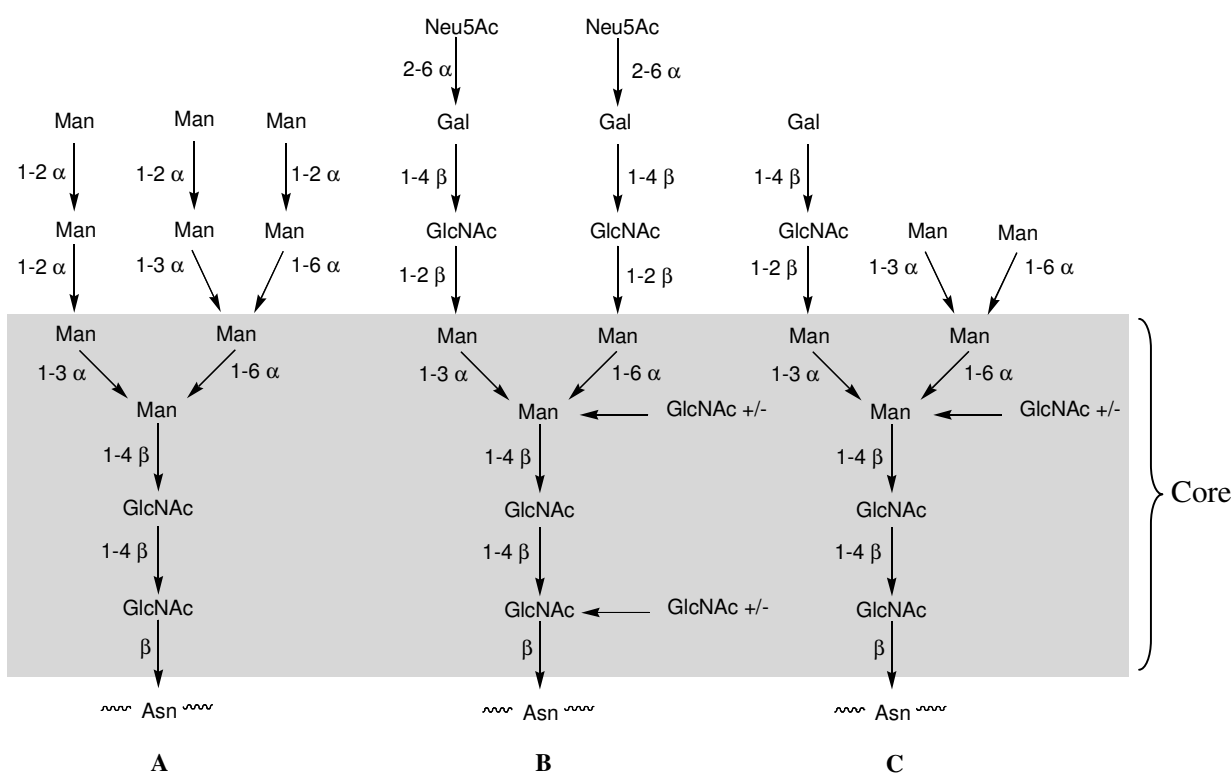


Figure 30: Types of *N*-glycans. (A) high mannose type; (B) complex type; (C) hybrid type (Core pentasaccharide shaded in gray).

These *N*-glycans are involved in various biological processes including cell-cell adhesion, immune response and cell signalling.^[59] To investigate the biological function of the carbohydrates found on cell surfaces, it has become important to mimic the natural structures. In this project, the trisaccharide unit from the core pentasaccharide Man-(β 1,4)-GlcNAc-(β 1,4)GlcNAc became a key target to be further developed into functional glycoconjugate mimetics. Hence, β -mannoside synthesis is quite tedious, the trisaccharide structure was altered and target Man-(α 1,4)-GlcNAc-(β 1,4)GlcNAc.

5.2 Synthesis

For the synthesis of the targeted trisaccharide three building blocks **I**, **24** and **39** were needed (Figure 31). First glycosylation of **24** and **39** should give disaccharide Man-(α 1,4)-GlcNAc which would be further reacted with building block **I** to yield the desired trisaccharide in the form of a glycosyl azide to allow eventual functionalisation and labelling at the reducing end.

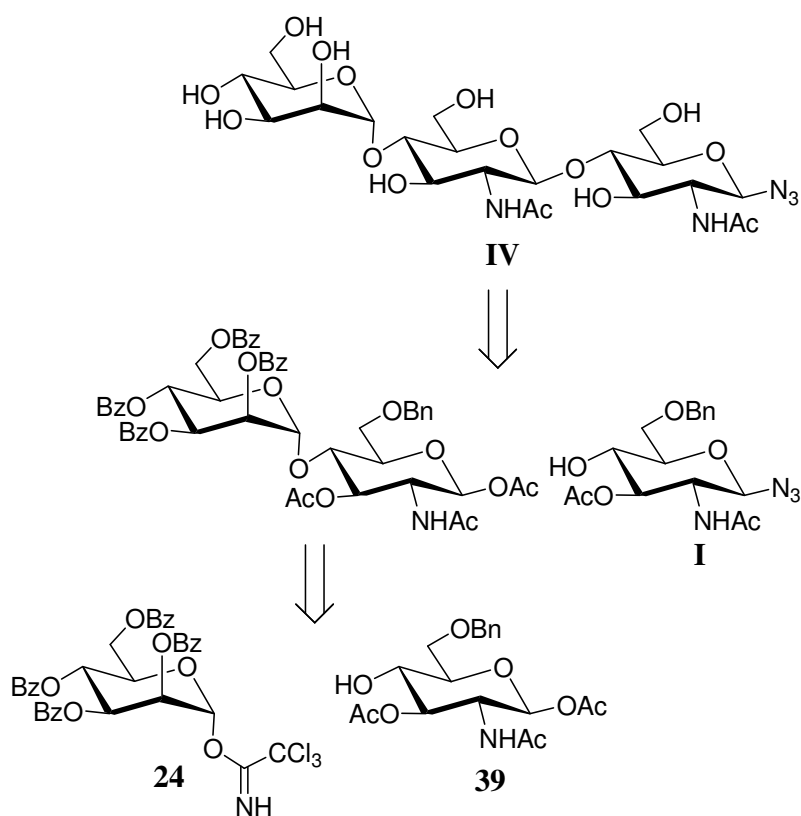
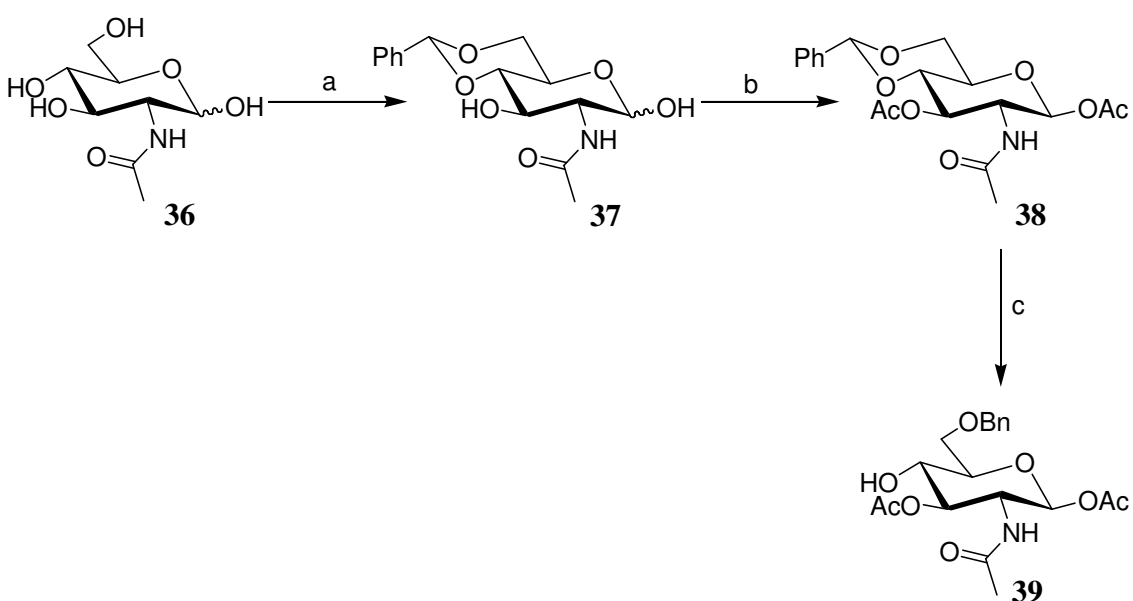


Figure 31: Retrosynthesis of the trisaccharide structure **IV**.

In the first attempt of synthesizing the above mentioned trisaccharide **IV**, the synthesis of building block **39** was started with *N*-acetyl glucosamine **36**. The free sugar **36** was first reacted with benzylidene dimethyl acetal using *p*-toluene sulphonic acid as an acidic catalyst to give **37** in poor yield. The 4,6-*O*-benzylidene-protected compound was then protected with acetyl groups using acetic anhydride and pyridine to give compound **38** in moderate yield. Then, fully protected **38**^[60] was subjected to reductive ring opening with sodium cyano boron hydride to yield the 4-OH-free GlcNAc derivative **39** in good yield.^[61] Interestingly, during silica gel chromatography evolution of gas was observed making initial filtration necessary through a small silica gel column.



Scheme 13: Synthesis of protected glycoside **39**. (a) PhCH(OCH₃)₂, *p*-TsOH, DMF, 60 °C, 3 h, 22 %; (b) Ac₂O, pyridine, RT, 16 h, 63 %; (c) Na(BH₃)CN, Et₂O·HCl, THF, 60 °C, 30 min, 78 %.

In the next step the alcohol **39** was subjected to mannosylation with the *O*-benzoyl-protected trichloroacetimidate **24** in presence of TMSOTf. The reaction was carried out over two days but no formation of the desired disaccharide **40** was observed (Figure 32). The reaction was also carried out using BF₃-etherate as Lewis acid but there was no trace of product. Also an addition of more equivalents of Lewis acid showed no results. The reason behind this failure might be the low nucleophilicity of the 4-hydroxy group in **39**. In addition the electron-withdrawing protecting groups in **39** intensify the problem.

Perhaps, use of electron-rich and electron-donating groups could enhance the reactivity of the 4-hydroxy group and allow eventual mannosylation.

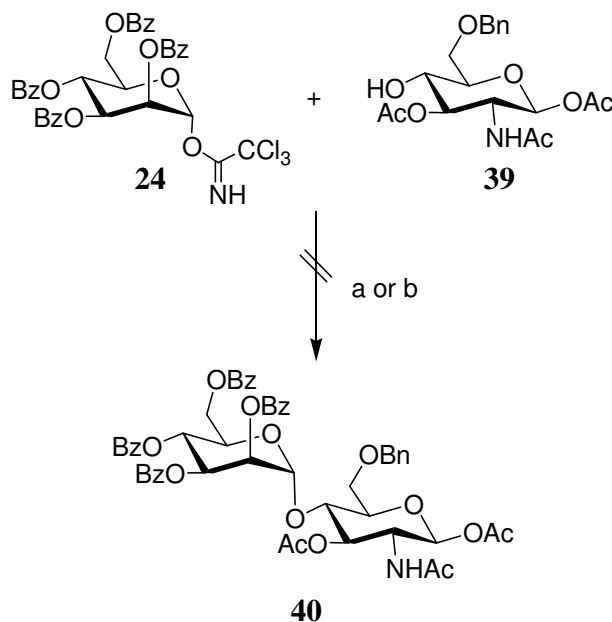


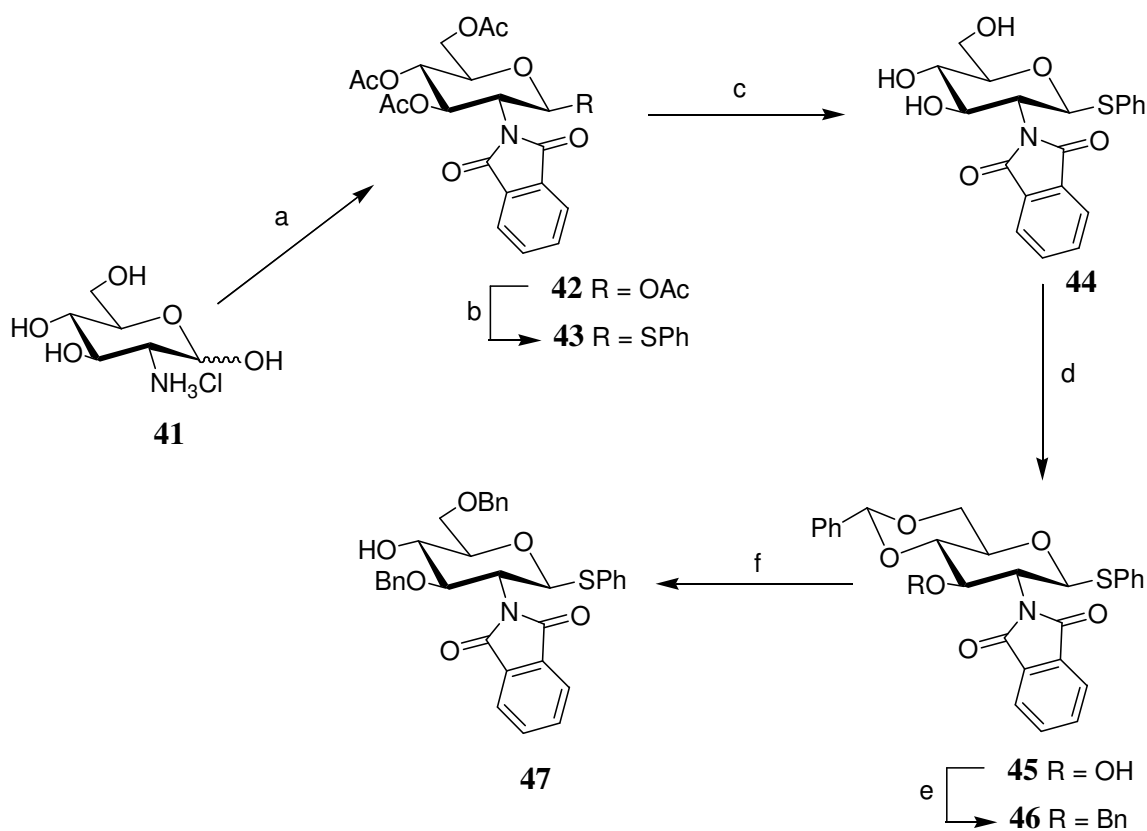
Figure 32: Attempted synthesis of disaccharide **40**. (a) TMSOTf, CH₂Cl₂, 0 °C → RT, 2 d; (b) BF₃·Et₂O, CH₂Cl₂, 0 °C → RT, 2 d.

Hereafter the synthetic plan could not take place. As discussed above the *N*-acetyl glucose amine was difficult to react being very electron deficient at 4-position.

5.3 Synthesis with electron-rich protecting group strategy

As discussed above, to enhance the reactivity of the 4-GlcNAc-hydroxy group, the protecting group pattern had to be changed and electron-donating protecting groups had to be employed. In a modified synthetic strategy, synthesis was started with glucosamine hydrochloride (Scheme 14). In the first step, the sugar **41** was treated with sodium methanolate solution followed by the reaction with phthalic anhydride. The 2-phthalimido-protected compound was then acetylated in pyridine with acetic anhydride to lead to **42**, which was then converted to the thioglycoside employing thiophenol and tin tetrachloride as promoter. The reaction worked fantastically giving thioglycoside **43** in very good yields. In the next reaction step, the thioglycoside was deprotected using sodium methanolate and glycoside **44** was obtained in pure form after chromatography. In order to get the 4-position free OH-free for this eventual glycosylation reaction, a

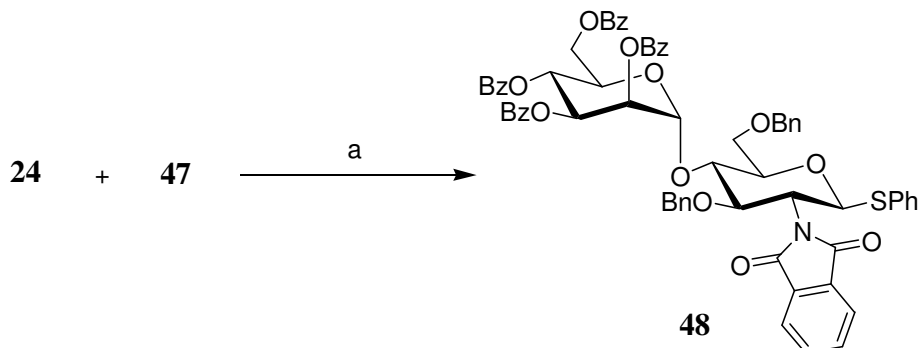
typical reaction sequence was carried out. Thus the unprotected thioglycoside **44** was first reacted with benzylidene dimethyl acetal to give **45** in good yields. Then the 3-position was benzyl-protected to yield **46**^[62], which was subjected to selective opening of the benzylidene acetal, which is possible according to two different reductive methods. In first method, triethyl silane^[63] was used and in the other method sodium cyano boron hydride to obtain the unmasked hydroxyl compound **47** in good yield. However, both methods worked well but the triethyl silane method can be preferred as the sodium cyano boron hydride requires the HCl gas formation (Scheme 14).



Scheme 14: Synthesis of protected thioglycoside **47**. (a) (i) CH₃ONa, CH₃OH, 60 °C; (ii) phthalic anhydride, (iii) Ac₂O, pyridine, 57 %; (b) PhSH, SnCl₄, CH₂Cl₂, RT, 16 h, 83 %; (c) CH₃ONa, CH₃OH, RT, 4 h, 94 %; (d) PhCH(OCH₃)₂, CSA, CH₃CN, 60 °C, 6 h, 81 %; (e) BnBr, NaH, TBABr, DMF, 0 °C → RT, 6 h, 56 %; (f) method (i) (CF₃CO)₂O, TES, TFA, 0 °C → RT, 2 h, 65 %; method (ii) Na(BH₃)CN, Et₂O·HCl, THF, 60 °C, 6 h, 71 %.

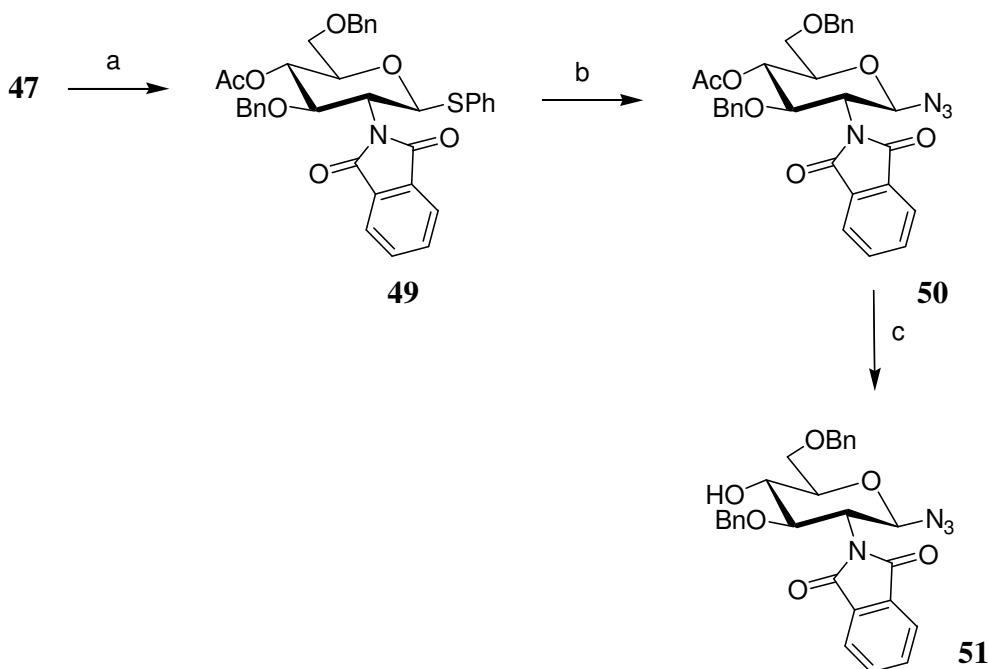
To synthesize the disaccharide building block, *O*-benzoyl-protected mannosyl trichloroacetimidate **23** was used as glycosyl donor in excess compared to the thioglycoside **47**. The glycosylation reaction was brought to completion by addition of

BF_3 -etherate until the glycosyl acceptor **47** was completely consumed according to TLC. Thus, oligosaccharide **48** was obtained in good yield (Scheme 15).



