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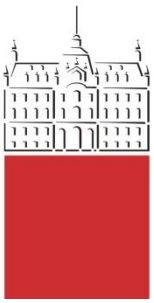

SYNTHESIS OF ALPHA-D-MANNOSYL GLYCOCONJUGATES AIMED TO TARGET MANNOSE-BINDING LECTINS

SINTEZA ALFA-D-MANOZILIRANIH GLIKOKONJUGATOV KOT LIGANDOV ZA LEKTINE, SPECIFIČNE ZA MANOZO

UNIFORM MASTER'S STUDY PROGRAMME PHARMACY

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This master thesis was performed at the Department of pharmaceutical chemistry at the Faculty of pharmacy under the supervision of Assoc. Prof. Dr. Marko Anderluh and at the Department of organic and industrial chemistry at the University of Milan under the supervision of Prof. Dr. Anna Bernardi.

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Posebna hvala moji družini, ki mi ves čas stoji ob strani in me bodri. Brez vas mi ne bi uspelo.

Statement

I hereby declare that this Master's thesis was done by me under supervision of Assoc. Prof. Dr. Marko Anderluh and co-supervision of Prof. Dr. Anna Bernardi.

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ABSTRACT

It has been known for some time that adhesion is of major importance in order for pathogenic bacteria or viruses to achieve infection. Adhesion is usually obtained by appendages present on cell surface named lectins, which are carbohydrate-binding proteins. As the explanation suggests, they bind to carbohydrates present on "partner" cell surfaces through carbohydrate recognition domains, and thus act as recognition sites between microbes and the host cells. In this thesis we will focus on two lectins, FimH, a region on type 1 fimbriae, present on most *E. coli* strains causing uroepithelial infections; and DC-SIGN - dendritic cell-specific ICAM3-grabbing non-integrin - present on dendritic cells, which is the first to come into contact with HIV-1 virus. Both lectins would be suitable targets for anti-adhesive compounds. Since both have shown specificity for D-mannose monosaccharide, we designed and synthesized mannose-based antagonists of both receptors by attaching appropriate hydrophobic groups to increase binding affinity.

In the first part of our thesis we synthesised a molecule following a T-shape structure with two hydrophobic groups, naphthalene-1-yl and 4-yl-benzoic acid, attached by a glycerol linker to the monosaccharide core. This showed to be very effective in increasing binding affinity to FimH with aromatic moieties occupying the "tyrosine gate" and lipophilic area around it. The second part was dedicated to forming a library of pseudo-mannosides. Four different compounds were successfully synthesised through a procedure that included the synthesis of a pseudo-mannoside scaffold to which five different amines were attached in a condensation reaction. One compound was lost during the final step of deprotection, but the other four compounds will be tested in a novel microarray using clickable polymers. They promise to be effective in binding to DC-SIGN since a pseudo-mannoside structure has been proven successful in targeting the receptor.

Anti-adhesive therapy is a promising branch of pharmaceutical chemistry which would forever change treating of infectious diseases. By using them as complementary drugs in anti-infection treatment we could slowly introduce them as an independent treatment or, more likely, preventative therapy and thus reduce the use of antibiotics.

Key words: adhesion, lectin, FimH, DC-SIGN, D-mannose, tyrosine gate, pseudo-mannoside

POVZETEK

Za razvoj infekcij je izjemno pomemben proces adhezije bakterijskih celic ali virusov na gostiteljske celice. Najpogosteje pri adheziji patogenov sodelujejo lektini, to so proteini, ki vežejo ogljikove hidrate prisotne na površini gostiteljevih celic in delujejo kot prepoznavna mesta med celico in okoljem. V magistrski nalogi se bomo osredotočili na dva lektina: na lektin FimH, ki je regija na pilusih tipa 1, prisotnih na večini sevov *E. coli* odgovornih za infekcije urogenitalnega trakta, ter lektin DC-SIGN, ki je prisoten na dendritičnih celicah in je prva makromolekula, ki pride v stik s številnimi patogeni kot npr. virusom HIV-1. Oba sta pokazala specifičnost za vezavo D-manoznih monosaharidov, zato smo se odločili za načrtovanje in sintezo antagonistov, ki vsebujejo D-manozo kot jedro na katerega so pripete primerne hidrofobne skupine, ki povečajo vezavno afiniteto.

V prvem delu naloge smo sintetizirali molekulo, ki je sledila konformaciji molekul v obliki črke T: na monosaharidno jedro smo preko glicerolnega ditančnika pripeli dve hidrofobni skupini, naftalen-1-il in 4-il-benzojsko kislino. Predvidevamo, da aromatske enote zasedejo »tirozinska vrata« v receptorju, kot tudi lipofilno območje okoli njih, in s tem povečajo vezavo spojine v vezavno mesto FimH. Drugi del je bil posvečen sintezi knjižnice psevdo-manozidov, kjer smo uspešno sintetizirali štiri različne spojine. Postopek je vključeval sintezo psevdo-manozidnega ogrodja na katerega smo z reakcijo kondenzacije vezali 5 različnih aminov. Žal smo eno spojino izgubili pri zadnjem koraku, to je pri odščiti zaščitnih skupin pripetih na D-manozo. Vezavno afiniteto ostalih štirih spojin bomo v prihodnosti testirali na novo razvitem testu, ki vključuje »click« polimere, kjer ravno zaradi psevdo-manozidne strukture pričakujemo pozitivne rezultate meritev vezave na DC-SIGN.

Z antiadhezivno terapijo bi lahko spremenili način zdravljenja infektivnih bolezni, zatorej je raziskovanje te smeri zelo perspektivno. Z uporabo antiadhezivov kot dopolnila k zdravljenju, bi jih lahko počasi vpeljali kot samostojno ali, bolj verjetno, kot preventivno terapijo ter s tem zmanjšali uporabo antibiotikov.

Ključne besede: adhezija, lektin, FimH, DC-SIGN, D-manoza, tirozinska vrata, psevdo-manozid

1. INTRODUCTION

1. 1. Bacterial adhesion

Adhesion of pathogenic organisms is mandatory in order for microbes, including bacteria, protozoa, viruses and fungi, to commence infection on host cells and cause disease. First and foremost adhesion must be obtained so that the pathogens are not eliminated by cleansing mechanisms present in the host, such as urinary flow in the urinary tract or airflow in the respiratory tract, and for the microbes to acquire sufficient nutrients to survive and consequently successfully infect the host. Secondly, by achieving proper adhesion, delivery of toxic agents and ultimately the penetration of microbes into the host tissue is facilitated, which again results in successful infection (1).

Typically adhesion of bacteria or viruses is achieved by specific proteinaceous appendages present on their surface called lectins. Their expression is a necessity for the pathogenesis of microbes as they are responsible for adhesion by recognizing complementary carbohydrates on the host's surface (2, 3). Adhesion of a microbe is accomplished in the following manner (**Figure 1**): the microbe first attaches to the host cell surface through weak non-specific interactions, which are not mediated by specific adhesion-receptor pairing but by overall physicochemical properties of the host and bacterial surface – charge and hydrophobicity. This is followed by primary adhesion, which is more specific, since it is intervened by specific interactions, but still allows the microbe to sample the hosts' surface by gliding or rolling on the surface. Both of these processes are transitory and are later strengthened by high affinity microbe-host interactions that depend on specific interaction between host cell receptors and bacterial surface molecules (4).

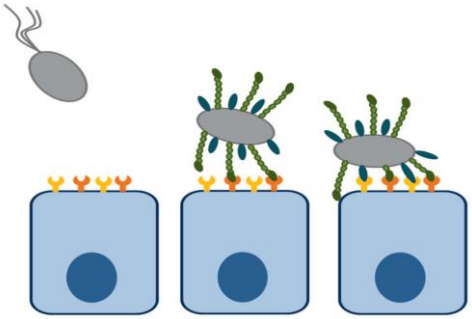


Figure 1: Adhesion of microbes to host cells.

(Adjusted from 4)

Expression of a given lectin on the host cell surface acts as an address indicator for the microbe which enables that same microbe to target a specific surface (5). The binding moiety of the lectin can vary in terms of its chemical identity from a protein, to sugar and a lipid (4).

1. 2. Lectins

Lectins are proteins specific for carbohydrate binding present on the surface of microbes or eukaryotic cells that act as recognition sites between the cell and the environment which surrounds it. Their main role is to promote cell to cell contact binding in a manner where one cell expresses lectins on its surface, which interacts with an array of carbohydrates expressed on another cell's surface. They reversibly and specifically bind monosaccharides, oligosaccharides or partial structures of saccharides and can be found in practically all organisms from viruses to animals. They contain one or more carbohydrate recognition domains (CRDs) which are responsible for binding carbohydrates. It is just in the carbohydrate recognition domain that they differ specifically, according to the type of monosaccharide they bind, and are divided into five groups; specific for:

a) D-mannose,
b) D-galactose and *N*-acetylgalactosamine,
c) *N*-acetylglucosamine,
d) L-fucose and
e) *N*-acetylneuraminic acid.

Lectins actually interact with complex oligosaccharide portions present in glycoconjugates, so this is only a simplification of natural specificities. They can be very specific, binding only one monosaccharide, or their specificity can be very low as in the case of "mannose-specific" lectins, which also bind L-fucose and other monosaccharides with lower, but comparable affinities (6). Two families of lectins are known: intracellular (L-type, P-type etc.) and extracellular family (galectins, C-type etc.), and it is the extracellular lectins, which are responsible for recognizing carbohydrate epitopes of pathogens and other cells, that are interesting as molecular targets (7). Further we will describe **FimH**, a region of type 1 fimbriae present on bacteria, and dendritic cell-specific ICAM3-grabbing non-

integrin – **DC-SIGN**, as a representative of C-type lectins. Both are among the most promising targets in the field of anti-adhesion therapy.