Scheme 15: Synthesis of disaccharide **48**. (a) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, CH_2Cl_2 , $0\text{ }^\circ\text{C} \rightarrow \text{RT}$, 16 h, 78 %.

According to the retrosynthetic approach, the disaccharide unit **48** was achieved by glycosylation of **24** and **47**. Next, trisaccharide synthesis demanded the synthesis of an appropriate glycosyl azide which was performed as follows. The orthogonally protected thioglycoside **47** was first 4-*O*-acetylated to get the corresponding thioglycoside **49** (Scheme 16).



Scheme 16: Synthesis of glycosyl azide **51** (a) Ac_2O , pyridine, 16 h, RT 85 %; (b) TMSN_3 , NIS, TfoH, 4 Å molecular sieves, CH_2Cl_2 , $-78\text{ }^\circ\text{C}$, 89 %; (c) CH_3ONa , CH_3OH , RT, 4 h, 86 %.

In the next step compound **49** was reacted with TMSN_3 in presence of NIS and triflic anhydride as described in the literature.^[64] When the reaction was carried out with freshly bought triflic anhydride there was no product formation observed. But when the reaction was carried out with NIS and triflic acid combination glycosylazide **50** was obtained in very good yields. This suggests that activation of thioglycosides is only possible with NIS/TfOH combination, not with Tf_2O .^[65] As triflic anhydride gets hydrolysed by moisture, it is possible that traces of triflic acid present in Tf_2O , yield to product formation. This is well explained by looking at the glycosylation mechanism mediated by NIS and triflic acid (Figure 33).

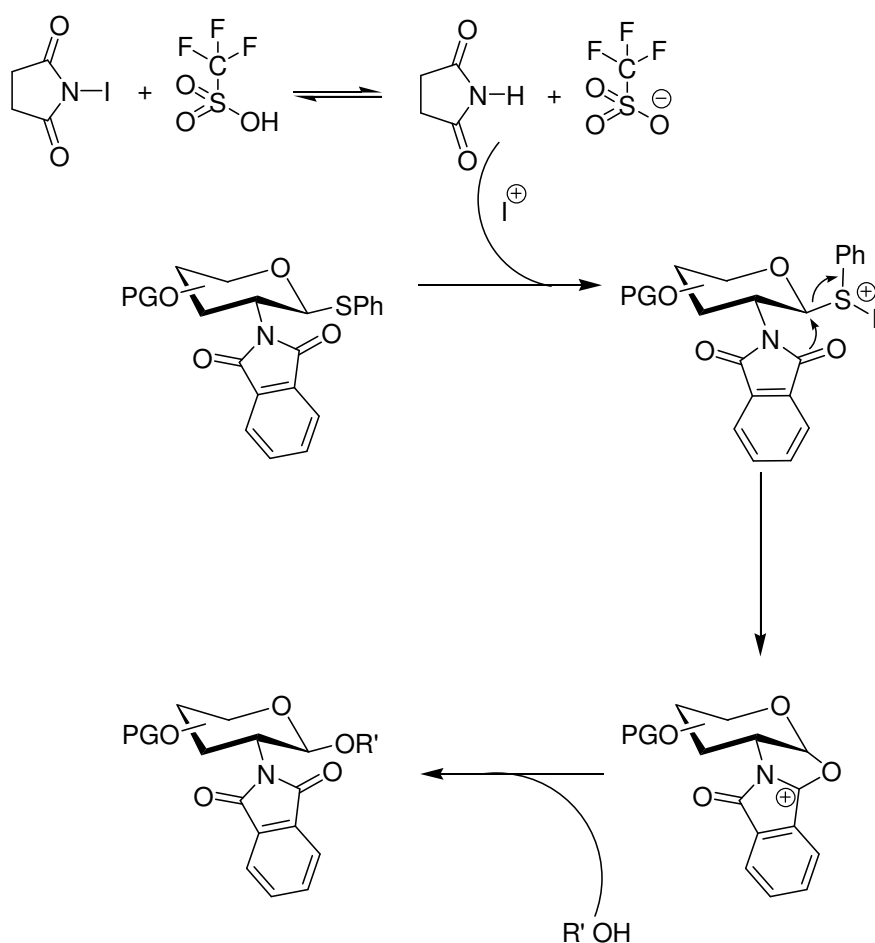
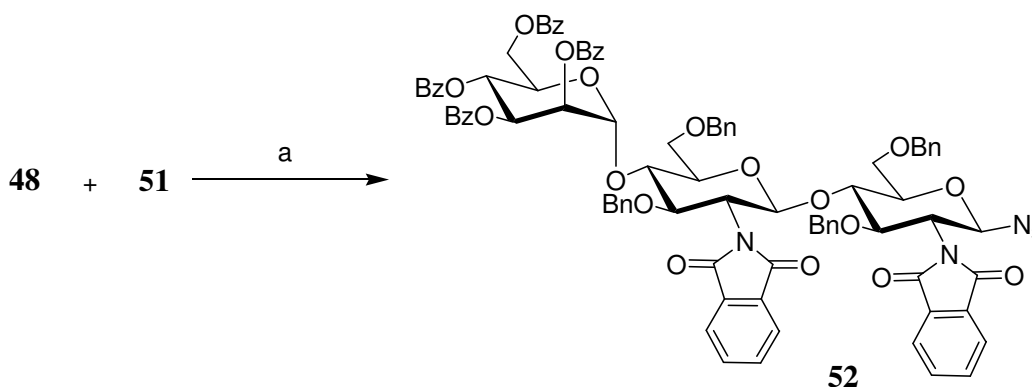


Figure 33: Mechanism of NIS/TfOH catalysed glycosylation.

In a glycosylation reaction promoted by NIS and TfOH, both reagents react with each other to deliver iodonium ions. The thioglycoside reacts with the iodonium ion and with neighbouring group participation of the 2-protecting group the thioaglycon is released.

Then the attack of a glycosyl acceptor at the anomeric position gives the glycosylated product. If triflic anhydride is used instead of triflic acid then there is no iodonium ion formation hence glycosylation does not occur. Finally, **51** was obtained after *O*-acetyl deprotection of **50** in good yield.

Upon having the disaccharide **47** and glycosyl azide **51** in hand a glycosylation reaction was performed in presence of NIS and TfOH to give trisaccharide **52** (Scheme 17). The structure **52** was confirmed by NMR spectroscopy and correct molecular mass was found in HRESI experiment.



Scheme 17: Synthesis of trisaccharide **52**. (a) NIS, TfOH, CH₂Cl₂, -70 °C, 30 min, 71 %.

5.4 GPI anchor mimic

GPI anchored proteins are a kind of membrane proteins which are anchored to the membrane via means of translipid modifications. They are glycosylphosphatidylinositol molecules present in parasites as well as mammals. The GPI anchors are classified into three different classes on the basis of their glycan structure: type-1 has Man α 1-6Man α 1-4GlcN α 1-6PI, type-2 has Man α 1-3-Man α 1-4GlcN α 1-6PI and type-3 has Man α 1-3Man α 1-4GlcN α 1-6PI structure. The core structure of GPI anchor is composed of single phospholipids and complex head group composed of phosphodiester-linked inositol, a glucose amine residue, mannose residues and phosphoethanolamine.^[66] The general structure contained by GPI anchors is depicted below (Figure 34). The GPI anchors have predicted have hydrolytic activity or serve as receptor or adhesive proteins. The defect in the biosynthesis of the GPIs is responsible for many disorders in humans. e.g. paraoxysmal nocturnal hemoglobinuria is caused by somatic defect in the first step of biosynthesis of GPIs in human stem cells.^[67]



Figure 34: General structural unit in GPI anchors (numbers indicate the position of attachment).

Here, it was attempted to synthesize a GPI anchor mimic from the synthesized trisaccharide **52**. The trisaccharide **52** contains two glucosamine moieties protected with phthalimido groups, which could be deprotected to have a free amine. On other hand, the anomeric azido group could be reacted with a lipid chain to form a mimic of GPI anchor, employing Staudinger ligation for example.

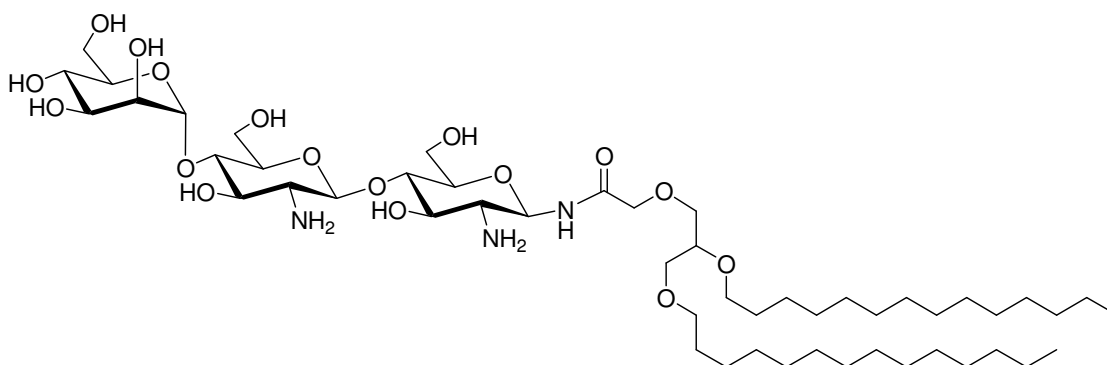
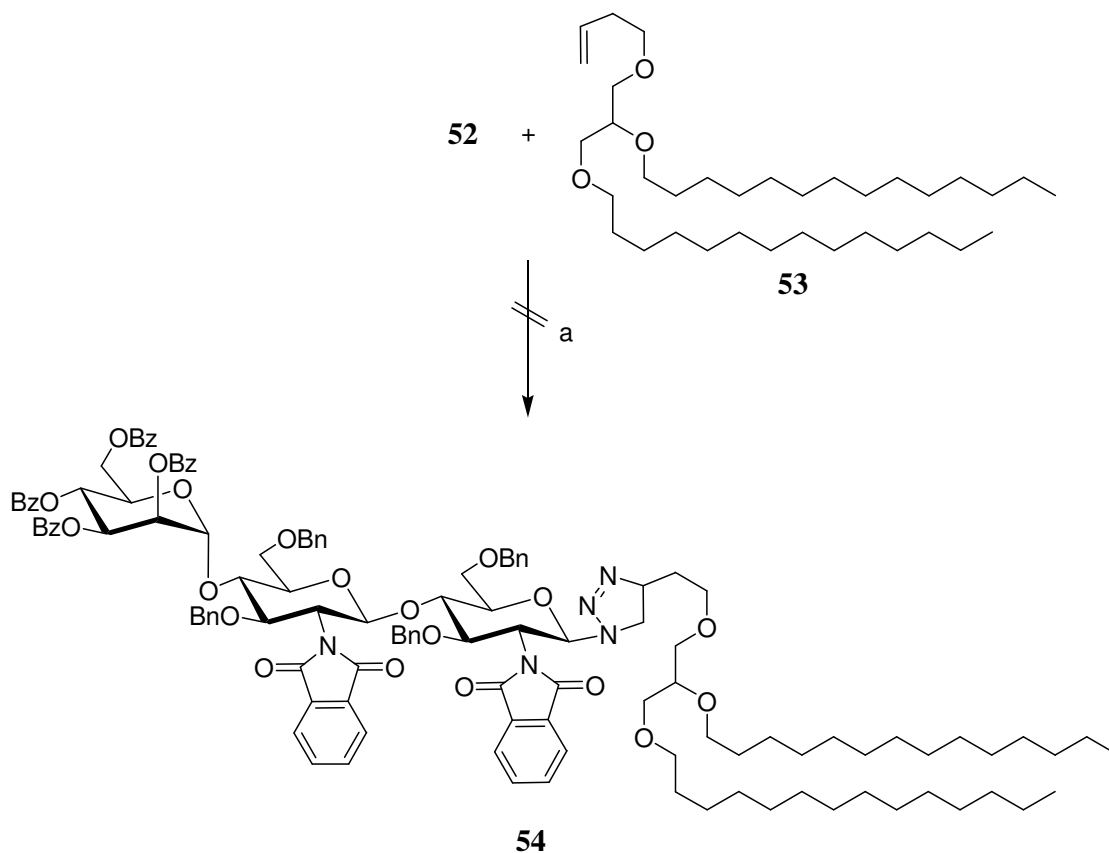


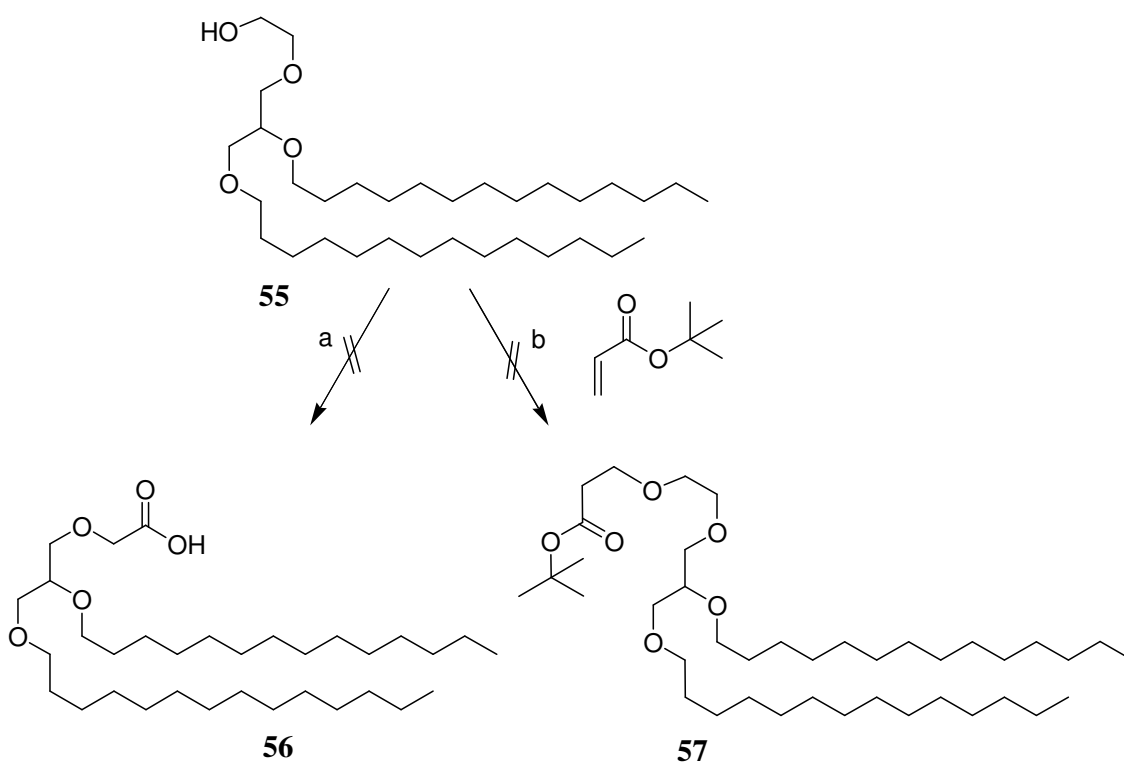
Figure 35: Synthetic GPI anchor mimic.

In addition the anomeric azide function suggests a Huisgen-type 1,3-dipolar cycloaddition reaction as an option to ligate **52** to alkenes and alkynes as reported in literature.^[68] Thus, the trisaccharide **52** was refluxed with the alkene **53** for three days but there was no conversion of reactant into product **54** (Scheme 18).



Scheme 18: Attempted synthesis of compound **54**. (a) CCl₄, reflux, 3 d, no reaction.

A second option, Staudinger ligation between a carboxylic acid and the azide **52** was targeted next. Therefore, it was attempted to oxidize^[69] alcohol **55** with pyridinium chloro chromate (PCC) and periodic acid but it did not give the desired carboxylic acid **56**. However, decomposition of reactant was observed. In another approach **55** was reacted with *tert*-butyl acrylate using DBU, but no reaction occurred (Scheme 19). In the reaction with sodium hydride as a base, product **57** was observed in MALDI-ToF-MS but could not be isolated.



Scheme 19: Attempted synthesis of compound **57** and **58**. (a) PCC, H_5IO_6 , CH_3CN , 12 h, RT, no reaction; (b) *tert*-butyl acrylate, method (i) DBU, CH_2Cl_2 , RT, 12 h, no reaction, method (ii) NaH, THF, 60 °C, 12 h, no reaction.

Once having a carboxylic acid at hand, it could be coupled to the trisaccharide **52** and followed deprotection of the resulting carbohydrate can serve as GPI anchor mimic bearing a lipid chain and $GlcNH_2$ moiety.

5.5 Perspective

The trisaccharide synthesized (**52**) is bearing a lot of functionalities which could allow this molecule to serve as different carbohydrate mimics.

- (a) As mentioned above the trisaccharide could be attached to the lipid chains and form a GPI anchor mimic or other glycolipid mimetics. It will be interesting to synthesize the non natural mimic and study in biological systems.
- (b) The molecule could also be attached to lipid chains at the GlcNH₂ functions and the anomeric azide could be ligated to a peptide leading a simple mimic of a lipopolysaccharide bearing a peptide chain as mimic of protein.

6. Biological studies and CD spectroscopy

6.1 Introduction

Escherichia coli is a Gram-negative bacteria present for example in gastrointestinal tracks of mammals. In colon it is known to be harmless but it is also associated with number of infectious diseases. The surface is carrying a number of proteinaceous appendages known as fimbriae which one is involved in an adhesion process by forming multivalent interactions with the glycocalyx of a host cell. Among others *E.coli* are utilizing so called type 1 fimbriae as adhesive organelles (Figure 36).



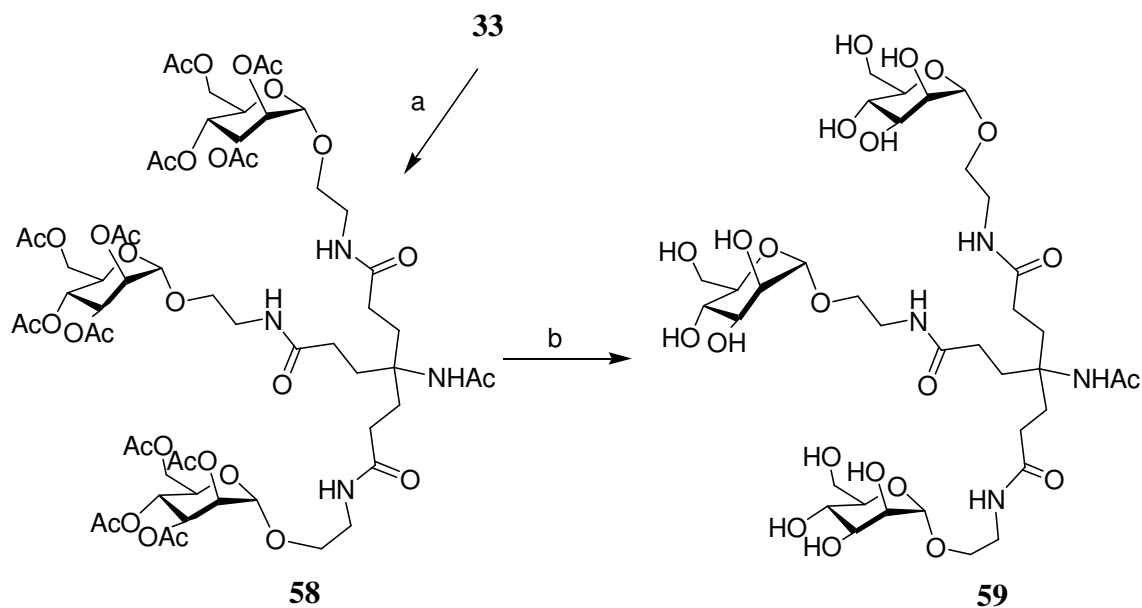
Figure 36: *Escherichia coli*.^[77]

The type 1 fimbriae are composed of four proteins, the largest part is making up for 98 % of the fimbriae is FimA, and additional proteins are FimG, FimF and FimH. FimH is a carbohydrate-specific lectin, which specifically binds to α -mannosyl residues.^[70] The crystal structure of FimH is known for a while, showing that at the tip of the protein there is a binding pocket for a single α -D-mannoside.^[71] Thus, FimH plays an important role in the adhesion process. It has been reported, a type 1 fimbriae-mediated adhesion is better with oligomannosides than when single α -D-mannosyl residues are addressed. Therefore, it has been proposed, that there are more than one binding sites for saccharides on FimH.