1. 2. 1. Fimbriae and FimH

Bacterial surface lectins are usually located at the tip of fimbriae (or pili) – these are polymorphic and consist of an assembly of hundreds of protein subunits of several kinds, with multiple copies of CRDs among them. They can be found in the form of fuzzy tangles that are made up of thin fibres with diameters of around 2 nm or as rigid structures with diameters between 5 and 10 nm. The structural protein, which represents the bulk of the organelle, acts as a scaffold on which a couple of adhesion receptors are exhibited, usually at the tip of the fimbria (5). Fibres of fimbriae are 1-2 μm long and 7 nm thick and are rod-like. Each fimbria individually consists of a base to which four building blocks are added during its growing (**Figure 2**). FimA is the major structural element and about 1000 copies of it are polymerized to form a right handed helical structure, also referred to as a "shaft", which is linked to a thick distal tip fibrillum with the diameter of 3 nm. This fibrillum is composed of two adapter proteins, FimF and FimG, and also a third one, FimH, which differs from the other two. FimH is responsible for the fimbria sugar binding activity since it is the only one capable of carbohydrate-binding (3, 5). They can be found in 95% of *E. coli* strains, mediating uroepithelial infections and are also produced by other species – *Klebsiella Pneumonia, Salmonella typhimurium* and *Salmonella Enteritidis*. Typically these type of fimbriae have higher affinity for oligosaccharides such as Manα3Manβ4GlcNAc or Manα6(Manα3)Manα6(Manα3)Man which are known to be constituents of cell surface glycoproteins. What is of utmost importance, FimH is the major virulence factor of uropathogenic *E. coli*, as it enables the attachment and further migration of bacteria on uroepithelial surface. Consequently, by inhibiting FimH binding to uroepithelial surface glycoproteins, one can eradicate bacteria with a mechanism substantially different from the common antibacterial therapy – via anti-adhesion mechanism.

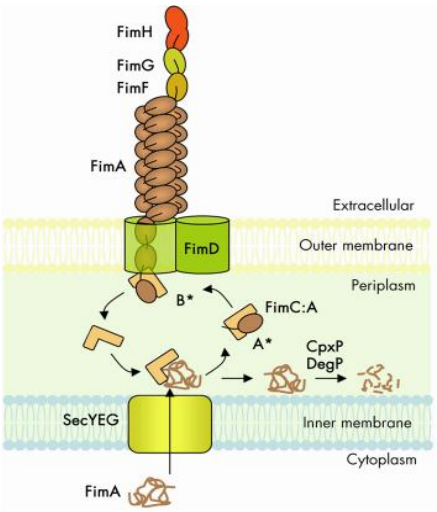


Figure 2: The chaperone-usher biosynthetic pathway, labelled accordingly (Fim cluster)

(Adjusted from 8)

The structure of the FimH subunit was studied by X-ray crystallography thus allowing a better insight into ligand binding mechanism and further structure-based design of potential FimH antagonists. While examining the 3D structure of the FimC-FimH complex with bound mannose, Hung and his group discovered that only the D-mannose α-anomer binds to the FimH (9). As depicted in **Figure 3**, this specific binding is a direct consequence of steric constraints and amino acid residues that form a hydrogen bond network by binding to hydroxyl groups of mannose at positions 2, 3, 4 and 6. Tyr48 and Tyr137 form "the tyrosine gate" - an entrance to the mannose-binding pocket, while the opposing part of the entrance is composed of Ile13 and Phe142 residues, which are responsible for hydrophobic interactions. This hydrophobic ridge forms around the site of binding and possibly helps direct the ligand into it (10). Replacing residues Asp54, Gln133, Asn135 and Asp140 results in total loss of activity (3).

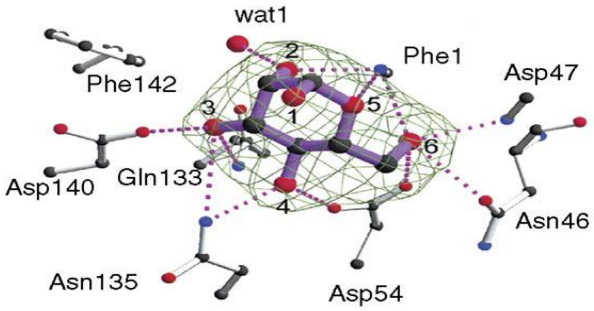


Figure 3: α-Mannose in FimH binding site

(Adjusted from 9)

1. 2. 2. Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN)

DC-SIGN belongs to the family of C-type lectins, whose main characteristic is the presence of a Ca^{2+} ion in its carbohydrate-recognition domain that enables binding of the "core monosaccharides", and can be found on dendritic cells (DCs). DCs are cells of the immune system that act as messengers between the innate and the adaptive immune system and are responsible for antigen presentation. They do so by processing antigen material and then presenting it on their surface to T cells of the immune system. Derived from the hematopoietic bone marrow progenitor cells, they transform into immature DCs which turn into mature DCs after binding antigens. DC-SIGN enables DC migration, antigen recognition and processing. It is also responsible for DCs adhesion to T cells via interaction with type I transmembrane glycoprotein ICAM-3. Several microbes use this mechanism to surpass the immune system – they bind to DC-DIGN and are internalized by DCs where they somehow escape the lytic process. Embedded in DC endosomes they survive in the host environment until target cells are reached to start the infection. It is believed that it is exactly the pathogen adhesion to DC-SIGN which enables this "Trojan horse" mechanism, which offers a rationale, that DC-SIGN antagonists might be used to prevent the infection even before its outbreak (11).

The binding site of DC-SIGN selectively binds monosaccharides with hydroxyl groups in position 3 and 4 in the equatorial orientation. The two hydroxyls form coordination bonds with Ca^{2+} ion, and hydrogen bonds with adjacent glutamic acid side chain carboxylates, thus forming a dense bonding network that selectively accommodates L-fucose and D-mannose (12). Described interactions in the binding site can be seen in **Figure 4**.

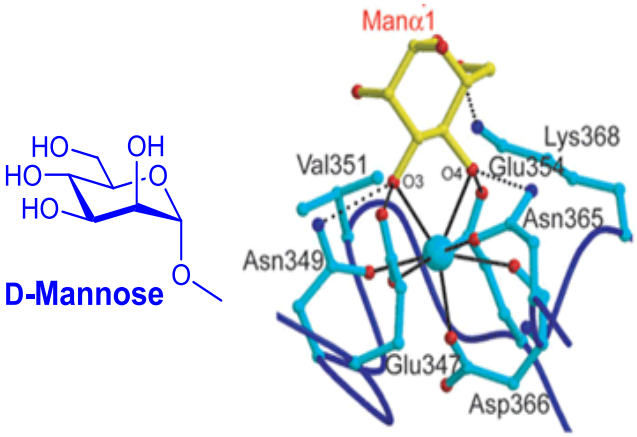


Figure 4: Binding of D-mannose to DC-SIGN

(Adjusted from 12)

1. 3. Glycoconjugates

By targeting adhesins like FimH and DC-SIGN with appropriate carbohydrates or their analogues, treatment or prevention of infections, such as urinary tract infection with *E. coli* or HIV-1 infection, would be possible. Saccharides and/or their analogues would be a perfect choice because the chance of them being immunogenic or toxic is very slim since they are normal constituents of cells. Compared to antibiotics already in use, the anti-adhesion molecules would not kill or arrest the growth of microbes, but would prevent the very first step of infection. Consequently, resistant strains are very unlikely to emerge or would be diluted with the majority of the microbes whose adhesion would be inhibited and would thus be shed from the host. Isolation of natural saccharides or their synthesis is both difficult and time-consuming, and their fast metabolism would probably prevent them from reaching target receptors. Glycoconjugates are the perfect answer to these problems. Structurally, they are a combination of carbohydrates and other biological molecules that are covalently linked to each other by glycosylation reaction. Macrolides, ergot alkaloids, anthracyclines, cardiac glycosides and many more pharmaceutical active compounds belong to the category of glycoconjugates. In size they range from small molecules to complex molecules linked to proteins and lipids called glycoproteins, glycolipids and proteoglycans, which are among the most important biomolecules in the cell. They can form a structurally very diverse group of molecules, since any of the hydroxyl groups can be glycosylated and give one of two stereoisomers, an α or β glycoside. But to form glycoconjugates only a small portion of the large array of monosaccharides is actually used (4, 13).

1. 3.1. Glycosides

Glycosides are a subgroup of glycoconjugates with a moiety linked to a sugar via a glycosidic bond. They have a structure of the cyclic acetal (**Figure 5**), that is with an aglycone component attached to the glycone moiety at the anomeric center.

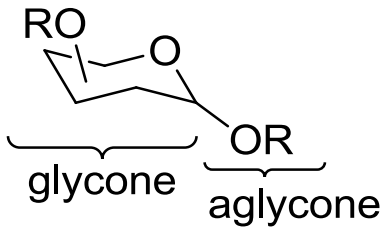


Figure 5: Structure of a glycoside

O-glycosides, N-glycosides, C-glycosides and S-glycosides are known, depending on the glycosidic bond they form.

In the glycoside forming process the first step that must be taken is protecting the hydroxyl groups of the sugar (14).

1. 3.1.1. Protecting groups and their elimination

The protection of saccharide hydroxyl groups is a paramount step in the glycosylation process since they contain several hydroxyl groups with different reactivity. Protecting groups can influence the reactivity of carbohydrate derivatives because of steric or electronic influences. The ones adjacent to the anomeric center will influence the stereoselectivity of glycosylation. Most frequently used protection groups for hydroxyls are acetals, ethers and esters, and among these, ester groups are most commonly employed. Peracetylation is accomplished with acetic anhydride in pyridine, and perbenzoylation with benzoyl chloride in pyridine at room temperature (**Figure 6**).

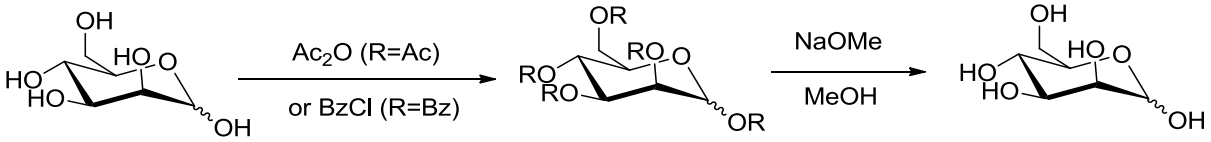


Figure 6: Scheme of protection and deprotection of the saccharide unit.