6.2 Glycopeptide analogs for bio-assaying

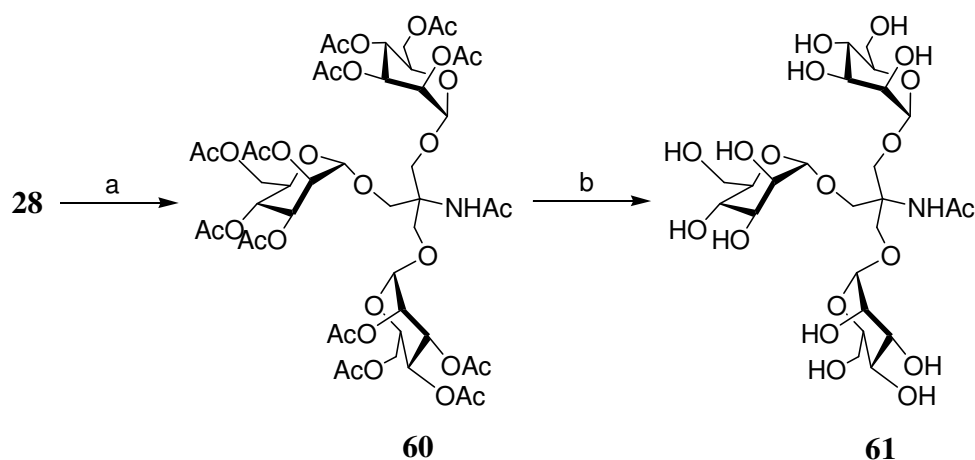
The herein prepared glycoclusters were interesting candidates in the bio-assay for FimH binding. The glycocluster carrying an amino functionality was acetylated to form a *N*-acetyl glycocluster in analogy to the glycopeptides to let the amide bond at the focal point resemble a peptide bond analog. Thus, glycocluster **59** can be considered as an analogue of glycopeptide **31** and glycocluster **61** can be considered as analogue of glycopeptide **32**. These glycoclusters are carrying three α -D-mannoside residues which make them suitable ligands for FimH as FimH is carrying the binding pocket for α -mannoside residues (CRD).

In the first reaction sequence, glycocluster **33**, obtained from the modified Staudinger reaction from (Chapter 4.2) was treated with morpholine in order to remove the Fmoc protecting group at the focal point. In the next reaction step the substrate was used without any purification and reacted with acetic anhydride in pyridine to give the *N*-acetylated glycocluster **58**. Then, the peracetylated glycocluster **58** was deprotected using sodium methanolate to give *N*-acetyl cluster molecule **59** in good yield (Scheme 20).



Scheme 20: Synthesis of glycocluster **59**. (a) (i) morpholine, CH_2Cl_2 , RT, 4 h, (ii) Ac_2O , pyridine, RT, 16 h, 68 %; (b) CH_3ONa , CH_3OH , 3 h, quant.

In a similar way, the glycocluster **28** was first acetylated with acetic anhydride in pyridine to form cluster molecule **60**. Then, the *O*-acetyl groups of cluster molecule **60** were cleaved using sodium methanolate to give *N*-acetylated glycocluster **61** in quantitative yield (Scheme 21).



Scheme 21: Synthesis of *N*-acetyl glycocluster **61**. (a) Ac_2O , pyridine, 16 h, RT, 80 %; (b) CH_3ONa , CH_3OH , 3 h, quant.

6.3 Competitive assay on FimH-binding

For studying the inhibitory potency of the mannosyl glycoclusters, fluorescent bacteria were used. The bacterium was subjected to gene transformation, which is coding for a fluorescent protein; the fluorescent protein encoded was 'Green fluorescent protein'.^[72] The inhibitory potencies of substances **59** and **61** were measured in a competitive bioassay. In the procedure developed for measuring the inhibitory potency, type-1-fimbriated, GFB-transfected *E. coli* and serially diluted cluster mannosides were added to the mannan-coated test surface and incubated for 45 min. The bacteria with no adhesion to the mannan surface were washed away with the inhibitor and the fluorescence of remaining GFB transfected bacteria was read. For the glycoclusters showing better inhibitory potency, less fluorescence was detected.^[73] A curve was drawn from the inhibition values of the serially diluted samples and deduced IC_{50} values were compared between the tested cluster mannosides and the literature-known inhibitor methyl α -D-mannoside (MeMan, Table 1).

Table 1: Comparison of IC_{50} values of glycocluster **59** and **61** with methyl- α -D-mannoside.

| | MeMan | 59 | 61 |
|------------------------------------|--------------|-----------|-----------|
| IC_{50} (μM) | 1119 | 319 | 222 |

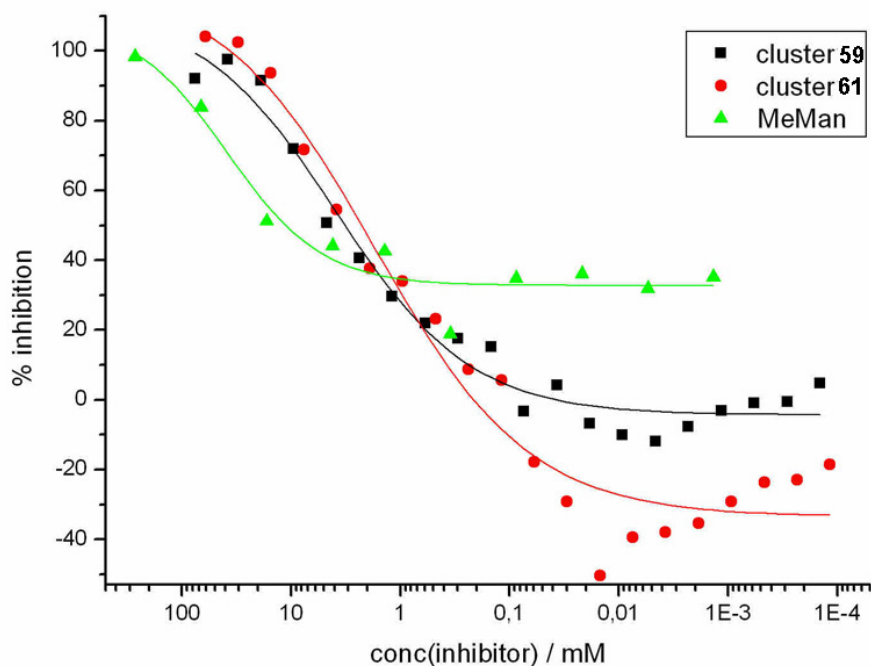


Figure 37: Sigmoidal fitting of the data to obtain IC_{50} values of **59**, **61** and methyl- α -D-mannoside.

6.4 Discussion

As shown in the Table 1, glycocluster **59** and **61** were studied in assay for FimH. The IC_{50} values were determined from the plot shown in Figure 37. Both of the glycoclusters were found to be inhibiting the tested bacterial adhesion process. The larger the IC_{50} is the lower is the inhibitory potency and vice versa. The glycoclusters **59** and **61** both showed a lower IC_{50} values as compared to MeMan.

Mirja Hartmann M.Sc. is thankfully acknowledged for carrying out the inhibition experiments.

6.5 CD spectroscopy of glycocluster peptides

Optical rotatory dispersion (ORD) and circular dichroism (CD) are widely used methods in the conformational analysis of biomolecules and in particular of proteins and peptides. Circular dichroism occurs when polarized light is interacting with the chromophores in an asymmetric environment. In proteins and peptides the main chromophores are the peptide bonds as well as aromatic side chains. In ORD different refractive indices are measured at different wavelengths, whereas in CD absorption is measured at different wavelengths of the optically active molecules. In optically active substances the absorption of left handed polarised and right handed polarised light is different. The circular polarised light forms an ellipsoid in the space. In CD spectroscopy the difference in absorption of left circular and right circular polarised light is measured. The absorption coefficient ϵ for polarised light is noted as $(\epsilon_L - \epsilon_R)$. It is named as ellipticity and represented by an equation:

$$\Theta(\lambda) = \text{const} \cdot (\epsilon_L - \epsilon_R) \cdot c \cdot d$$

For spectral analysis of peptides wavelength range 180 to 250 nm (far-UV-CD spectroscopy). In this wavelength range $n \rightarrow \pi$ and $\pi \rightarrow \pi^*$ transitions are observed but they are very small. The CD spectra of peptides are very sensitive because of the chirality in the structure.^[74]

In peptides and proteins have domains where highly ordered secondary structures such as α -helix or β -plated sheets are predominant. The optical transitions of the amide bond can split into multiple transitions, depending on the orientation of the peptide bonds to each other. The wavelength and intensity of the transitions can increase or decrease, thereby giving characteristic spectra for α -helices, β - sheets and β -turns.^[75]

6.6 Discussion

Formation of a secondary structure is a very common phenomenon among the natural products such as proteins and nucleic acids. The transmembrane proteins in the lipid bilayer membrane are widely assumed to be α -helical.^[76]

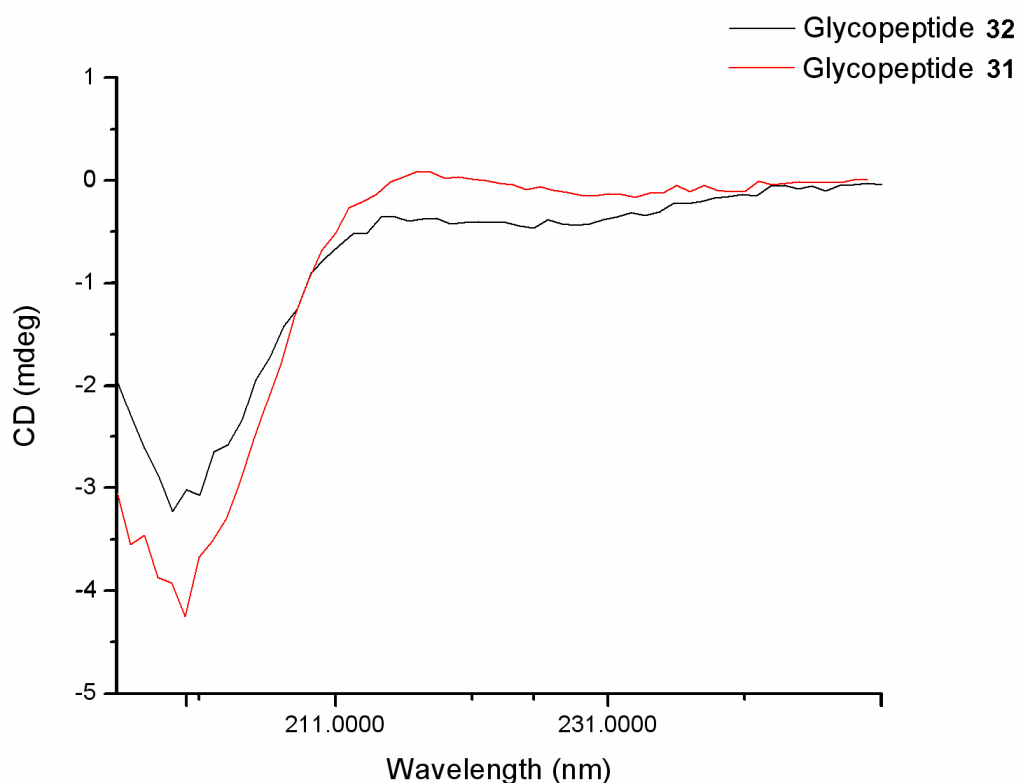


Figure 38: CD spectra of glycoclusters **31** and **32** in water.

As already discussed earlier in this thesis, two glycocluster peptides **31** and **33** synthesized as transmembrane protein mimic. When the CD spectrum was measured of these molecules it was found that both of these glycocluster peptides did not form any secondary structure. However, it was found out that they were conformed in a random coil structure which was interpreted from Figure 38. The glycocluster peptide **31** was observed to show higher absorption than that of **32**. The glycocluster **31** has additionally three peptide bonds in the glycocluster part, the carbohydrates are attached to the trivalent wedge; they must be contributing to the higher absorption. However, the initial finding of the NMR studies was confirmed by the CD spectrum suggesting the formation of a random coil.

Prof. Dr. Grötzinger and Dipl. Biochem. Sascha Jung are thankfully acknowledged for their help in carrying out the CD spectroscopy and interpretation of the data.

7. Summary

7.1 Summary

In order to mimic natural glycoconjugates, dendrimer chemistry provides a possibility to prepare branched cluster glycosides as oligosaccharide mimetics. In this thesis, synthesis of two glycopeptides **31** and **32** is described which were synthesized as transmembrane protein mimics and coined as ‘glyocluster peptides’.

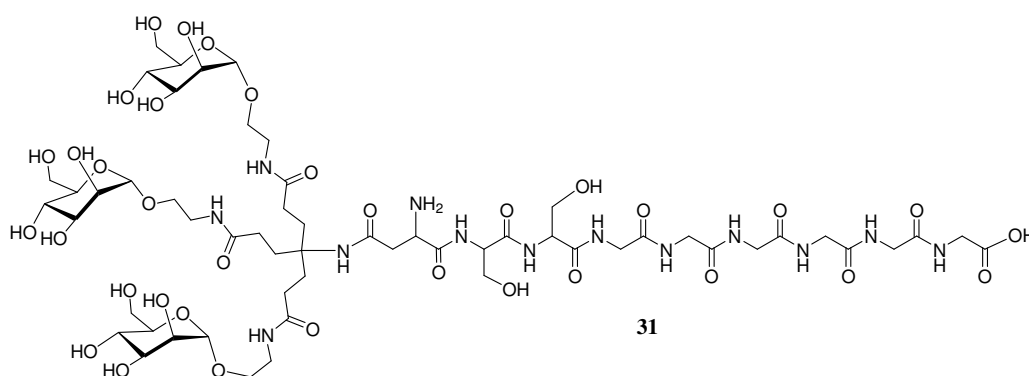


Figure 39: Glyocluster peptide **31** as transmembrane protein mimic.

The glyocluster part of this molecule represents the extracellular highly branched oligosaccharide structures whereas the peptide chain represents the transmembrane protein part composed of lipophilic amino acids like glycine. To synthesize these glyocluster peptides, a convergent approach was followed where a tailored octapeptide **6** was synthesized via solid phase synthesis; the glyoclusters **21** and **30** were then coupled to this model peptide on solid phase. Glyoclusters **21** and **30** carrying α -D-mannoside residues and aspartic acid at the focal point of the dendritic wedge were employed in the synthesis (Figure 41).

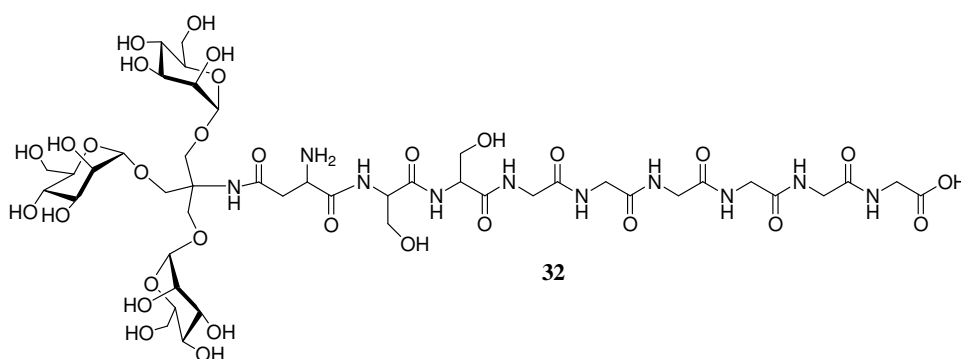


Figure 40: Glyocluster peptide **32** as transmembrane protein mimic.

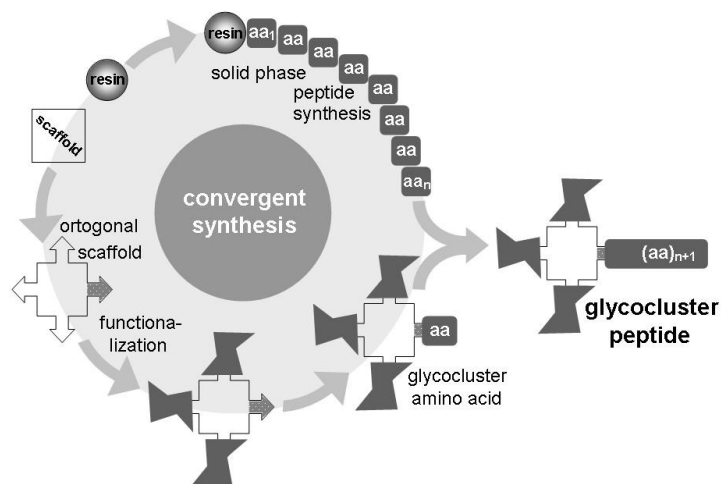


Figure 41: This cartoon highlights the chosen approach to glycocluster peptides. The convergent synthesis relies on one hand on solid phase peptide synthesis, and on the other hand on the orthogonal derivatization of an appropriate tetrafunctional scaffold molecule.

In order to build different glycoclusters an approach was employed which utilized a modified Staudinger reaction, which successfully yielded two glycoclusters **33** and **35** (Figure 42). This approach gave successful coupling reaction between multivalent carboxylic acid and two different mannosyl azides in presence of trimethyl phosphine, which can prove useful in the synthesis multivalent glycoconjugate synthesis. In the advantageous method one can save reaction steps and time and the notorious problem of *O*→*N* acetyl group migration can be avoided.

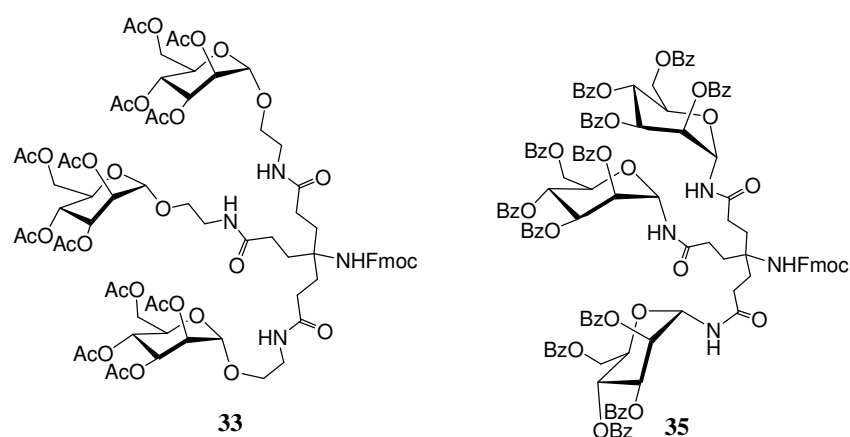


Figure 42: Synthesized glycoclusters **33** and **35** via modified Staudinger reaction.