(Adjusted from 15)

Esters are deprotected using a well-known transesterification reaction (**Figure 7**) called **the Zemplén procedure**, catalyzed by a sodium methoxide as a base catalyst (16).

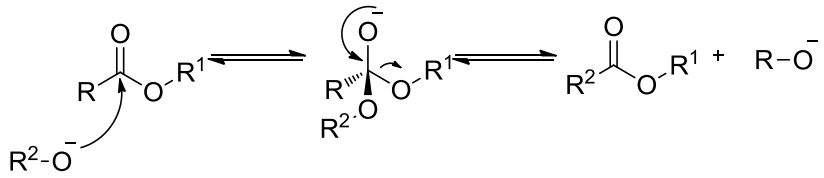


Figure 7: Mechanism of the Zemplén reaction

(Adjusted from 17)

1. 3.1.2. Selective deprotection and activation of anomeric carbon

Another important step in glycosylation is the selective deprotection of the anomeric center, especially in the case where trichloroacetimidates are used as glycosyl donors. The selective deprotection can be done using a milder nucleophile, for example by using one equivalent of hydrazine acetate/hydrazine hydrate in *N,N'*-dimethylformamide or piperidine and 2-aminoethanol. In general, anomeric debenzoylations take longer than the respective deacetylations (18).

After selective deprotection has been completed, activation of the anomeric carbon follows, by attaching a suitable leaving group to the anomeric carbon. Most commonly used leaving groups are trichloroacetimidate, bromide, chloride and oxazoline, and most glycosylations are perfomed by either **Koenigs-Knorr reaction** or the **trichloroacetimidate method** (19). In the Koenigs-Knorr reaction (**Figure 8**) the glycosyl donor is a glycosyl bromide or a glycosyl chloride and silver salts are used as promoters of the reaction (20).

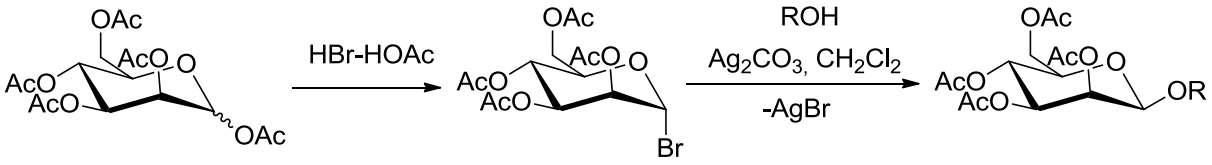


Figure 8: Scheme of the Koenigs-Knorr method

(Adjusted from 21)

For a long time the Koenigs-Knorr type reactions were the only possible methods for glycosylation, but they have two major disadvantages – the intrinsic lability of glycosyl halides and the use of heavy metal salts (Ag) in equimolar amounts as promotors. That is why the trichloroacetimidate method by Schmidt et. al. is widely used for glycosylation nowadays. The anomeric oxygen is derivatized with a group known as a good leaving group (**Firgure 9**) that is activated by a Lewis acid, such as trimethylsilyl trifluoromethanesulfonate (TMSOTf), in the glycosylation process. Both anomeric glycosyl trichloroacetimidates can be obtained in pure form, depending on the base used for deprotonation of the reducing sugar (22).

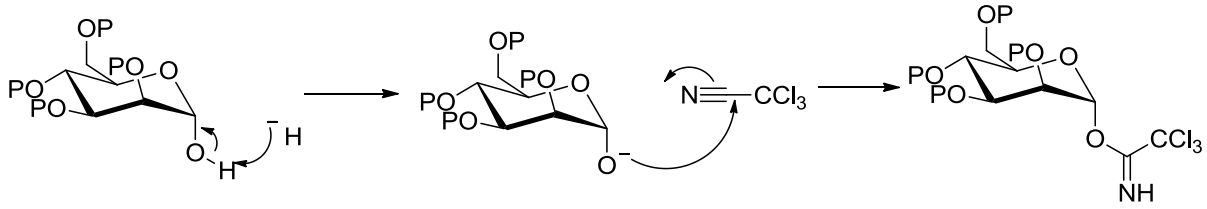


Figure 9: Mechanism of the formation of the trichloroacetimidate (Schimdt et. al.)

(Adjusted from 23)

1. 3.1.3. Glycosylation reaction

Glycosylation is a reaction involving a glycosyl donor and a glycosyl acceptor which are coupled by an activator (in the case of the trichloroacetimidate method this is a Lewis acid). The leaving group on the glycosyl donor is activated by an activator and forms an oxocarbenium ion. The anomeric carbon with an electrophilic character is left after the oxocarbenium ion is eliminated from the molecule. The glycosidic bond is formed when the nucleophilic hydroxyl group of the glycosyl acceptor attacks the anormeric carbon. Attack of the nucleophile can occur on either diastereomeric face of the oxocarbenium. In the absence of other effects, the α-glycoside product is favoured, due to the anomeric effect (24, 25). However, mixtures of anomers are usually obtained. The steric outcome of glycosylation reactions can be controlled by an ester protecting group at position 2 of the sugar ring. When the oxocarbenium is formed, the adjacent carbonyl stabilizes the positive charge (anchimeric assistance), thus shielding one of the faces of the

cation. Attack of the nuclefile occurs *trans* to the 2-substituent, giving α-glycosides for *manno* sugar acceptors (**Figure 10**), or β-glycosides for *gluco/galacto* sugars.

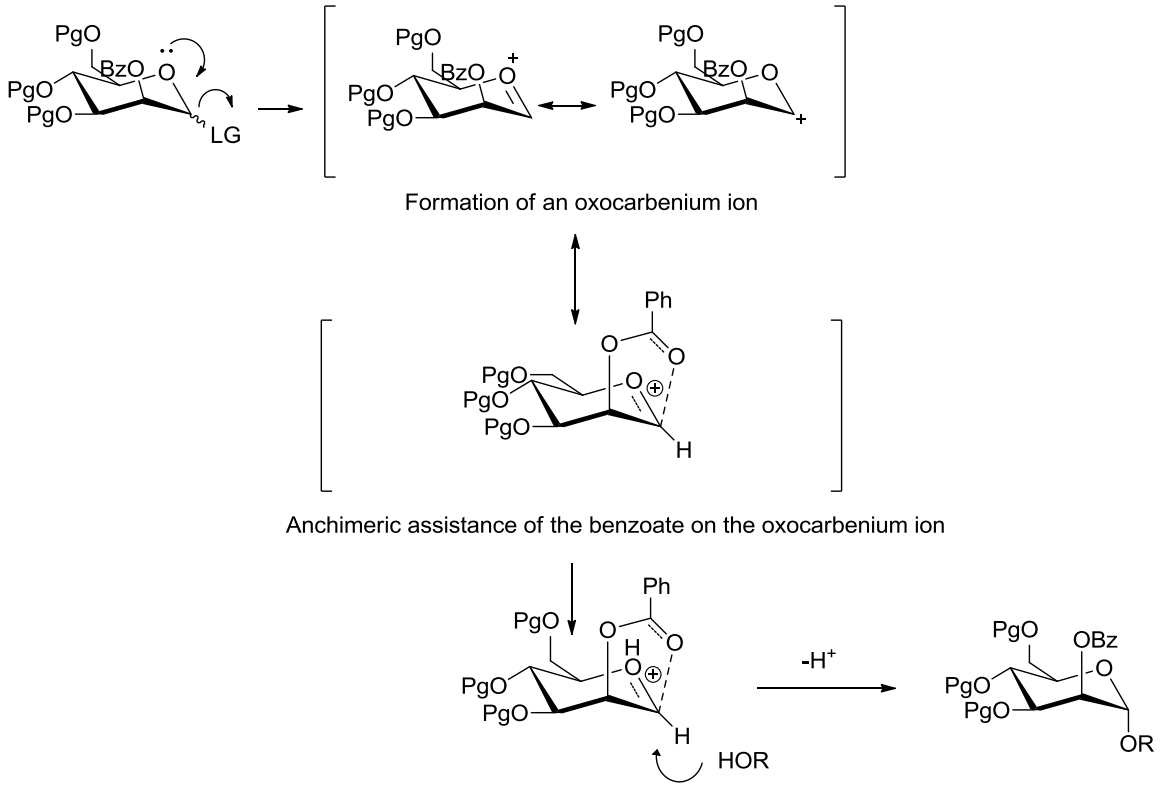


Figure 10: Mechanism of glycosylation of D-mannose 2-benzoate

1. 4. Antagonists of D-mannose based receptors

Previously designed FimH antagonists (**Figure 11**) followed a glycomimetic design, that is, a monosaccharide core with non-glycon moiety attached. The monosaccharide selectively anchors the ligand to the binding site and the non-glycon moiety enhances binding affinity (11).