In the other part of this work, a trisaccharide was synthesized based on the structure $\text{Man}(1-4\alpha)\text{GlcN}(1-4\beta)\text{GlcN}$ which belongs to the core pentasaccharide structure in *N*-glycans. It was also attempted towards to ligate the trisaccharide to a lipid chain to produce a GPI anchor mimic (Figure 43). This method needs formation of carboxylic acid of the alkyl chains which could be ligated to the trisaccharide through azide.

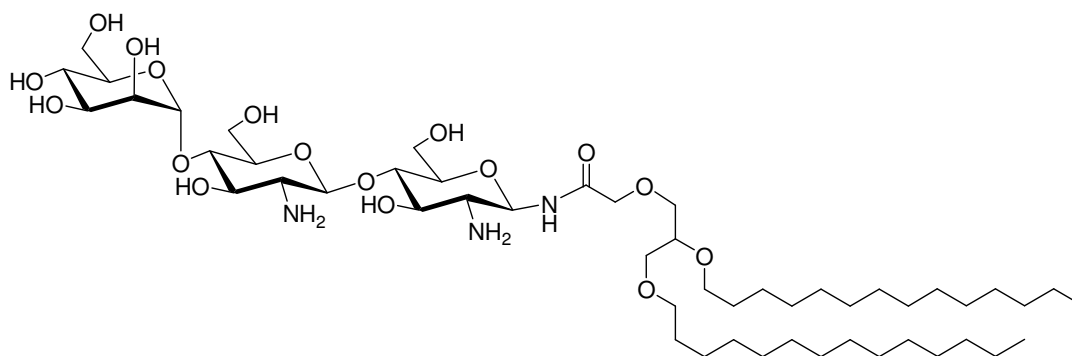


Figure 43: GPI anchor mimic from trisaccharide **52**.

To conclude, in this work synthesis of glycocluster peptides was successfully carried out yielding a glycoconjugate mimic which could prove useful for investigations in glycobiology. Here, a short nonapeptide was employed as a simple mimic of a transmembrane protein portion. Solid phase synthesis of glycocluster peptides was optimized and can eventually be used in synthesis of other glycocluster peptides with longer and structurally varied peptide chains. The facile synthesis of glycoclusters via modified Staudinger reaction can be utilized for the synthesis of further glycocluster peptides. Glycocluster peptides **31** and **32** were subjected to several NMR experiments, showing a formation of random coil. The random coil conformation was confirmed by CD spectroscopy showing a characteristic CD spectrum. Glycoclusters **59** and **61** in an inhibition assay as inhibitors of mannose-specific bacterial adhesion. Both of them showed inhibition and could thus be used as inhibitors of type-1-fimbriae mediated adhesion. While the versatile trisaccharide **52** unit can eventually be modified in different ways to lead a variety of functional glycoconjugate mimics. further potential lies in targeting the synthesis of GPI anchor mimetics and lipopolysaccharide mimetics. To study the biology of these synthetic made glycoconjugates will help to unravel the biological secrets of glycobiology.

7.2 Zusammenfassung

Die Dendrimerchemie bietet die Möglichkeit, verzweigte Glycocluster herzustellen um die natürlich vorkommenden multivalenten Oligosaccharidstrukturen in Glykokonjugaten zu mimikrieren. In dieser Arbeit wird die Synthese der Glycopeptide **31** und **32** beschrieben, die als Transmembranprotein-Mimetika gedacht und als „Glycocluster-Peptide“ bezeichnet wurden.

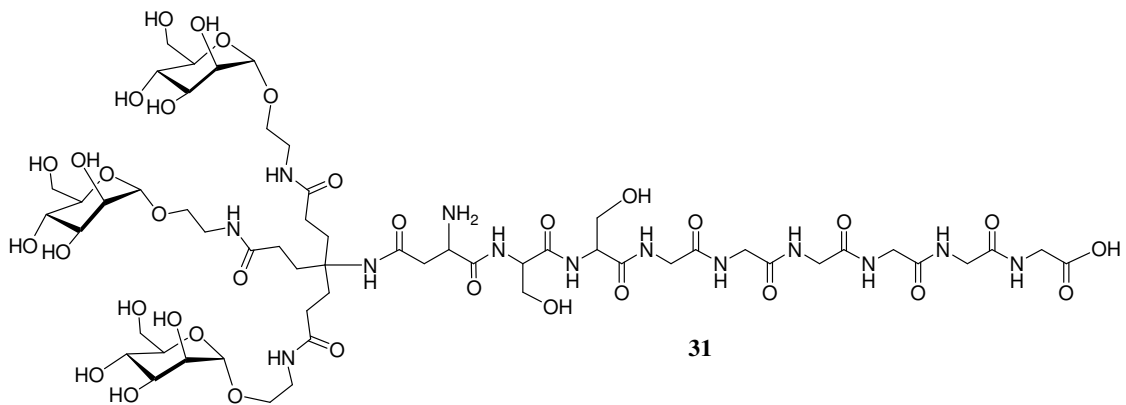


Figure 44: Glycoclusterpeptid **31** als Transmembranprotein-Mimetikum.

Der Bereich des Glycoclusters dieser Moleküle soll einen Teil der extrazellulär exponierten hochverzweigten Oligosaccharide repräsentieren, während die Peptidkette einen Bereich eines lipophilen Transmembranproteins verkörpern soll. Zur Synthese der Glycocluster-Peptide wurde ein konvergenter Ansatz verfolgt, wobei ein Octapeptid **6** mittels Festphasensynthese dargestellt und mit den Glycoclustern **21** und **30** verknüpft wurde. Bei **21** und **30** handelt es sich um α -D-mannosyliertetrivalente Dendronen, die am fokalen Punkt mit Asparaginsäure funktionalisiert wurden (Figure 46).

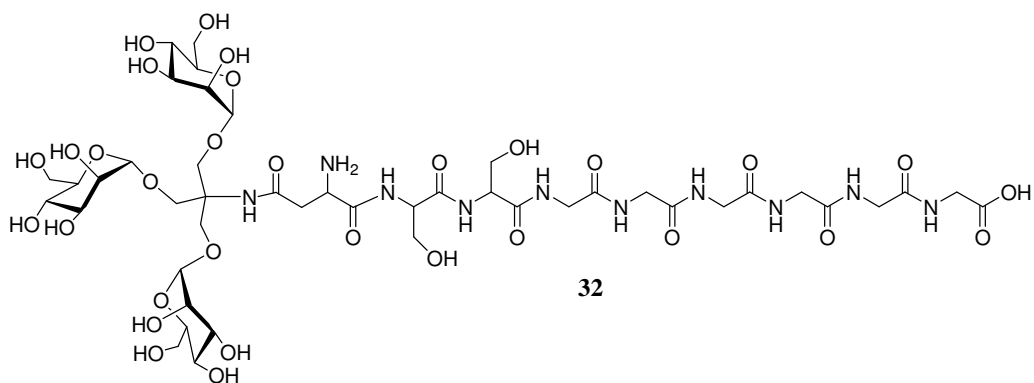


Figure 45: Glycoclusterpeptid **32** als Transmembranprotein-Mimetikum.

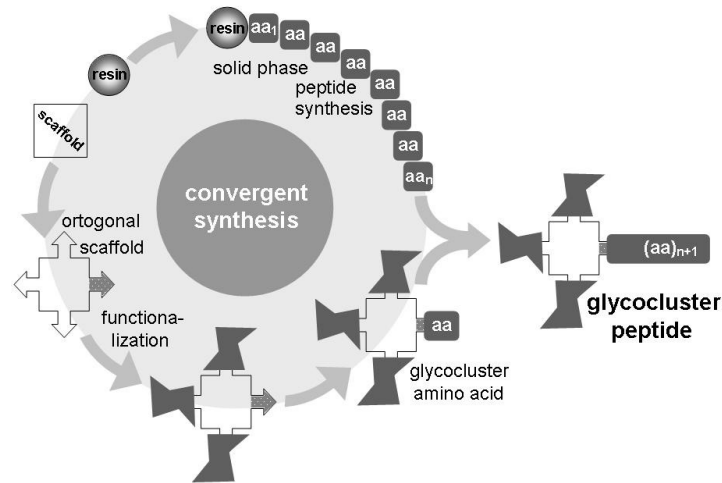


Figure 46: Darstellung des gewählten Weges zur Glyocluster-Peptidsynthese. Der konvergente Ansatz basiert zum einen auf der Festphasensynthese der Peptide und andererseits auf der orthogonalen Derivatisierung geeigneter Kernmoleküle.

Um verschiedene Glyocluster aufzubauen wurde eine modifizierte Staudinger-Reaktion gewählt, die erfolgreich die Glyocluster **33** und **35** lieferte (Figure 47). Diese wurden durch eine erfolgreiche Kupplungsreaktionen zwischen multivalenten Carbonsäuren und zwei verschiedenen Mannosylaziden in der Gegenwart von Trimethylphosphin erhalten, das sich als sinnvoller Zusatz in der multivalenten Glyocluster-Synthese erweisen kann. Diese sehr vorteilhafte Methode kann nicht nur Zeit und Stufen sparen, sondern auch das Problem der *O*→*N*-Acetylgruppenwanderung vermeiden.

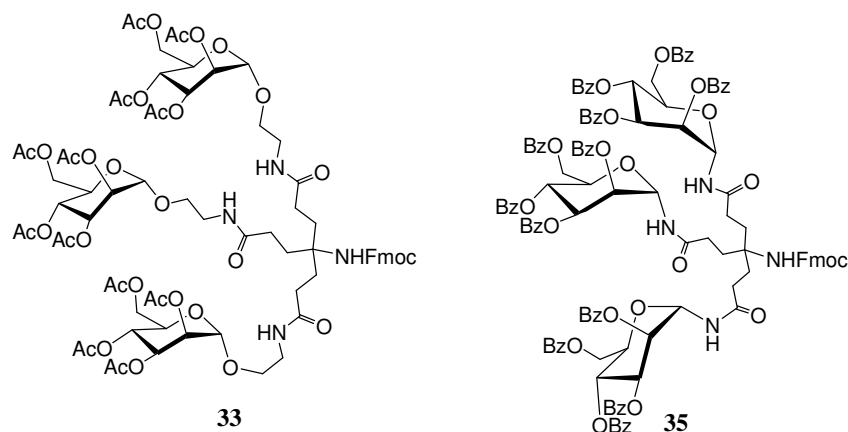


Figure 47: Durch Staudinger Reaktion synthetisierte Glyocluster **33** and **35**.

In dem anderen Teil der Arbeit wurde ein Trisaccharid auf der Basis von Man(1-4 α)GlcN(1-4 β)GlcN synthetisiert, das zu der Kern-Pentasaccharid-Struktur in *N*-Glycanen gehört. Es wurde versucht, das Trisaccharid mit einer Lipidkette zu verknüpfen um einen GPI-Anker zu mimikrieren (Figure 48). Diese Methode benötigt die Bildung einer Carbonsäure der Lipidkette um diese über das Azid mit dem Trisaccharid zu verbinden.

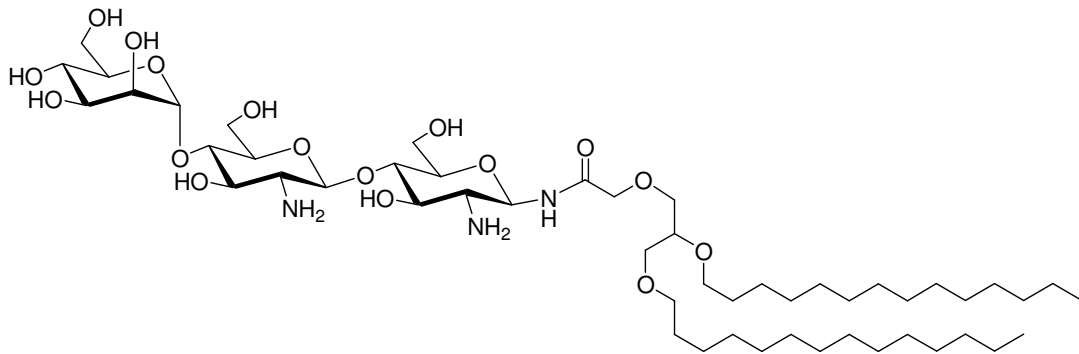


Figure 48: GPI-Anker Mimetikum des Trisaccharids **52**.

Zusammenfassend wurde in dieser Arbeit die Synthese neuer Glycocluster-Peptide erfolgreich durchgeführt, wobei ein Glycokonjugat-Mimetikum erhalten wurde, das sich als sinnvoll für glycobiologische Untersuchungen erweisen könnte. Es wurde hier ein kurzes Nonapeptid als einfaches Modell eines Transmembranproteins genutzt. Die Festphasensynthese für Glycocluster-Peptide wurde optimiert und konnte in der Synthese von mehreren Glycocluster-Peptiden mit längeren künstlichen Peptidketten genutzt werden. Die einfache Synthese von Glycoclustern mittels Staudinger-Reaktion kann für die Darstellung auch anderer Glycocluster-Mimetika genutzt werden. Beide Strukturen wurden NMR-Experimenten unterzogen, die eine Zufallsknäuel-Struktur der Kette nahelegten. Diese Konformation wurde mit CD-Spektroskopie bestätigt. Die Analoga der Glycocluster **59** und **61** wurden als Inhibitoren Typ-1-Fimbrien vermittelter Adhäsion in einem Inhibition-Assay getestet. Beide zeigten Inhibition und könnten weiter zur Untersuchung von Adhäsionsmechanismen genutzt werden. Eine weitere Option ist die Synthese von GPI-Ankern und Lipopolysacchariden, die sich schnell mittels der hier hergestellten Verbindungen mimikrieren lassen sollten. Biologische Untersuchungen mit diesen synthetischen Glycokonjugaten können helfen, biologische Vorgänge im Zusammenhang mit der Glycocalyx-Biochemie zu entschlüsseln.

8. Experimental part

8.1 General methods and material

All reactions were carried out under normal atmospheric conditions unless otherwise mentioned. The reactions under Schlenk conditions were carried out in nitrogen atmosphere and the apparatus was heated before use.

Dry solvents were produced by refluxing the solvent several hours with the noted drying agents under nitrogen atmosphere followed by distillation. The other solvents were purchased dry from the producer.

| | |
|-----------------|-----------------------------|
| Dichloromethane | over calcium hydride |
| Methanol | over magnesium |
| DMF | dry from producer |
| Acetonitrile | over calcium hydride |
| Diethyl ether | over sodium-potassium alloy |
| THF | over sodium-potassium alloy |

All reactions were followed by TLC on silica gel 60 GF₂₅₄ from Merck. The detection of the carbohydrate containing substances was carried out by charring with 10 % ethanolic solution in sulfuric acid solution and followed by heat treatment. UV active substances were detected by a UV lamp from CAMAG with 254 nm wavelength.

Flash chromatography was performed on silica gel 60 (0.040-0.063 mm, 230-400 mesh) from Merck using distilled solvents. For the size exclusion chromatography Sephadex LH-20 from Pharmacia was used with distilled methanol as eluent. The organic solutions were concentrated using a rotary evaporator at water bath temperature < 45 °C. Aqueous solutions were concentrated by lyophilisation on freeze dryer.

¹H and ¹³C NMR spectra were recorded at DRX-500 (500.13 MHz for ¹H, 125.47 MHz for ¹³C) and AV-600 (600.13 MHz for ¹H, 120.91 MHz for ¹³C) from Bruker. Chemical shifts are given in ppm relative to internal TMS (0.00 ppm for ¹H and ¹³C) and when samples were measured in D₂O the spectra were calibrated referring to internal HOD (4.63 for ¹H). Further one dimensional spectra (DEPT), two dimensional ¹H-¹H-COSY,

^1H - ^{13}C -COSY (HSQC) as well as HMBC and NOESY experiments were measured for complete signal assignment whenever necessary.

The sugar residues were numbered from 1 to 6 with the anomeric position being number 1, the remaining part of the molecule was numbered along their longest chains. The MALDI-ToF mass spectra were recorded on Bruker Biflex and ESI-MS on Finnigan MAT 95. For MALDI-ToF measurements the samples were prepared as solutions in methanol/water, with a concentration of 1 mg/mL solution. Compounds were co-crystallized with either 2,5-dihydroxy benzoic acid (DHB) or α -cyano-4-hydroxycinnamic acid (CCA). The mass peaks obtained with all the samples were calibrated in reference to the $[\text{M}+\text{H}]^+$ peaks of angiotensin II (1046.54), angiotensin I (1296.69), bombesin (1619.82), and to the $[2\text{M}+\text{H}]^+$ peak of CCA (380.02). High resolution ESI mass spectra were measured with an Applied Biosystems Mariner ESI-ToF 5280. The DMF (peptide synthesis grade), DIPEA, HATU and Fmoc-Gly-OH were purchased from Fluka. The other amino acids, Fmoc-Asp-O^tBu-OH and Fmoc-Ser-O^tBu-OH were purchased from Novabiochem. The Wang resin (loading capacity 0.9 mmol/g) was purchased from Advanced Chemtech.

Optical rotation values were recorded at Perkin-Elmer-polarimeter (Na-D line 589 nm, 20 °C, cell length 10 cm) in the noted solvent. CD-experiments were carried out with a Jasco J-720 spectropolarimeter (Japan) at room temperature, using a tandem-quartz cuvette with a light path of 1 mm (Helma GmbH & Co. KG, Germany). Measurement was done within a spectral width from 250 nm to 195 nm and performed ten accumulations.

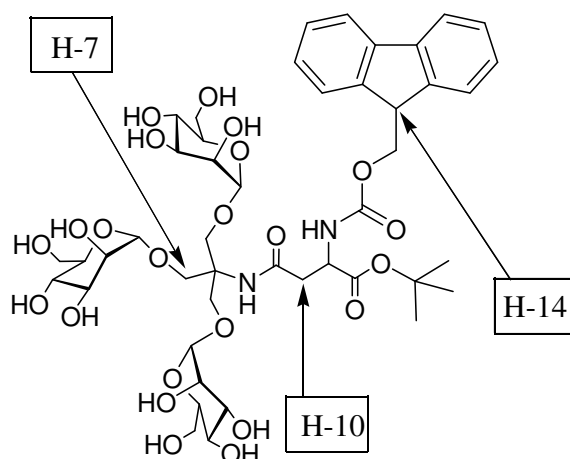


Figure 49: ^1H NMR assignment of molecules.

8.2 General procedures

8.2.1 General coupling procedure for peptides

Before each peptide coupling step the resin was treated with a solution of piperidine in DMF (20 %, 5 ml) for 10 min. The same treatment was repeated to assure complete cleavage of Fmoc protection and then the resin was repeatedly washed with DMF (five times with 5 ml). For peptide coupling, the respective amino acid derivative (4 eq relative to the peptide on resin), HBTU (3.6 eq) and HOBT (4 eq) were dissolved in DMF (2-3 ml) and DIPEA (4 eq) was added. This solution was shaken for 5 min and then the activated amino acid derivative was transferred to the resin. This mixture was kept under N₂ bubbling for 3-4 hrs at room temperature. Completion of the coupling reaction was confirmed by the 'Kaiser test'. Kaiser's test is negative, in the absence of any blue dye on the resin.

8.2.2 General procedure for *tert*-butyl deprotection

The *tert*-butyl ester to be cleaved is dissolved in a mixture of TFA and H₂O (7:3). The reaction mixture is stirred at room temperature for 1-1.5 hrs, then concentrated in vacuo and lyophilized to give the corresponding carboxylic acid which can be employed without further purification.

8.2.3 Kaiser test for confirmation of peptide coupling

A few beads of resin were taken in a test tube and washed several times with ethanol. Then two drops of 5% Ninhydrin solution in ethanol was added to it followed by a drop each of phenol solution in ethanol (80 g phenol in 20 ml ethanol) and potassium cyanide solution in pyridine (2 ml of 0.001 M aq. solution of KCN in 98 ml of pyridine). Then the beads were heated up to 110 °C for 5 min. The presence of primary amine groups was indicated by blue resin beads.

8.2.4 Standard protocol for the cleavage of peptide from resin

For Fmoc-deprotection the resin was first treated with piperidine in DMF (20 %, 5 ml) for 20 min and was subsequently washed with DMF (5 ml, three times) and CH₂Cl₂ (5 ml, three times). The resin was slurred in a mixture of TFA and CH₂Cl₂ (4:1, 5 ml) and kept for 30 min. Then the resin was filtered through a sintered funnel and washed with TFA-CH₂Cl₂ (4:1, 3 ml three times). The combined washings were evaporated in vacuo, then dissolved in H₂O and passed through a syringe filter to remove insoluble impurities. Lyophilisation furnished the respective glycocluster peptide.

8.2.5 General procedure for *O*-acetyl / *O*-benzoyl deprotection

The protected sugar was dissolved in 10 ml of absolute methanol under nitrogen atmosphere. To the above solution, 100 µl of 1 M sodium methanolate solution was added and reaction was allowed to run for 3-4 hrs. The reaction mixture was neutralised by IR-120 resin. The resin was filtered off and filtrate was evaporated under reduced pressure. The substrate was subjected to lyophilisation.

8.3 Synthesis of octapeptide

8.3.1 Loading the resin with Fmoc-Gly-OH (1)

In a round bottom flask 2 g of Wang resin was suspended in 30 ml of 9:1 DCM/DMF. In a separate flask 1.34 g of Fmoc-Gly-OH (2.5 eq relative to resin) was dissolved in 4 ml of DMF along with 610 mg of HOBt (2.5 eq relative to resin). This solution was added to the resin and in a separate flask 218 mg (0.1 eq relative to Fmoc-Gly-OH) of DMAP in 2 ml of DMF was dissolved. Then 700 μ l (1.0 eq relative to Fmoc-Gly-OH) of DIC was added to the resin mixture and then added the DMAP solution. The flask was equipped with a drying tube. The reaction mixture was agitated on mechanical shaker for 3 hrs at room temperature. Then 140 μ l (2 eq relative to the resin) of acetic anhydride and 170 μ l (2 eq relative to the resin) pyridine was added to it and mixed for additional 30 min to end-cap the unreacted hydroxyl groups. The resin was filtered on a sintered filter and washed for 3 times with DMF followed by 3 times with DCM and then 3 times with MeOH. Then the resin was dried in vacuo to a constant weight. The amount of resin was found to be 2.66 g out of which 666 mg (0.45 mmol of Fmoc-Gly-OH) used for the peptide synthesis.

Peptide load found: 0.60 mmol/g.

8.3.2 Synthesis of peptide (2) and (3)

A mixture of 535 mg (4 eq) of Fmoc-Gly-OH, 243 mg (4 eq) of HOBt and 614 mg (3.6 eq) of HBTU was dissolved in 2 ml of DMF and 310 μ l of DIPEA was added to it. The mixture was stirred for 5 min and then transferred to resin.

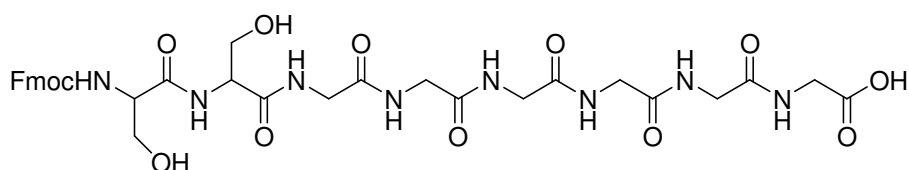
8.3.3 Preparation of peptide (4)

A mixture of 740 mg (4 eq) of Fmoc-Gly-Gly-Gly-OH, 243 mg (4 eq) of HOBt and 614 mg (3.6 eq) of HBTU was dissolved in 2 ml of DMF and 310 μ l of DIPEA was added to it. The mixture was stirred for 5 min and then transferred to resin.

8.3.4 Preparation of peptide (5) and (6)

A mixture of 383 mg (4 eq) of Fmoc-Ser-O^tBu-OH, 243 mg (4 eq) of HOBt and 614 mg (3.6 eq) of HBTU was dissolved in 2 ml of DMF and 310 μ l of DIPEA was added to it. The mixture was stirred for 5 min and then transferred to resin.

8.3.5 N-Fluorenylmethoxycarbonyl -Ser-Ser-Gly-Gly-Gly-Gly-OH (6)



The octapeptide **6** (50 mg of resin) was cleaved using 8.2.4 to receive the Fmoc-protected octapeptide without *tert*-butyl groups.

Peptide load found: 20 mg (0.46 mmol/g).

¹H NMR (600 MHz, DMSO-D₆, TMS): δ = 8.11 (t, 4 H, J = 5.44 Hz, 4 NHCH₂), 8.04 (t, 2 H, J = 7.84 Hz, CONHCH, CONHCH₂), 7.88 (d, 2 H, J = 7.52 Hz, Ar-H), 7.73 (dd~t, 2 H, J = 7.29 Hz, Ar-H), 7.42 (dd~t, 2 H, J = 7.48 Hz, Ar-H), 7.36 (m_c, 1 H, CHCONH), 7.35 (dd~t, 2 H, J = 7.47 Hz, Ar-H), 4.29 (m_c, 3 H, 2 COOCH₂, 1 CONHCH), 4.23 (t, 1 H, J = 6.86 Hz, OCH₂CH), 4.18 (m_c, 1 H, CONHCH), 3.76 (m_c, 12 H, NHCH₂CO), 3.61 (m_c, 4 H, CHCH₂OH) ppm;

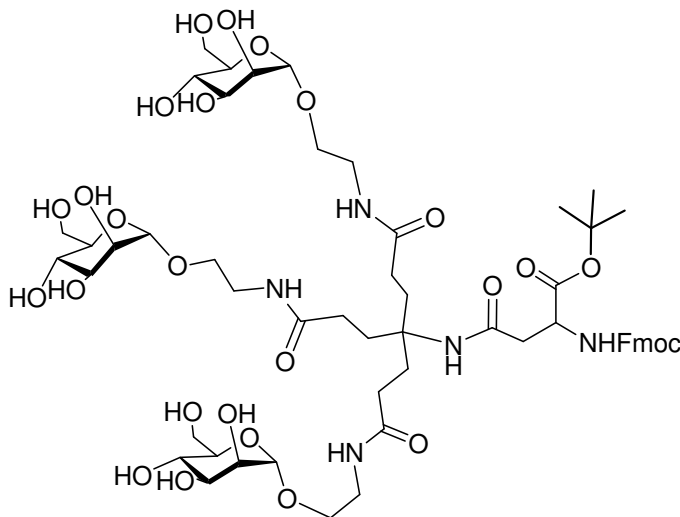
¹³C NMR (150 MHz, DMSO-D₆, TMS): δ = 171.0 (CO), 170.5 (CO), 169.2 (CO), 169.1 (2 \times CO), 169.0 (CO), 169.0 (3 \times CO), 155.9 (NHCOOCH₂), 143.8 (Ar-C), 140.7 (Ar-C), 129.4 (Ar-C), 128.8 (Ar-C), 127.1 (Ar-C), 121.2 (Ar-C), 65.8 (COOCH₂), 61.8 (CHCH₂OH), 61.2 (CHCH₂OH), 56.7 (CONHCH), 55.2 (CONHCH), 46.6 (OCH₂CH), 42.2 (NHCH₂CO), 42.0 (2 \times NHCH₂CO), 41.7 (NHCH₂CO), 40.7 (2 \times NHCH₂CO) ppm;

HRESI-MS m/z : [M+K]⁺ calcd. for C₃₃H₄₀N₈O₁₃, 795.2352; found, 795.2244.

8.4 Synthesis of glycocluster amino acid with ethylene spacer

3-Cascade:*N*-fluorenylmethoxycarbonyl-aspartyl-aminomethane[3]:propane-(1-amidoethyl- α -D-mannopyranoside)-*tert*-butylester- (20)

In a round bottom flask, Fmoc-protected cluster mannoside **18** (172 mg, 0.138 mmol) was dissolved in absolute DMF (10 ml) under inert atmosphere. Then morpholine (260 μ l) was added and the mixture was stirred at room temperature for 2 h before the solvent was evaporated in vacuo to get the amine **19** (ESI-MS m/z : $[M+Na]^+$ calcd. for,



$C_{37}H_{62}N_4O_{21}$, 885.3804; found, 885.3754). Without any further purification compound **19** was mixed with Fmoc-Asp-O^tBu-OH (142 mg, 0.345 mmol) and HATU (131 mg, 0.345 mmol) and this mixture was dissolved in absolute DMF (15 ml) under inert atmosphere and cooled to 0 °C. Then DIPEA (58 μ l, 0.345 mmol) was added and the reaction mixture was allowed to stir at room temperature for 4 h. The solvent was evaporated under reduced pressure and the residue was subjected to gel chromatography. Further purification by HPLC resulted in the title compound as white lyophilisate.

HPLC: t_R = 41.98 min [250/4 LiChrosorb 7 μ m C8, A = water, B = acetonitrile, 20 % B \rightarrow 50 % B, 75 min, 1 ml/min].

Yield: 63 mg, 0.05 mmol, 36 %.

$[\alpha]_D^{20} = +1.8^\circ$ ($c = 0.8$, CH₃OH).

¹H NMR (600 MHz, CD₃OD, TMS): δ = 7.82 (d, 2 H, $J = 7.48$ Hz, Ar-H), 7.71 (m_c, 2 H, Ar-H), 7.43 (t, 2 H, $J = 7.53$ Hz, Ar-H), 7.35 (m_c, 2 H, Ar-H), 4.79 (d, 3 H, $J_{1,2} = 1.58$ Hz, 3 H-1), 4.45 (dd, 1 H, $J = 5.05$, $J = 8.20$ Hz, H-15), 4.41 (dd, 1 H,

$J = 7.02$, $J = 10.51$ Hz, H-17), 4.36 (m_c, 1 H, H-17'), 4.26 (m_c, 1 H, H-18), 3.87 (dd, 3 H, $J_{5,6} = 2.33$, $J_{6,6} = 11.74$ Hz, 3 H-6), 3.85 (dd, 3 H, $J_{1,2} = 1.71$, $J_{2,3} = 3.38$ Hz, 3 H-2), 3.78 (m_c, 3 H, 3 H-7), 3.74 (m_c, 6 H, 3 H-3 and 3 H-6'), 3.64 (dd~t, $J_{3,4} = 9.7$, $J_{4,5} = 9.3$ Hz, 3 H-4), 3.59-3.54 (m, 6 H, 3 H-5 and 3 H-7'), 3.46-3.42 (m, 3 H, H-8), 3.38 (m_c, 3 H, 3 H-8'), 2.78 (dd, 1 H, H-14), 2.63 (dd, 1 H, H-14'), 2.22 (m_c, 6 H, H-10), 2.01 (m_c, 6 H, H-11), 1.5 (s, 9 H, CCH₃);

¹³C NMR (150 MHz, CD₃OD, TMS): $\delta = 175.9$ (C-9), 172.3 (C-13), 171.7 (C-19), 158.5 (C-16), 145.3 (Ar-C), 142.6 (Ar-C), 128.9 (Ar-C), 128.2 (Ar-C), 126.2 (Ar-C), 120.9 (Ar-C), 101.7 (C-1), 83.3 (C-20), 74.8 (C-5), 72.6 (C-3), 72.1 (C-2), 68.8 (C-4), 68.2 (C-17), 67.3 (C-7), 63.1 (C-6), 59.4 (C-12), 53.4 (C-15), 48.4 (C-18), 40.4 (C-8), 39.5 (C-14), 32.0 (C-11), 31.4 (C-10), 28.3 (C-CCH₃) ppm;

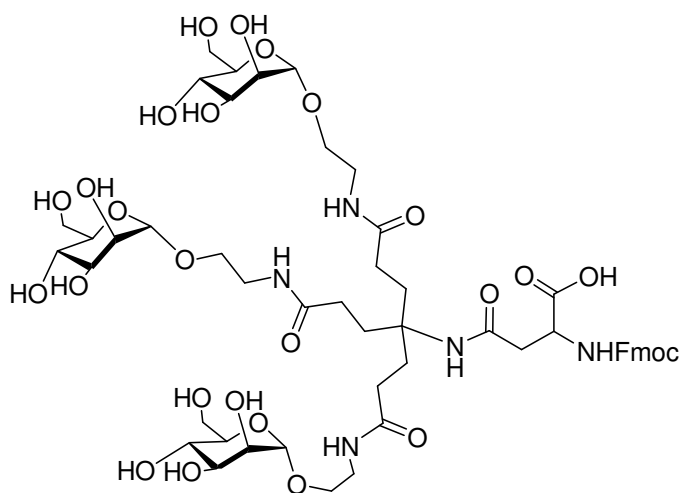
HRESI-MS m/z : [M+Na]⁺ calcd. for C₅₇H₈₅N₅O₂₆, 1278.5380; found, 1278.5633.

3-Cascade:*N*-fluorenylmethoxycarbonyl-aspartyl-aminomethane[3]:propane-(1-amidoethyl- α -D-mannopyranoside) (21)

The *tert*-butyl ester **20** (40 mg, 0.031 mmol) was deprotected by general procedure 8.1.2 to give corresponding carboxylic acid after lyophilisation.

Yield: 36 mg, 0.029 mmol, 95 %.

$[\alpha]_D^{20} = +1.2^\circ$ ($c = 0.8$ CH₃OH).



¹H NMR (600 MHz, CD₃OD, TMS): $\delta = 7.78$ (d, 2 H, $J = 7.49$ Hz, Ar-H), 7.76 (m_c, 2 H, Ar-H), 7.38 (m_c, 2 H, Ar-H), 7.31 (m_c, 2 H, Ar-H), 4.76 (d, 3 H, $J_{1,2} = 1.54$ Hz, 3 H-1), 4.49 (m_c, 1 H, H-15), 4.34 (m_c, 2 H, H-17, H-17'), 4.23 (m_c, 1 H, H-18), 3.83 (dd, 3 H, $J_{1,2} = 1.30$, $J_{5,6} = 11.74$ Hz, 3 H-6 and 3 H-2), 3.76-3.69 (m, 9 H, 3 H-6', 3 H-3 and

3 H-7), 3.61 (dd~t, $J_{4,5} = 9.40$ Hz, H-4), 3.56-3.49 (m, 6 H, 3 H-5 and 3 H-7'), 3.42-3.32 (m, 6 H, H-8), 2.76 (m_c, 1 H, H-14), 2.18 (m_c, 1 H, H-14'), 2.18 (m_c, 6 H, H-10), 2.96 (m_c, 6 H, H-11) ppm;

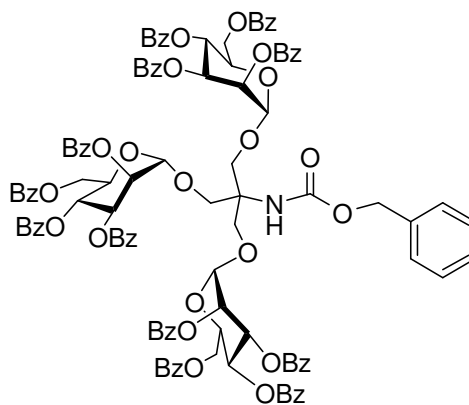
¹³C NMR (150 MHz, CD₃OD): $\delta = 176.1$ (C-19, C-9), 145.3 (Ar-C), 142.6 (Ar-C), 128.8 (Ar-C), 128.3 (Ar-C), 126.3 (Ar-C), 120.9 (Ar-C), 101.7 (C-1), 74.8 (C-5), 72.6 (C-3), 72.1 (C-2), 68.8 (C-4, C-17), 67.2 (C-7), 63.0 (C-6), 59.4 (C-12), 55.9 (C-15), 48.4 (C-18), 40.4 (C-8, C-14), 31.9 (C-11), 31.4 (C-10) ppm;

HRESI-MS m/z : $[M+Na]^+$ calcd. for C₅₃H₇₇N₅O₂₆, 1222.4754; found, 1222.4847.

8.5 Synthesis of glycocluster from tris(hydroxymethyl)aminomethane (TRIS)

N-(Benzyloxycarbonyl)tris[[*(2,3,4,6*-tetra-*O*-benzoyl- α -D-mannopyranosyl)oxy]methyl]methylamine (26)

In a Schlenk flask compound **25** (231 mg, 0.90 mmol) and mannosyl trichloroacetimidate **23** (5.03 g, 6.70 mmol) were dissolved in absolute CH₂Cl₂ (50 ml) under inert atmosphere. The reaction mixture was cooled to 0 °C and BF₃·Et₂O (800 μ l, 7.30 mmol) was added dropwise to the solution. Then the reaction mixture was stirred at room temperature for 16 h. A saturated aq. solution of NaHCO₃ was added and the aqueous phase was extracted with CH₂Cl₂



(100 ml) for three times. The combined organic phases were washed with brine, dried over MgSO₄ and concentrated in vacuo after filtration. The flash chromatography with 30 % ethyl acetate in cyclohexane gave pure product as colourless foam.

Yield: 1.19 g, 0.60 mmol, 66 %.

^1H NMR (600 MHz, CDCl_3 , TMS): δ = 8.13 (dd, 6 H, Ar-H), 7.97 (dd, 6 H, Ar-H), 7.86 (dd, 6 H, Ar-H), 7.75 (dd, 6 H, Ar-H), 7.53 (m, 6 H, Ar-H), 7.43-7.36 (m, 12 H, Ar-H), 7.32-7.25 (m, 11 H, Ar-H), 7.19 (dd, 6 H, Ar-H), 7.10 (dd, 6 H, Ar-H), 6.20 (dd~t, 3 H, $J_{3,4}$ = 10.10 Hz, 3 H-4), 5.94 (dd, 3 H, $J_{2,3}$ = 3.22, $J_{3,4}$ = 10.12 Hz, 3 H-3), 5.79 (dd, 3 H, $J_{2,3}$ = 3.22, $J_{1,2}$ = 1.76 Hz, 3 H-2), 5.30 (d, 3 H, $J_{1,2}$ = 1.82 Hz, 3 H-1), 5.18 (d, 2 H, J = 2.31 Hz, H-10), 4.80-4.77 (m, 3 H, 3 H-6) 4.61-4.56 (m, 6 H, 3 H-6', 3 H-5), 4.47 (d, 3 H, J = 10.34 Hz, H-7), 4.08 (d, 3 H, J = 10.38 Hz, H-7') ppm;

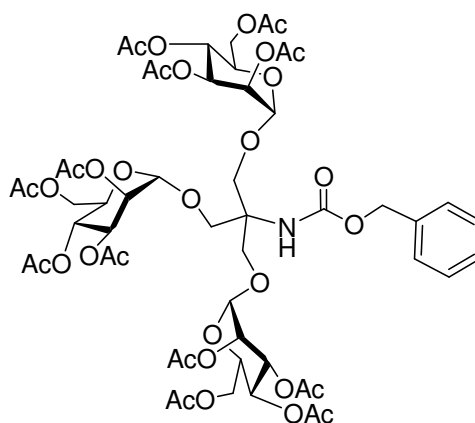
^{13}C NMR (150 MHz, CDCl_3 , TMS): δ = 166.2 ($\underline{\text{C}}$ OPh), 166.5 ($\underline{\text{C}}$ OPh), 166.4 ($\underline{\text{C}}$ OPh), 166.3 ($\underline{\text{C}}$ OPh), 155.3 (C-9), 136.1 (Ar-C), 133.3 (Ar-C), 133.2 (Ar-C), 133.0 (Ar-C), 132.9 (Ar-C), 130.1 (Ar-C), 129.9 (Ar-C), 129.8 (Ar-C), 129.8 (Ar-C), 129.7 (Ar-C), 129.4 (Ar-C), 129.1 (Ar-C), 128.9 (Ar-C), 128.6 (Ar-C), 128.5 (Ar-C), 128.4 (Ar-C), 128.3 (Ar-C), 128.2 (Ar-C), 99.8 (C-1), 70.4 (C-3), 70.2 (C-2), 69.7 (C-5), 67.5 (C-7), 67.1 (C-10), 66.5 (C-4), 62.7 (C-6), 58.8 (C-8) ppm;

HRESI-MS m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{114}\text{H}_{95}\text{NO}_{32}$, 2012.5735; found, 2013.5849.