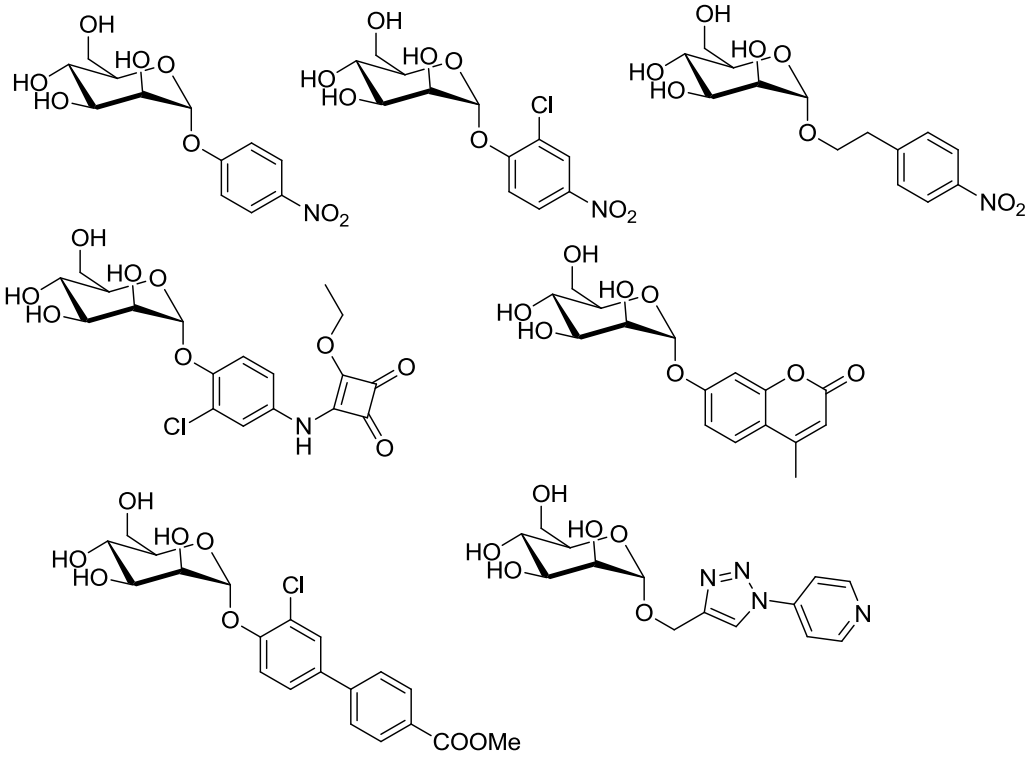


Figure 11: FimH antagonists

(Adjusted from 26-32)

DC-SIGN antagonists which were reported thus far have been 2-C-substituted branched D-mannose analogues (**figure 12**) (33), pseudo-1,2-mannobiosides which have the second D-mannose unit replaced by a cyclohexanediol that is conformationally constrained (**figure 13**) (34) and pseudo-trisaccharides by replacing the middle mannose unit with the cyclohexanediol (**figure 14**) (35). **Figure 15** depicts a few mannose-based glycoconjugates designed by Obermajer et al. (36) that are also a basis for some of the compounds presented in this thesis.

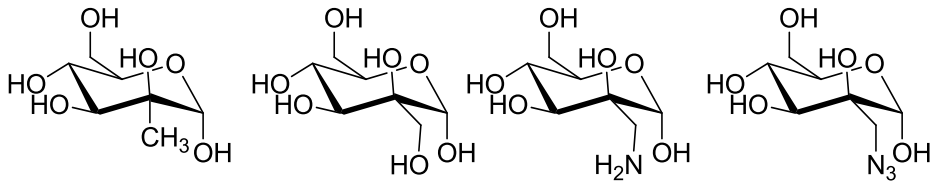


Figure 12: 2-C-substituted D-mannose analogues

(Adjusted from 33)

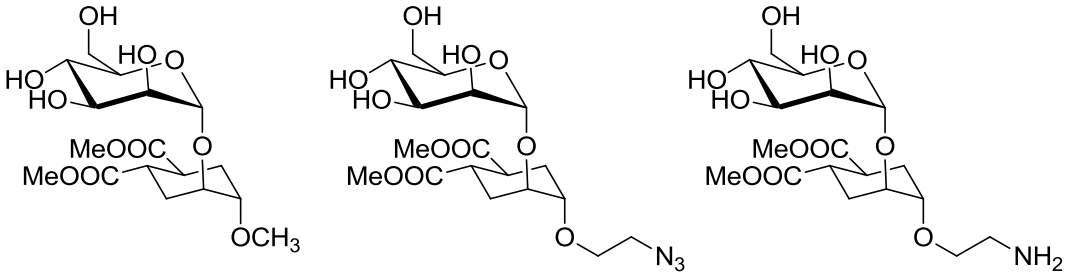


Figure 13: Pseudo-1,2-mannobiosides

(Adjusted from 34)

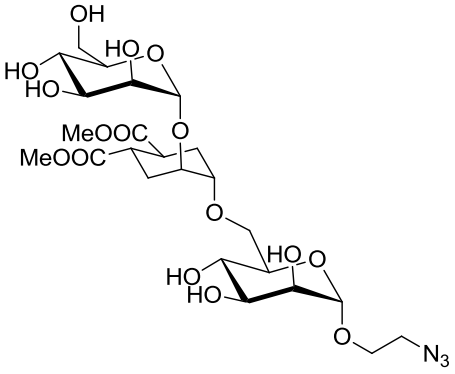


Figure 14: Pseudo-trisaccharides

(Adjusted from 35)

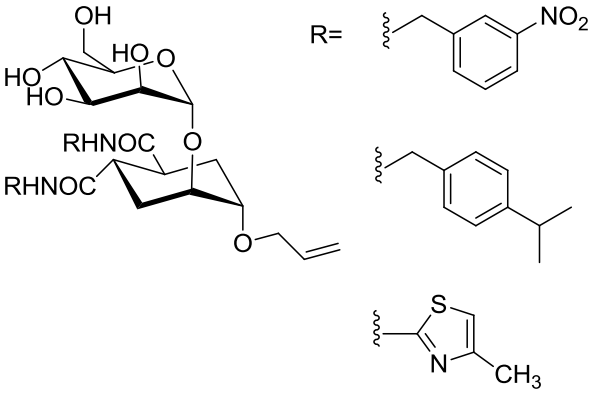


Figure 15: Mannose-based glycoconjugates

(Adjusted from 36)

2. OBJECTIVES

The goal of the experimental work is the design and synthesis of α-D-mannosyl glycoconjugates aimed to target mannose-binding lectins. Experimental work will be divided into two parts; one will be performed at the Department of pharmaceutical chemistry at the Faculty of pharmacy at the University of Ljubljana under the supervision of Assoc. Prof. Dr. Marko Anderluh, and the other at the Department of organic and industrial chemistry at the University of Milan under the supervision of Prof. Dr. Anna Bernardi.