Elemental analysis for $\text{C}_{114}\text{H}_{95}\text{NO}_{32}$: calcd. C 68.77, H 4.81, N 0.70; found C 67.73, H 4.79, N 1.05 %.

***N*-(Benzyloxycarbonyl)tris[[*(2,3,4,6*-tetra-*O*-acetyl- α -D-mannopyranosyl)oxy]methyl]methylamine (**23**)**

The TRIS-Cbz **22** (73 mg, 0.28 mmol) was taken with 1-*O*-(2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl)-trichloroacetimidate **25** (773 mg, 1.57 mmol) and dissolved in freshly distilled CH_2Cl_2 under nitrogen atmosphere. The reaction mixture was cooled to 0 °C followed by the addition of $\text{BF}_3\cdot\text{Et}_2\text{O}$ (300 μl). The reaction mixture was allowed to stir at room temperature for 14 h. Then, saturated solution of NaHCO_3 was added to the reaction mixture and the



aqueous phase was extracted for three times with CH_2Cl_2 . The combined organic phase was washed with brine and dried over MgSO_4 followed by evaporation under reduced

pressure. The residue was subjected to silica gel chromatography with 20 % ethyl acetate in toluene. The pure compound was isolated as slightly coloured glass.

Yield: 196 mg, 0.157 mmol, 56 %.

$[\alpha]_D^{20} = +4.8^\circ$ ($c = 0.8$, CHCl_3).

^1H NMR (600 MHz, CDCl_3 , TMS): $\delta = 7.36$ (m, 5 H, Ar-H), 5.28 (dd-t, 3 H, $J_{3,4} = 9.91$ Hz, 3 H-4), 5.24 (dd, 3 H, $J_{2,3} = 3.41$, $J_{3,4} = 9.99$ Hz, 3 H-3), 5.19 (dd, 3 H, $J_{1,2} = 1.75$, $J_{2,3} = 3.38$ Hz, 3 H-2), 5.05 (m, 2 H, H-10), 4.85 (d, 3 H, $J_{1,2} = 1.64$ Hz, 3 H-1), 4.31 (dd, $J_{5,6} = 5.05$, $J_{6,6'} = 12.36$ Hz, 3 H-6), 4.14 (m, 6 H, 3 H-6', 3 H-7), 3.71 (d, 3 H, $J = 10.19$ Hz, 3 H-7'), 2.14 (s, 9 H, COCH_3), 2.10 (s, 9 H, COCH_3), 2.10 (s, 9 H, COCH_3), 2.02 (s, 9 H, COCH_3) ppm;

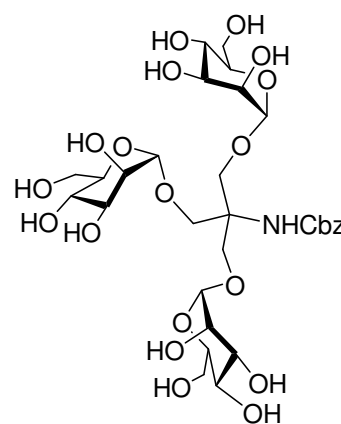
^{13}C NMR (150 MHz, CDCl_3 , TMS): $\delta = 170.7$ (COCH_3), 169.9 (COCH_3), 169.8 (COCH_3), 169.6 (COCH_3), 154.9 (NHCOOCH_2), 140.9 (Ar-C), 129.8 (Ar-C), 128.6 (Ar-C), 128.23 (Ar-C), 98.5 (C-1), 69.2 (C-2), 69.1 (C-5), 69.0 (C-3), 66.9 (C-10), 66.8 (C-7), 65.9 (C-4), 62.2 (C-6), 58.43 (C-8), 20.8 (COCH_3), 20.7 (COCH_3), 20.6 ($2 \times \text{COCH}_3$) ppm;

HRESI-MS: $m/z = [\text{M} + \text{Na}]^+$ calcd. for $\text{C}_{54}\text{H}_{71}\text{NO}_{32}$, 1268.3857; found, 1268.3878.

***N*-(Benzyloxycarbonyl)tris[(α -D-mannopyranosyl)oxy]methyl]methylamine (27)**

The glycocluster **23** (100 mg, 0.080 mmol) was deprotected by following general procedure for acetyl deprotection 8.2.5 to give white foam after lyophilisation.

Yield: 52 mg, 0.070 mmol, 88 %.



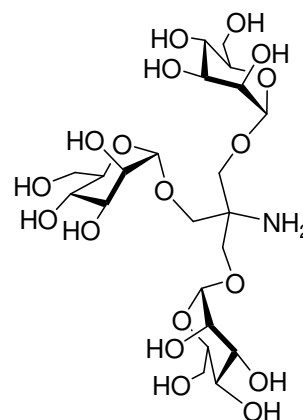
^1H NMR (600 MHz, CD_3OD , TMS): $\delta = 7.39$ (m_c , 4 H, Ar-H), 7.34-7.31 (m , 1 H, Ar-H), 5.71 (d , 1 H, $J = 2.43$ Hz, H-10), 5.07 (d , 1 H, $J = 12.35$ Hz, H-10'), 4.78 (d , 3 H, $J_{1,2} = 1.73$ Hz, 3 H-1), 3.98 (dd , 3 H, 3 H-7), 3.88 (dd , 3 H, $J_{5,6} = 5.4$, $J_{6,6'} = 9.74$ Hz, 3 H-6) 3.85 (d , $J_{1,2} = 1.72$ Hz, $J_{2,3} = 3.36$ Hz, 3 H-2), 3.74 (m_c , 9 H, 3 H-3, 3 H-6', 3 H-7'), 3.64 (m_c , 9 H, 3 H-4, 3 H-5) ppm;

^{13}C NMR (150 MHz, CD_3OD , TMS): $\delta = 158.1$ (C-9), 139.7 (Ar-C), 129.5 (Ar-C), 129.0 (Ar-C), 128.8 (Ar-C), 102.4 (C-1), 74.9 (C-5), 72.7 (C-3), 72.0 (C-2), 68.8 (C-4), 67.4 (C-7, C-10), 62.9 (C-6), 59.7 (C-8) ppm;

MALDI-ToF-MS m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{30}\text{H}_{47}\text{NO}_{20}$, 764.7; found, 764.9.

Tris[(α -D-mannopyranosyloxy)methyl]methylamine (**28**)

The Cbz-protected cluster **27** (246 mg, 0.33 mmol) was dissolved in absolute methanol (10 ml) and a catalytic amount of Pd on activated charcoal was added. The round bottom flask was equipped with a H_2 gas balloon and allowed to stir at room temperature for 12 h. The reaction mixture was filtered through Celite and concentrated in vacuo. After lyophilisation with H_2O the title compound was isolated as white foam.



Yield: 192 mg, 0.31 mmol, 95 %.

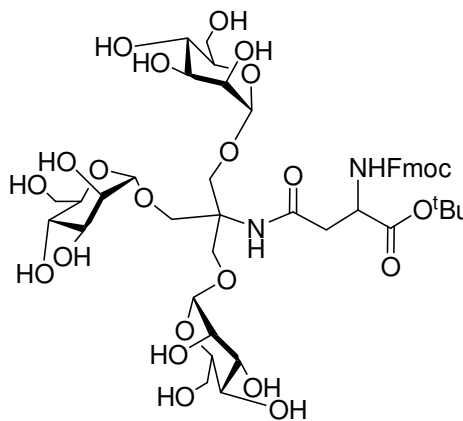
^1H NMR (500 MHz, DMSO-D_6 , TMS): $\delta = 4.66$ (q , 7 H, 7 OH), 4.54 (d , 3 H, $J_{1,2} = 1.33$ Hz, 3 H-1), 4.48 (d , 3 H, 3 OH), 4.38 (t , 3 H, 1 OH , NH_2), 3.71 (d , $J = 9.69$ Hz, 3 H-7), 3.63-3.59 (m , 6 H, 3 H-2, 3 H-6), 3.49 (m_c , 6 H, $J = 9.84$ Hz, 3 H-6, 3 H-7'), 3.44 (dd , 3 H, $J_{2,3} = 5.7$, $J_{3,4} = 8.17$ Hz, 3 H-3), 3.41 (dd , 3 H, $J = 9.27$ Hz, 3 H-4), 3.34 (m_c , 3 H, H-5) ppm;

^{13}C NMR (125 MHz, DMSO-D_6 , TMS): $\delta = 100.7$ (C-1), 73.7 (C-5), 70.9 (C-3), 70.17 (C-2), 66.8 (C-4), 65.3 (C-7), 60.9 (C-6), 58.0 (C-8) ppm;

HRESI-MS m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{22}\text{H}_{41}\text{NO}_{18}$, 630.2221; found, 630.2171.

***N*-(*N*-Fluorenylmethoxycarbonyl-aspartyl-*tert*-butylester)tris[[(2,3,4,6-tetra-*O*-benzoyl- α -D-mannopyranosyl)oxy]methyl]methylamide (29)**

In a Schlenk flask **28** (75 mg, 0.12 mmol), Fmoc-Asp-O^tBu (103 mg, 0.25 mmol), and HATU (95 mg, 0.25 mmol) were dissolved in absolute DMF (10 ml). The solution was cooled to 0 °C for 10 min. Then DIPEA (42 μ l, 0.25 mmol) was added and the reaction mixture was allowed to stir at room temperature for 4 h. The solvent was evaporated in vacuo and the residue was subjected to gel chromatography. Further purification by HPLC gave pure product as white lypophilisate.



HPLC: t_R = 44.93 min [250/4 LiChrosorb 7 μ m C8, A = water, B = acetonitrile, 20 % B \rightarrow 50 % B, 75 min, 1 ml/min].

Yield = 46 mg, 0.045 mmol, 37 %.

$[\alpha]_D^{20} = +4.8^\circ$ ($c=0.8$, CH₃OH).

¹H NMR (600 MHz, CD₃OD, TMS): δ = 7.82 (d, 2 H, J = 7.47 Hz, Ar-H), 7.73-7.71 (m, 2 H, Ar-H), 7.43 (t, 2 H, J = 7.34 Hz, Ar-H), 7.35 (t, 2 H, J = 7.48 Hz, Ar-H), 4.79 (d, 3 H, $J_{1,2} = 1.57$ Hz, 3 H-1), 4.45-4.41 (m, 2 H, 1 H of H-13, H-11), 4.36 (t, 1 H, H-13'), 4.30 (t, 1 H, J = 6.76, 7.05 Hz, H-14), 3.99 (d, 3 H, J = 9.57 Hz, H-7), 3.90-3.87 (m, 6 H, 3 H-2, 3 H-6), 3.84 (d, 3 H, J = 9.74 Hz, H-7'), 3.77-3.74 (m, 3 H, $J_{6,6'} = 11.8$ Hz, H-6'), 3.74-3.72 (dd, 3 H, $J_{2,3} = 3.40$, $J_{3,4} = 9.23$ Hz, 3 H-3), 3.65-3.62 (m, 6 H, H-5, H-4), 2.79 (dd, 1 H, H-10), 2.73 (dd, 1 H, H-10'), 1.50 (s, 9 H, CCH₃) ppm;

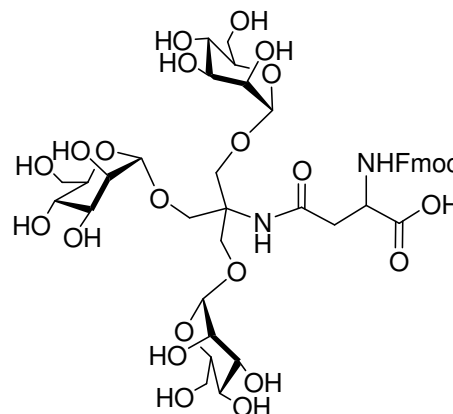
¹³C NMR (150 MHz, CD₃OD, TMS): δ = 172.4 (C-9), 172.2 (C-15), 158.4 (C-12), 145.3 (Ar-C), 142.6 (Ar-C), 128.8 (Ar-C), 128.2 (Ar-C), 126.4 (Ar-C), 120.9 (Ar-C), 102.4 (C-1), 83.2 (CCH₃), 74.9 (C-5), 72.8 (C-3), 71.9 (C-2), 68.9 (C-4), 68.3 (C-13), 67.4 (C-7), 62.9 (C-6), 60.8 (C-8), 53.1 (C-11), 48.4 (C-14), 38.9 (C-10), 28.3 (CCH₃) ppm;

HRESI-MS m/z : $[M+Na]^+$ calcd. for $C_{45}H_{67}N_2O_{23}$, 1023.3798; found, 1023.3806.

***N*-(*N*-Fluorenylmethoxycarbonyl-aspartic acid)tris[(2,3,4,6-tetra-*O*-benzoyl- α -D-mannopyranosyl)oxy]methyl]methylamide (30)**

The *tert*-butyl ester **29** (57 mg, 0.056 mmol) was deprotected following general procedure 8.2.2. After lyophilisation corresponding carboxylic acid was isolated as white lyophilisate.

Yield: 50 mg, 0.052 mmol, 94 %.



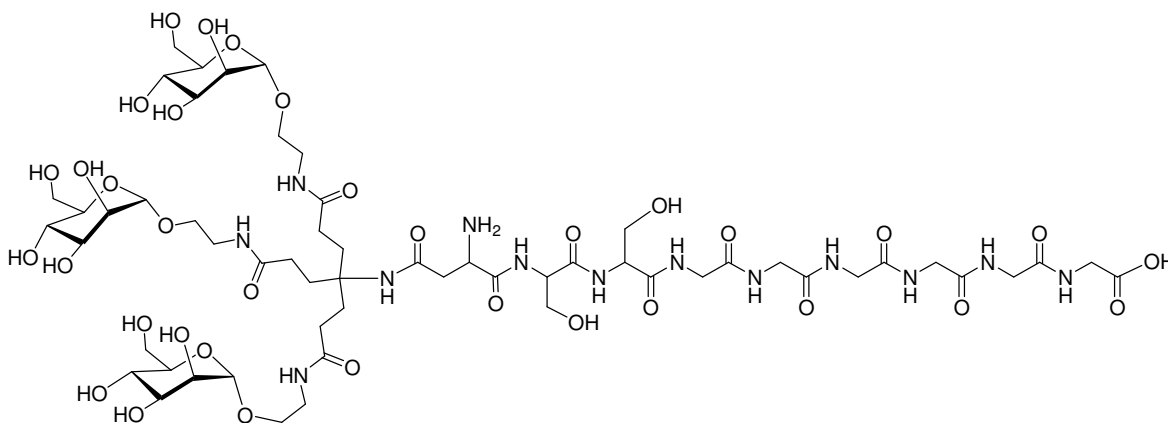
^1H NMR (600 MHz, CD_3OD , TMS): δ = 7.82 (d, 2 H, J = 7.45 Hz, Ar-H), 7.61 (m_c, 2 H, Ar-H), 7.42 (t, 2 H, J = 7.51 Hz, Ar-H), 7.35 (t, 2 H, J = 7.45 Hz, Ar-H), 4.79 (d, 3 H, $J_{1,2}$ = 1.70 Hz, 3 H-1), 4.55 (m_c, 1 H, H-11), 4.43-4.40 (m, 1 H, H-13), 4.35 (m_c, 1 H, H-13'), 4.30 (dd~t, 1 H, J = 6.81, J = 7.09 Hz, H-14), 4.02 (d, 3 H, J = 7.87 Hz, H-7), 3.88 (m_c, 6 H, 3 H-2, 3 H-6), 3.80-3.73 (m, 9 H, 3 H-3, 3 H-6' and 3 H-7'), 3.66-3.63 (m, 6 H, 3 H-4, 3 H-5), 2.85 (m_c, 1 H, H-10), 2.79 (m_c, 1 H, H-10') ppm;

^{13}C NMR (150 MHz, CD_3OD , TMS): δ = 174.7 (C-15), 172.4 (C-9), 158.4 (C-12), 145.3 (Ar-C), 142.6 (Ar-C), 128.8 (Ar-C), 128.2 (Ar-C), 126.4 (Ar-C), 120.9 (Ar-C), 102.4 (C-1), 74.8 (C-5), 72.7 (C-3), 71.9 (C-2), 68.9 (C-4), 68.3 (C-13), 67.1 (C-7), 62.9 (C-6), 60.8 (C-8), 52.1 (C-11), 48.4 (C-14), 39.1 (C-10) ppm;

MALDI-ToF-MS m/z : $[M+Na]^+$ calcd. for $C_{41}H_{56}N_2O_{23}$, 967.3; found, 967.5.

8.6 Synthesis of glycocluster peptides

Synthesis of glycocluster peptide (31)



Resin-bound peptide **6** (32 mg, 0.015 mmol) was swelled in the glass apparatus used for peptide coupling. In a round bottom flask glycocluster **21** (27 mg, 0.022 mmol) and HATU (8 mg, 0.022 mmol) were dissolved in DMF (2 ml) and DIPEA (5 μ l) was added. The mixture was shaken for 5 min and then added to the resin. After 24 h the glycocluster peptide was cleaved using standard procedure 8.2.4 to give **31**.

Yield: 21 mg, 0.014 mmol, 93 %.

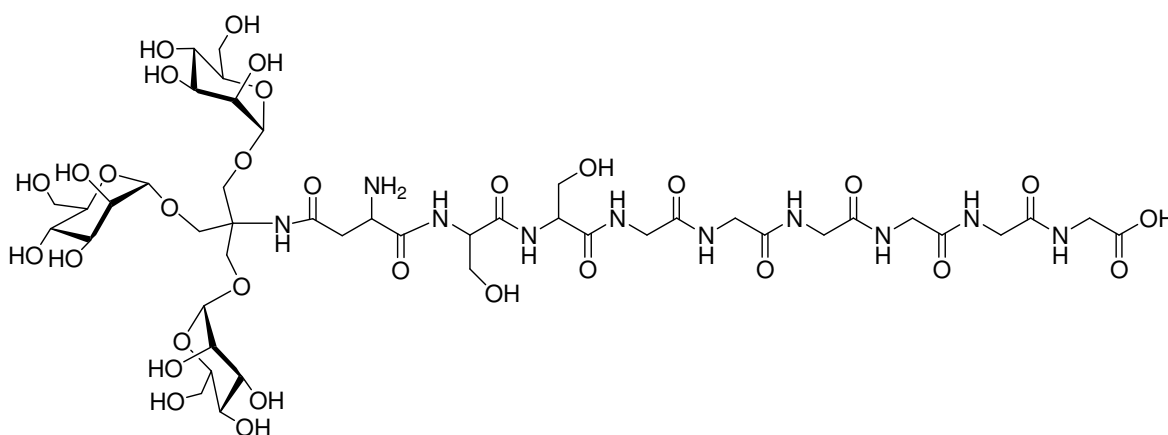
^1H NMR (600 MHz, DMSO- D_6 , TMS): δ = 8.58 (m_c, 1 H, C-16-NH), 8.16 (t, 1 H, C-30-NH), 8.15 (m_c, 5 H, C-18-NH, C-22-NH, C-24-NH, C-26-NH, C-28-NH), 7.95 (t, 1 H, C-20-NH), 7.80 (t, 3 H, C-8-NH), 7.62 (s, 1 H, C-12-NH), 4.62 (s, 3 H, H-1), 4.45 (m_c, 1 H, H-17), 4.36 (m_c, 1 H, H-19), 4.16 (m_c, 1 H, H-15), 3.76 (m_c, 12 H, 2 H-21, 2 H-23, 2 H-25, 2 H-27, 2 H-29, 2 H-31), 3.67-3.62 (m, 6 H, 3 H-2, 3 H-6), 3.58-3.44 (m, 13 H, 3 H-3, 3 H-6', 2 C-17-CH₂, 2 C-19-CH₂, 3 H-7), 3.40-3.36 (m, 6 H, 3 H-7', 3 H-4), 3.32 (m_c, 3 H, 3 H-5), 3.25 (m_c, 3 H, 3 H-8), 3.18 (m, 3 H, 3 H-8'), 2.67 (m_c, 1 H, H-14), 2.55 (m_c, 1 H, H-14'), 2.04 (m_c, 6 H, H-10), 1.83 (m_c, 6 H, H-11) ppm;

^{13}C NMR (150 MHz, DMSO- D_6): δ = 169.7, 169.1, 169.1, 169.0, 168.8, 168.8 (C-9, C-13, C-16, C-18, C-20, C-22, C-24, C-26, C-28, C-30, C-32), 99.9 (C-1), 73.9 (C-5), 72.1 (C-2), 70.9 (C-3), 67.0 (C-4), 65.3 (C-7), 61.6 (C-17-CH₂, C-19-CH₂), 61.2 (C-6), 57.5

(C-12), 53.5 (C-17), 53.4 (C-19), 49.2 (C-15), 41.7 (C-21), 40.7 (C-23, C-25, C-27, C-29, C-31), 38.5 (C-8), 34.3 (C-14), 30.1 (C-10), 29.5 (C-11) ppm;

MALDI-ToF-MS m/z : $[M+Na]^+$ calcd. for $C_{56}H_{95}N_{13}O_{34}$, 1516.6; found, 1516.9.

Synthesis of glycocluster peptide (32)



Resin-bound peptide **6** (30 mg, 0.014 mmol) was swelled in DMF for 1 h in a round bottom flask, glycocluster **30** (27 mg, 0.028 mmol) and HATU (11 mg, 0.028 mmol) were dissolved in DMF (2 ml) and DIPEA (5 μ l) was added. The mixture was shaken for 5 min and added to the resin. After 24 h the glycocluster peptide was cleaved using standard procedure 8.2.4 to give **32**.

Yield: 14 mg, 0.011 mmol, 82 %.

^1H NMR (600 MHz, $\text{DMSO-}D_6$, TMS): δ = 8.64 (d, 1 H, C-12-NH), 8.24 (d, 1 H, C-14-NH), 8.13 (m_c, 6 H, C-16-NH, C-18-NH, C-20-NH, C-22-NH, C-24-NH, C-26-NH), 7.81 (s, 1 H, C-8-NH), 4.56 (s, 3 H, 3 H-1), 4.47 (m_c, 1 H, H-13), 4.32 (m_c, 1 H, H-15), 4.13 (m_c, 1 H, H-11), 3.77 (m_c, 12 H, 1 H-19, 2 H-21, 2 H-23, 2 H-25, 2 H-27, 3 H-7), 3.63 (m_c, 14 H, 3 H-2, 3 H-6, 3 H-7', 1 C-13-CH₂, 2 C-15-CH₂, 2 H-17, 1 H-19), 3.53-3.41 (m, 10 H, 3 H-3, 3 H-4, 3 H-6', 1 C-13-CH₂), 3.31 (m_c, 5 H, 3 H-5, 2 C-11-NH₂), 2.77 (m_c, 1 H, H-10), 2.59 (m_c, 1 H, H-10')

^{13}C NMR (150 MHz, $\text{DMSO-}D_6$): δ = 170.9 (C-28), 170.2, 169.7, 169.1, 169.0 (C-9, C-12, C-14, C-16, C-18, C-20, C-22, C-24, C-26), 100.7 (C-1), 73.8 (C-5), 70.9 (C-3), 70.0

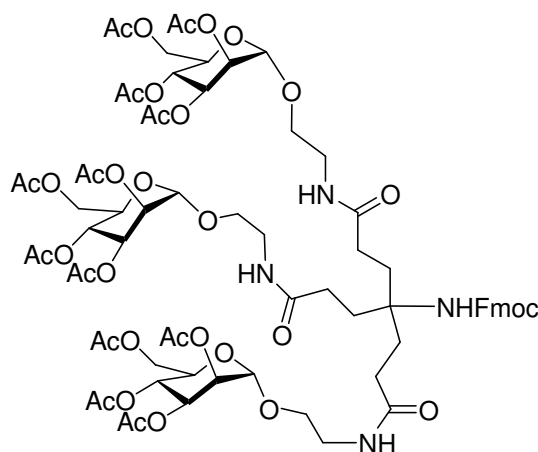
(C-2), 66.9 (C-4), 65.5 (C-7), 61.6, 61.7 (C-13-CH₂, C-15-CH₂), 60.9 (C-6), 59.2 (C-8), 55.4 (C-15), 54.7 (C-13), 49.1 (C-11), 42.1, 41.9, 41.7, 40.6 (C-17, C-19, C-21, C-23, C-25, C-27), 36.2 (C-10) ppm;

MALDI-ToF-MS m/z : [M+Na]⁺ calcd. for C₄₄H₇₂N₁₀O₃₁, 1261.4; found, 1261.5.

8.7 Glycocluster synthesis via Staudinger reaction

3-Cascade:*N*-fluorenylmethoxycarbonyl-aminomethane[3]:propane-(1-amidoethyl-2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranoside) (33)

In a Schlenk flask, Fmoc-triacid **17** (110 mg, 0.234 mmol) with mannosyl azide **11** (584 mg, 1.40 mmol) and HOBt (104 mg, 772 mmol) was taken and dried under vacuum for about 1 h. Then the above mixture was dissolved in 20 ml of absolute THF under inert atmosphere. To the solution, DIC (81 μ l, 772 mmol) was added and stirred for 10 min. Then trimethyl phosphine 1 M solution (2.41 ml, 2.1 mmol) at -70 °C was added. The reaction was stirred for 12 h and then reaction was quenched by addition of water. The aqueous phase was washed with CH₂Cl₂ and dried over MgSO₄. The silica gel column chromatography with 60 % ethyl acetate in cyclohexane gave pure product as white foam.



Yield: 194 mg, 0.122 mmol, 52 %.

$[\alpha]_D^{20} = +2.9^\circ$ ($c = 0.8$, CH₂Cl₂).

¹H NMR (600 MHz, CDCl₃, TMS): $\delta = 7.75$ (d, 2 H, $J = 7.15$ Hz, Ar-H), 7.62-7.61 (m, 2 H, Ar-H), 7.39 (m_c, 2 H, Ar-H), 7.30 (m_c, 2 H, Ar-H), 5.29 (dd, 3 H, $J_{2,3} = 3.32$, $J_{3,4} = 10.0$ Hz, 3 H-3), 5.26 (d, 3 H, $J_{3,4} = 9.77$ Hz, H-4), 5.24 (dd, 3 H, $J_{1,2} = 1.76$, $J_{2,3} = 3.29$ Hz, 3 H-2), 4.83 (d, 3 H, $J_{1,2} = 1.59$ Hz, 3 H-1), 4.38 (br. m, 2 H, 2 H-14), 4.23

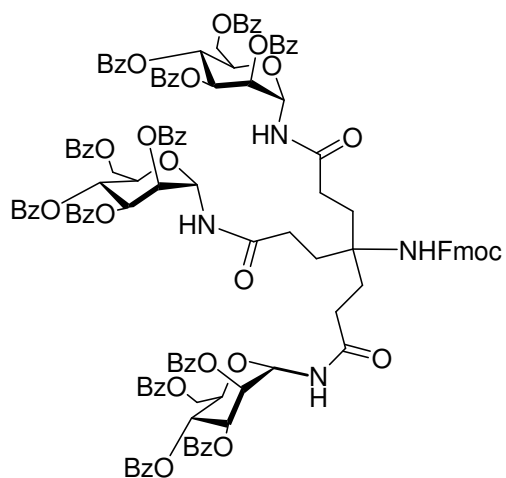
(dd, 3 H, $J_{5,6} = 5.59$, $J_{6,6'} = 12.26$ Hz, 3 H-6), 4.18 (t, 1 H, $J = 6.67$ Hz, H-15), 4.13 (dd, 3 H, $J_{5,6'} = 2.53$, $J_{6,6'} = 12.30$ Hz, 3 H-6'), 3.98 (ddd, $J_{4,5} = 9.55$, $J_{5,6} = 5.45$, $J_{5,6'} = 2.51$ Hz, 3 H-5), 3.76 (m_c, 3 H, 3 H-7), 3.57-3.50 (m, 6 H, 3 H-7', 3 H-8), 3.41-3.36 (m, 3 H, 3 H-8'), 2.21 (m_c, 6 H, 6 H-10), 2.14 (s, 9 H, COCH₃), 2.09 (s, 9 H, COCH₃), 2.03 (s, 9 H, COCH₃), 1.98 (m_c, 6 H, 6 H-11), 1.96 (s, 9 H, COCH₃) ppm;

¹³C NMR (150 MHz, CDCl₃, TMS): $\delta = 173.3$ (C-9), 170.7 (COCH₃), 170.1 (2×COCH₃), 169.7 (COCH₃), 154.8 (C-13), 144.1 (Ar-C), 144.4 (Ar-C), 127.6 (Ar-C), 127.0 (Ar-C), 125.1 (Ar-C), 120.0 (Ar-C), 97.4 (C-1), 69.5 (C-2), 69.2 (C-3), 68.8 (C-5), 67.5 (C-8), 67.0 (C-7), 66.2 (C-4), 65.8 (C-14), 62.6 (C-6), 57.2 (C-12), 47.5 (C-15), 31.2 (C-10), 30.7 (C-11), 21.0 (COCH₃), 20.8 (COCH₃), 20.7 (COCH₃), 20.7 (COCH₃) ppm;

HRESI-MS: m/z : [M+Na]⁺ calcd. for C₇₃H₉₆N₄O₃, 1611.5753; found, 1611.5917.

3-Cascade:*N*-fluorenylmethoxycarbonyl aminomethane[3]:propane-(2,3,4,6-tetra-*O*-benzoyl- α -D-mannopyranoside) (35)

In a Schlenk flask, Fmoc-triacid **17** (59 mg, 0.12 mmol) with mannosyl azide **34** (336 μ mol, 0.540 mmol) and HOBT (49 mg, 0.36 mmol) was taken and dried under vacuum for about 1 h. Then the above mixture was dissolved in 20 ml of absolute THF under inert atmosphere. To the solution, DIC (55 μ l, 0.36 mmol) was added and stirred for 10 min. Then 1 M trimethyl phosphine solution (540 μ l, 540 mmol) was added at -70 °C. The reaction was stirred overnight and then



quenched by addition of water. The aqueous phase was thrice extracted with CH₂Cl₂ and combined organic phases were dried over MgSO₄. The silica gel column chromatography with ethyl acetate / cyclohexane (1:1) followed by LH-20 column with CH₂Cl₂/CH₃OH (1:1) gave pure product as a white crystalline compound.

Yield: 89 mg, 0.040 mmol, 34 %.

$$[\alpha]_D^{20} = +0.6^\circ (c = 0.9, \text{CH}_2\text{Cl}_2).$$

^1H NMR (600 MHz, CDCl_3 , TMS): δ = 8.04 (m_c, 6 H, Ar-H), 7.96 (m_c, 5 H, Ar-H), 7.90 (m_c, 6 H, Ar-H), 7.74 (m_c, 5 H, Ar-H), 7.60 (m_c, 2 H, Ar-H), 7.53-7.47 (m, 10 H, Ar-H), 7.40-7.30 (m, 20 H, Ar-H), 7.26 (m_c, 6 H, Ar-H), 7.20 (m_c, 8 H, Ar-H), 6.85 (br. m, 3 H, NH-C-8), 6.02 (dd-t, 3 H, $J = 10.03$ Hz, 3 H-4), 5.81 (d, 3 H, $J_{2,3} = 4.05$ Hz, 3 H-2), 5.74 (d, 3 H, $J = 9.53$ Hz, 3 H-1), 5.68 (dd, 3 H, $J_{2,3} = 3.16$, $J_{3,4} = 10.13$ Hz, 3 H-3), 5.02 (m_c, 1 H, H-13), 4.64 (dd, 3 H $J_{5,6} = 2.53$, $J_{6,6'} = 12.42$ Hz, 3 H-6), 4.35 (dd, 3 H, $J_{5,6} = 3.74$, $J_{6,6'} = 12.19$ Hz, 3 H-6'), 4.18 (m_c, 3 H, H-5), 3.82 (m_c, 2 H, H-12), 1.82 (m_c, 6 H, H-8), 1.75 (m_c, 6 H, H-9) ppm;

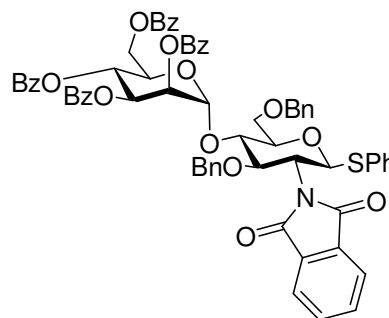
^{13}C NMR (150 MHz, CDCl_3 , TMS): δ = 172.6 (CO), 171.1 (CO), 166.1 (CO), 165.6 (CO), 165.4 (CO), 165.3 (CO), 157.0 (C-11), 143.9 (Ar-C), 143.7 (Ar-C), 141.3 (Ar-C), 141.3 (Ar-C), 133.6 (Ar-C), 133.4 (Ar-C), 133.2 (Ar-C), 132.9 (Ar-C), 129.9 (Ar-C), 129.9 (Ar-C), 129.8 (Ar-C), 129.8 (Ar-C), 129.1 (Ar-C), 128.9 (Ar-C), 128.9 (Ar-C), 128.6 (Ar-C), 128.4 (Ar-C), 128.4 (Ar-C), 128.3 (Ar-C), 127.6 (Ar-C), 127.0 (Ar-C), 124.8 (Ar-C), 119.8 (Ar-C), 76.6 (C-1), 74.1 (C-5), 72.6 (C-3), 70.9 (C-2), 66.3 (C-4), 65.7 (C-12), 62.4 (C-6), 57.1 (C-10), 47.2 (C-13), 31.3 (C-8), 30.5 (C-9) ppm;

HRESI-MS: m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{127}\text{H}_{108}\text{N}_4\text{O}_{32}$, 2201.6980; found, 2224.8521.

2,3,4,6-Tetra-*O*-benzoyl- α -D-mannosyl-(1 \rightarrow 4)-1-thiophenyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- α -D-glucopyranoside (48)

The thioglycoside **47** (281 mg, 0.481 mmol) with *O*-benzoyl protected mannosyl trichloroacetimidate **24** (0.90 g, 1.21 mmol) was dissolved in freshly distilled dichloromethane under inert atmosphere. The solution was cooled to 0 °C and $\text{BF}_3\cdot\text{Et}_2\text{O}$ (250 μl) was added to the reaction mixture. The reaction mixture was allowed to stir at room temperature for another 4 h and $\text{BF}_3\cdot\text{Et}_2\text{O}$ (100 μl) was given to it. After 2 h of additional stirring

another portion of $\text{BF}_3\cdot\text{Et}_2\text{O}$ was added and reaction mixture was allowed to stir for another 10 h. To the reaction mixture saturated solution of NaHCO_3 was added and the aqueous phase was washed with CH_2Cl_2 for three times. The organic phase was dried



over MgSO₄ and evaporated in vacuo. The residue was subjected to silica gel chromatography with 50 % ethyl acetate in cyclohexane to give pure disaccharide as slightly coloured syrup.

Yield: 436 mg, 0.376 mmol, 78 %.

$[\alpha]_D^{20} = +1.4^\circ$ ($c = 0.8$, CH₂Cl₂).

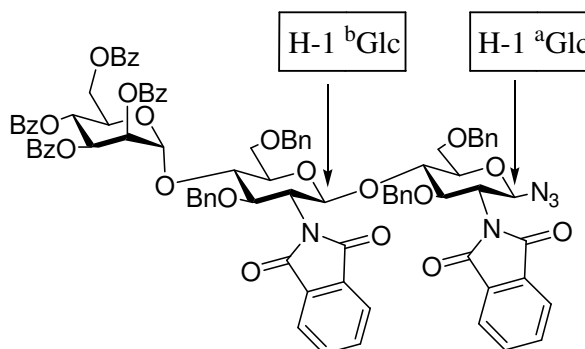
¹H NMR (600 MHz, CDCl₃, TMS): $\delta = 8.08$ (m_c, 2 H, Ar-H), 8.07-8.02 (m, 2 H, Ar-H), 7.93 (m_c, 4 H, Ar-H), 7.78 (m_c, 2 H, Ar-H), 7.67 (m_c, 2 H, Ar-H), 7.56 (m_c, 2 H, Ar-H), 7.51 (m_c, 2 H, Ar-H), 7.40-7.38 (m, 6 H, Ar-H), 7.37-7.32 (m, 6 H, Ar-H), 7.27-7.11 (m, 6 H, Ar-H), 7.00 (m_c, 2 H, Ar-H), 6.77 (m_c, 2 H, Ar-H), 6.67 (m_c, 1 H, Ar-H), 6.09 (dd~t, 1 H, $J = 10.08$ Hz, H-4 Man), 5.89 (dd, 1 H, $J_{2,3} = 3.14$, $J_{3,4} = 10.13$ Hz, H-3 Man), 5.79 (dd, 1 H, $J_{1,2} = 1.96$ Hz, 3.06 Hz, H-2 Man), 5.58 (d, 1 H, $J_{1,2} = 1.88$ Hz, H-1 Man), 5.55 (d, 1 H, $J_{1,2} = 10.47$ Hz, H-1 Glc), 4.93 (d, 1 H, $J = 12.07$ Hz, 1 H of OCH₂Ph), 4.62 (m_c, 3 H, H-3 Glc, 1 H of CH₂OCH₂Ph), 4.53 (m_c, 1 H, H-6 Man), 4.52-4.50 (m, 1 H, H-5 Man), 4.46 (d, 1 H, $J = 12.10$ Hz, 1 H of OCH₂Ph), 4.36 (dd, 1 H, $J_{6,6'} = 12.13$, $J_{5,6} = 3.81$ Hz, H-6' Man), 4.32 (dd~t, 1 H, $J = 10.34$ Hz, H-2 Glc), 4.12 (dd, 1 H, $J = 8.82$ Hz, H-4 Glc), 3.96 (m_c, 2 H, H-6, H-6' Glc), 3.87-3.84 (m, 1 H, H-5 Glc) ppm;

¹³C NMR (150 MHz, CDCl₃, TMS): $\delta = 166.1$ (CO), 165.5 (CO), 165.4 (CO), 165.1 (CO), 138.1 (C_{CON}), 137.6 (C_{CON}), 133.9 (Ar-C), 133.5 (Ar-C), 133.4 (Ar-C), 133.4 (Ar-C), 133.1 (Ar-C), 133.0 (Ar-C), 132.7 (2×Ar-C), 132.0 (Ar-C), 131.6 (Ar-C), 130.0 (Ar-C), 130.0 (Ar-C), 129.8 (2×Ar-C), 129.8 (2×Ar-C), 129.8 (2×Ar-C), 129.8 (2×Ar-C), 129.3 (Ar-C), 129.1 (Ar-C), 128.8 (3×Ar-C), 128.6 (Ar-C), 128.5 (3×Ar-C), 128.5 (3×Ar-C), 128.4 (3×Ar-C), 128.3 (Ar-C), 127.9 (3×Ar-C), 127.7 (3×Ar-C), 127.6 (Ar-C), 127.6 (3×Ar-C), 127.1 (Ar-C), 99.2 (C-1 Man), 83.5 (C-1 Glc), 81.8 (C-3 Glc), 79.0 (C-5 Glc), 78.0 (C-4 Glc), 75.2 (OCH₂Ph), 73.6 (CH₂OCH₂Ph), 70.5 (C-2 Man), 67.0 (C-3 Man), 69.8 (C-5 Man), 69.2 (C-6 Glc), 66.7 (C-4 Man), 62.8 (C-6 Man), 54.9 (C-2 Glc) ppm;

HRESI-MS: m/z : [M+Na]⁺ calcd. for C₆₈H₅₇NO₁₅S, 1182.3347; found, 1182.3187.

2,3,4,6-Tetra-*O*-benzoyl- α -D-mannosyl-(1 \rightarrow 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-1- α -D-glucopyranosyl-(1 \rightarrow 4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- α -D-glucopyranosyl azide (52)

The disaccharide **48** (354 mg, 0.305 mmol) and glycosyl azide **51** (114 mg, 0.221 mmol) were taken in a Schlenk flask and dried under reduced pressure for 1 hr. The above mixture was dissolved in anhydrous CH₂Cl₂ and 4 Å molecular sieves were added to it. The solution was cooled to -70 °C and NIS (115 mg, 0.691 mmol)



was added to it with further stirring for 10 min followed by the addition of TfOH (12 μ l). The reaction mixture was stirred for 30 min and filtered through celite to remove the molecular sieves. The organic layer was diluted with 50 ml of CH₂Cl₂ and washed with saturated solution of Na₂S₂O₃ and aqueous layer was extracted for three times, washed with brine. The organic phase was dried over MgSO₄ and concentrated in vacuo. The residue was purified by flash chromatography with 40 % ethyl acetate in cyclohexane.

Yield: 246 mg, 0.157 mmol, 71 %.

$$[\alpha]_D^{20} = +0.6^\circ (c = 0.8, \text{CHCl}_3).$$

¹H NMR (600 MHz, CDCl₃, TMS): δ = 8.06 (m_c, 2 H, Ar-H), 7.96-7.94 (m, 4 H, Ar-H), 7.80 (m_c, 3 H, Ar-H), 7.70-7.66 (m, 5 H, Ar-H), 7.57-7.48 (m, 4 H, Ar-H), 7.43-7.37 (m, 8 H, Ar-H), 7.36-7.32 (m, 5 H, Ar-H), 7.27-7.19 (m, 6 H, Ar-H), 7.15 (m_c, 1 H, Ar-H), 7.02 (m_c, 2 H, Ar-H), 6.94 (m_c, 2 H, Ar-H), 6.81-6.75 (m, 5 H, Ar-H), 6.99 (m_c, 1 H, Ar-H), 6.10 (dd~t, 1 H, J = 10.14 Hz, H-4 Man), 5.92 (dd, 1 H, $J_{2,3}$ = 3.10, $J_{3,4}$ = 10.20 Hz, H-3 Man), 5.79 (dd, 1 H, $J_{1,2}$ = 1.95, $J_{2,3}$ = 3.03 Hz, H-2 Man), 5.59 (d, 1 H, $J_{1,2}$ = 1.82 Hz, H-1 Man), 5.31 (d, 1 H, $J_{1,2}$ = 8.40 Hz, H-1 ^bGlc), 5.15 (d, 1 H, $J_{1,2}$ = 9.38 Hz, H-1 ^aGlc), 4.95 (d, 1 H, J = 12.05 Hz, OCH₂Ph), 4.82 (d, 1 H, J = 12.82 Hz, OCH₂Ph), 4.63 (d, 3 H, J = 11.47 Hz, OCH₂Ph), 4.58 (m_c, 2 H, H-6' Man, H-3 ^bGlc), 4.54-4.46 (m, 4 H, 3 OCH₂Ph, H-5 Man), 4.38 (dd, 1 H, $J_{5,6}$ = 3.96, $J_{6,6'}$ = 12.31 Hz, H-6 Man), 4.27 (m_c, 2 H, H-2 ^bGlc, H-3 ^aGlc), 4.18 (t, 1 H, J = 8.85 Hz, H-4 ^bGlc), 4.15 (t, 1 H, J = 8.45 Hz, H-4 ^aGlc), 4.06 (dd, 1 H, J = 10.43 Hz, H-2 ^aGlc),

3.81 (d, 2 H, $J = 2.54$ Hz, H-6 Glc), 3.60 (dd, $J = 11.13$ Hz, H-6 Glc), 3.46 (dd, $J_{5,6} = 3.53$, $J_{6,6'} = 11.28$ Hz, H-6' ^bGlc), 3.42-3.35 (m, 2 H, H-5 ^aGlc, H-5 ^bGlc) ppm;

¹³C NMR (150 MHz, CDCl₃, TMS): $\delta = 166.1$ (CO), 165.6 (CO), 165.4 (CO), 165.1 (CO), 138.4 (Ar-C), 138.2 (Ar-C), 138.1 (Ar-C), 138.0 (Ar-C), 133.4.0 (Ar-C), 133.8 (Ar-C), 133.4 (Ar-C), 133.3 (Ar-C), 133.3. (Ar-C), 133.1 (Ar-C), 133.0 (Ar-C), 131.6 (Ar-C), 130.0 (Ar-C), 129.9 (Ar-C), 129.8 (2×Ar-C), 129.7 (Ar-C), 129.4 (Ar-C), 129.2 (Ar-C), 128.5 (2×Ar-C), 128.4(2×Ar-C), 128.3 (Ar-C), 128.2 (Ar-C), 128.1 (Ar-C), 128.0 (Ar-C), 127.9 (Ar-C), 127.7 (3×Ar-C), 127.6 (Ar-C), 127.5 (Ar-C), 127.4 (Ar-C), 127.3 (Ar-C), 127.1 (Ar-C), 127.0 (Ar-C), 98.9 (C-1 Man), 96.8 (C-1 ^bGlc), 85.6 (C-1 ^aGlc), 80.7 (C-3 ^bGlc), 77.5 (C-4 ^bGlc), 76.8 (C-5 ^bGlc), 75.0 (C-3 ^aGlc), 74.9 (OCH₂Ph), 74.7 (C-5 ^aGlc), 74.6 (OCH₂Ph), 73.0 (OCH₂Ph), 73.4 (OCH₂Ph), 70.6 (C-2 Man), 70.0 (C-5 Man), 69.8 (C-3 Man), 67.9 (C-6 ^bGlc), 66.7 (C-4 Man), 63.3 (C-6 ^aGlc), 62.8 (C-6 Man), 56.5 (C-2 ^bGlc), 55.3 (C-2 ^aGlc) ppm;

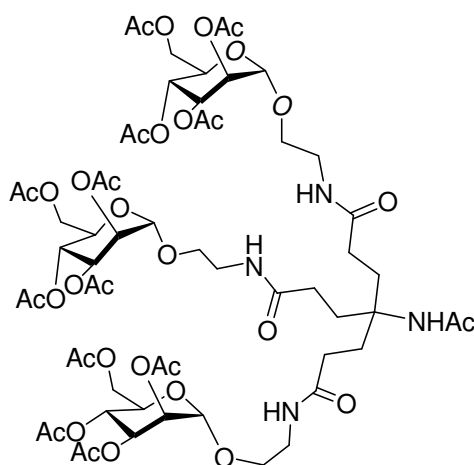
HRESI-MS: m/z : [M+Na]⁺ calcd. for C₉₀H₇₇N₅O₂₁, 1587.5850; found, 1587.5212.

Elemental analysis for C₉₀H₇₇N₅O₂₁: C 69.09 H 4.49, N 4.48; found C 68.98, H 5.16, N 4.55 %.

8.8 Synthesis of glycopeptide analogues

3-Cascade:*N*-acetyl-aminomethane[3]:propane-(1-amidoethyl-2,3,4,6 tetra-*O*-acetyl- α -D-mannopyranoside) (58)

The *O*-acetylated glycocluster **33** (80 mg, 5.03 mmol) was dissolved in absolute dichloromethane (5 ml) and morpholine (500 μ l) was added to it under inert atmosphere. The reaction mixture was stirred at room temperature for 4 h and then evaporated in vacuo. The residue was dried under high vacuum overnight; it was dissolved in acetic anhydride (1 ml) in pyridine (3 ml). The reaction mixture was stirred at room temperature for 16 h and concentrated in vacuo. The residue was subjected to silica gel chromatography with 60 % methanol in ethyl acetate to give pure product as slightly coloured syrup.



Yield: 47 mg, 0.034 mmol, 68 %.

$[\alpha]_D^{20} = +2.1^\circ$ ($c = 1.0$ CH₂Cl₂).

¹H NMR (600 MHz, CDCl₃, TMS): $\delta = 6.98$ (t, 3 H, $J = 5.48$ Hz, 3 C-8-NH), 5.33-5.27 (m, 6 H, 3 H-3, 3 H-4), 5.26 (dd, 3 H, $J_{1,2} = 1.84$, $J_{2,3} = 3.30$ Hz, 3 H-2), 4.84 (d, 3 H, $J_{1,2} = 1.70$ Hz, 3 H-1), 4.24 (dd, 3 H, $J_{5,6} = 5.51$, $J_{6,6'} = 12.26$ Hz, 3 H-6), 4.14 (dd, $J_{5,6'} = 2.49$, $J_{6,6'} = 12.23$ Hz, 3 H-6'), 4.02-3.99 (m, 3 H, 3 H-5), 3.77 (m_c, 3 H, 3 H-7), 3.55 (m_c, 6 H, 3 H-7', 3 H-8), 3.51-3.46 (m, 3 H, 3 H-8'), 2.24 (t, 6 H, 6 H-10), 2.16 (s, 9 H, COCH₃), 2.10 (s, 9 H, COCH₃), 2.06 (s, 9 H, COCH₃), 2.02 (t, 6 H, H-11), 2.0 (s, 9 H, COCH₃), 1.94 (s, 3 H, NHCOCH₃) ppm;

¹³C NMR (150 MHz, CDCl₃, TMS): $\delta = 173.8$ (C-13), 170.8 (COCH₃), 170.6 (COCH₃), 170.3 (COCH₃), 170.2 (COCH₃), 169.7 (COCH₃), 97.5 (C-1), 69.5 (C-4), 69.3 (C-3), 68.7 (C-5), 67.0 (C-7), 66.1 (C-2), 62.5 (C-6), 58.06 (C-12), 38.9 (C-8), 31.5 (C-11), 30.9

(C-10), 24.1 (NHCOCH₃), 20.9 (COCH₃), 20.7 (COCH₃), 20.7 (COCH₃), 20.6 (COCH₃) ppm;

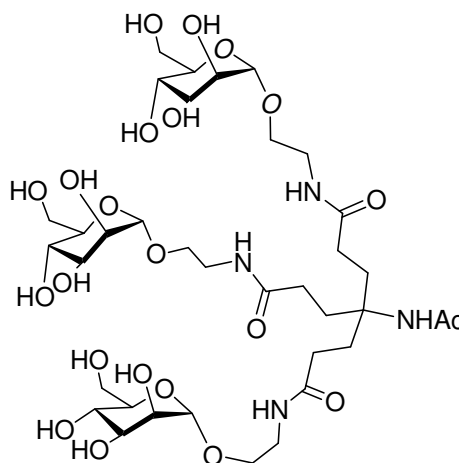
HRESI-MS: m/z : [M+Na]⁺ calcd. for C₆₀H₈₈N₄O₃₄, 1176.3595; found, 1176.3557.

3-Cascade:*N*-acetyl-aminomethane[3]:propane-(1-amidoethyl- α -D-mannopyranoside) (**59**)

The acetylated glycocluster **58** (30 mg, 0.021 mmol) was deprotected according to general procedure 8.2.5 to receive white foam after lyophilisation. Purity of the product was confirmed by HPLC.

Yield: 19 mg, 0.021 mmol, quant.

$[\alpha]_D^{20} = +1.5^\circ$ ($c = 1.0$, CH₃OH).



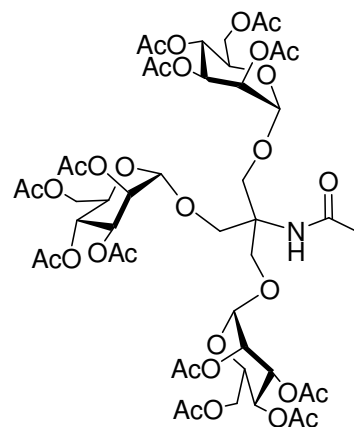
¹H NMR (600 MHz, CD₃OD, TMS): $\delta = 4.81$ (d, 3 H, $J_{1,2} = 1.66$ Hz, 3 H-1), 3.88 (dd, 1 H, $J_{5,6} = 2.27$, $J_{6,6'} = 11.70$ Hz, 3 H-6), 3.86 (dd, 3 H, $J_{2,3} = 1.71$, $J_{3,4} = 3.37$ Hz, 3 H-2), 3.81 (m_c, 3 H, 3 H-7), 3.75 (dd, 6 H, $J_{5,6'} = 6.62$, $J_{6,6'} = 11.63$ Hz, 3 H-6'), 3.74 (dd~t, $J_{2,3} = 3.58$, $J_{3,4} = 9.75$ Hz, 3 H-3), 3.64 (dd~t, 3 H, $J = 9.76$ Hz, 3 H-4), 3.61-3.57 (m, 6 H, 3 H-5, 3 H-7'), 3.49-3.46 (m, 3 H, 3 H-8), 3.43-3.40 (m, 3 H, 3 H-8'), 2.24 (m_c, 6 H, 3 H-10), 2.03 (m_c, 6 H, 3 H-11), 2.01 (s, 3 H, COCH₃) ppm;

¹³C NMR (150 MHz, CD₃OD, TMS): $\delta = 176.1$ (C-9, C-13), 101.7 (C-1), 74.8 (C-5), 72.6 (C-3), 72.1 (C-2), 68.9 (C-4), 67.2 (C-7), 63.0 (C-6), 59.3 (C-12), 40.5 (C-8), 31.8 (C-11), 31.2 (C-10), 23.5 (COCH₃) ppm;

HRESI-MS: m/z : [M+Na]⁺ calcd. for, C₃₆H₆₄N₄O₂₂, 927.3910; found, 927.3995.

***N*-(Acetoxycarbonyl)tris[[2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyl]oxy]methyl]methylamine (**60**)**

In a round bottom flask, **28** (50 mg, 0.082 mmol) was dissolved in pyridine (2 ml) under nitrogen atmosphere. To the above solution was added acetic anhydride (1 ml) and stirred overnight at room temperature. The reaction mixture was concentrated and applied to flash chromatography with 80 % ethyl acetate in cyclohexane to give compound **60** as colourless foam.



Yield: 76 mg, 0.066 mmol, 80 %.

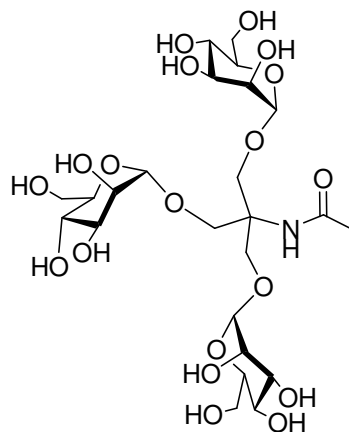
^1H NMR (500 MHz, CDCl_3 , TMS): δ = 5.63 (s, 3 H, NHCOCH_3), 5.29 (dd~t, 3 H, J = 9.86 Hz, 3 H-4), 5.25 (dd, 3 H, $J_{2,3}$ = 3.38, $J_{3,4}$ = 9.99 Hz, 3 H-3), 5.19 (dd, 3 H, $J_{1,2}$ = 1.74, $J_{2,3}$ = 3.35 Hz, 3 H-2), 4.85 (d, $J_{1,2}$ = 1.62 Hz, 3 H-1), 4.33 (dd, 3 H, $J_{5,6}$ = 12.40, $J_{6,6'}$ = 4.96 Hz, 3 H-6), 4.15-4.12 (m, 6 H, 3 H-6', 3 H-7), 3.98-3.95 (m, 3 H, 3 H-5), 3.75 (d, 3 H, J = 10.51 Hz, 3 H-7'), 2.15 (s, 9 H, COCH_3), 2.12 (s, 9 H, COCH_3), 2.05 (s, 9 H, COCH_3), 2.02 (s, 3 H, NHCOCH_3), 2.00 (s, 9 H, COCH_3) ppm;

^{13}C NMR (125 MHz, CDCl_3 , TMS): δ = 170.8 (C-9), 170.7 (COCH_3), 170.0 (COCH_3), 169.9 (COCH_3), 169.6 (COCH_3), 98.3 (C-1), 69.1 (C-2), 69.0 (C-5), 69.0 (C-3), 66.4 (C-7), 65.8 (C-4), 62.1 (C-6), 59.2 (C-8), 24.1 (NHCOCH_3), 20.8 (COCH_3), 20.7 (COCH_3), 20.6 ($2\times\text{COCH}_3$) ppm;

HRESI-MS: m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{48}\text{H}_{67}\text{NO}_{31}$, 1176.3595; found, 1176.3557.

***N*-(Acetoxycarbonyl)tris[α -D-mannopyranosyloxy)methyl]methylamine
3-mer (61)**

The acetylated glycocluster **60** (66 mg, 0.057 mmol) was dissolved in absolute methanol (3 ml). To the above solution 0.1 M solution of sodium methanolate in methanol was added (200 μ l). The reaction mixture was allowed to stir for 3 h and neutralized with Amberlite IR-120, filtered and solvent was evaporated in vacuo. After lyophilisation pure product was isolate as white lypophilisate. Purity of the product was confirmed by HPLC.



Yield: 37 mg, 0.056 mmol, quant.

$[\alpha]_D^{20} = + 2.1^\circ$ ($c = 1.0$, CH_3OH).

^1H NMR (600 MHz, CD_3OD , TMS): $\delta = 4.78$ (d, 3 H, $J_{1,2} = 1.69$ Hz, 3 H-1), 3.99 (d, $J = 9.72$ Hz, 3 H-7), 3.88 (dd, 3 H, $J_{5,6} = 1.97$, $J_{6,6'} = 11.82$ Hz, 3 H-6), 3.86 (dd, 3 H, $J_{1,2} = 1.74$, $J_{2,3} = 3.39$ Hz, 3 H-2), 3.80 (d, 3 H, $J = 9.72$ Hz, 3 H-7'), 3.77 (dd, 3 H, $J_{5,6'} = 5.63$, $J_{6,6'} = 11.97$ Hz, 3 H-6'), 3.73 (dd, 3 H, $J_{2,3} = 3.41$, $J_{3,4} = 8.92$ Hz, 3 H-3), 3.67-3.62 (m, 6 H, 3 H-4, 3 H-5), 2.00 (s, 3 H, NHCOCH_3) ppm;

^{13}C NMR (150 MHz, CD_3OD , TMS): $\delta = 173.7$ (NHCOCH_3), 102.3 (C-1), 74.8 (C-5), 72.6 (C-3), 71.9 (C-2), 68.5 (C-4), 66.9 (C-7), 62.8 (C-6), 60.7 (C-8), 23.5 (NHCOCH_3) ppm;

MALDI-ToF-MS m/z : $[\text{M}+\text{Na}]^+$ calcd. for $\text{C}_{24}\text{H}_{23}\text{NO}_{19}$, 673.1; found, 673.4.

8.9 Competitive assay on FimH binding

For the measurement of assay, 96-well microtiter plates were coated with the mannose polymer mannan by treating each well with 100 μ l of mannan solution (2 mg/mL in carbonate buffer) and letting the wells dry out over night. The uncoated well surface was blocked with BSA by treating the wells with a 5 % BSA solution in PBS. The coated and blocked wells were then incubated with a mixture of the serially diluted inhibitor in question and the GFP-transfected *E. coli* bacteria (altogether 100 μ l). The fluorescence of the GFP was read in PSB-filled wells. Each incubation step was followed by three washings with PBST.

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