In Ljubljana α-D-mannose will be used as a basic building block for the attachment of fragments with the intention of increasing affinity to targeting lectins (DC-SIGN, FimH). Previously known antagonists of mannose based lectins followed the plan consisting of a core monosaccharide - α-D-mannose, and an aglycon structure attached for hydrophobic interactions (**Figure 18**).

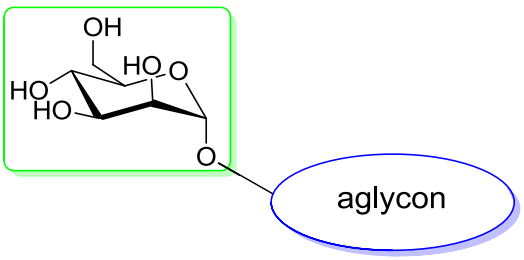


Figure 16: General structure of previously known antagonists

We will try to construct antagonists with an additional hydrophobic group attached, to form a sort of T-shaped structure which would better accommodate into lipophilic pockets that surround the mannose binding site (**Figure 19**).

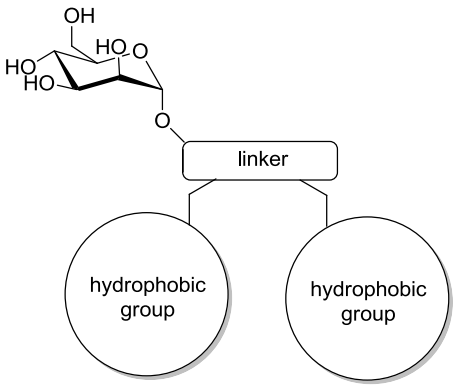


Figure 17: General structure of synthesised antagonists

As a linker we will use either 1,3-aminopropan-2-ol or glycerol, and hydrophobic groups will consist of mostly aryls. Final compounds will be tested on their affinity for target lectins (FimH and DC-SIGN) and so their therapeutic potential will be evaluated. Below two general procedures of forming glycoconjugates are depicted, one with the 1,3-aminopropan-2-ol (**Figure 20**) and the other with the glycerol (**Figure 21**) linker. Reaction conditions are described in the picture.

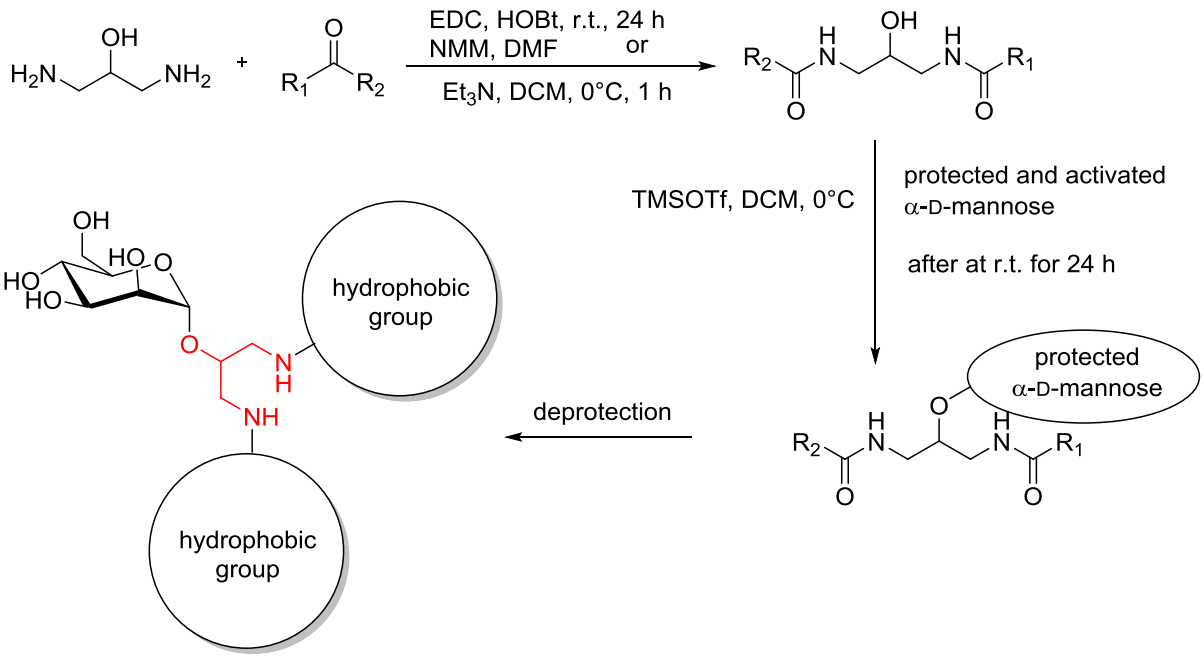


Figure 18: Scheme of the general procedure of forming glycoconjugates with the 1,3-aminopropan-2-ol linker

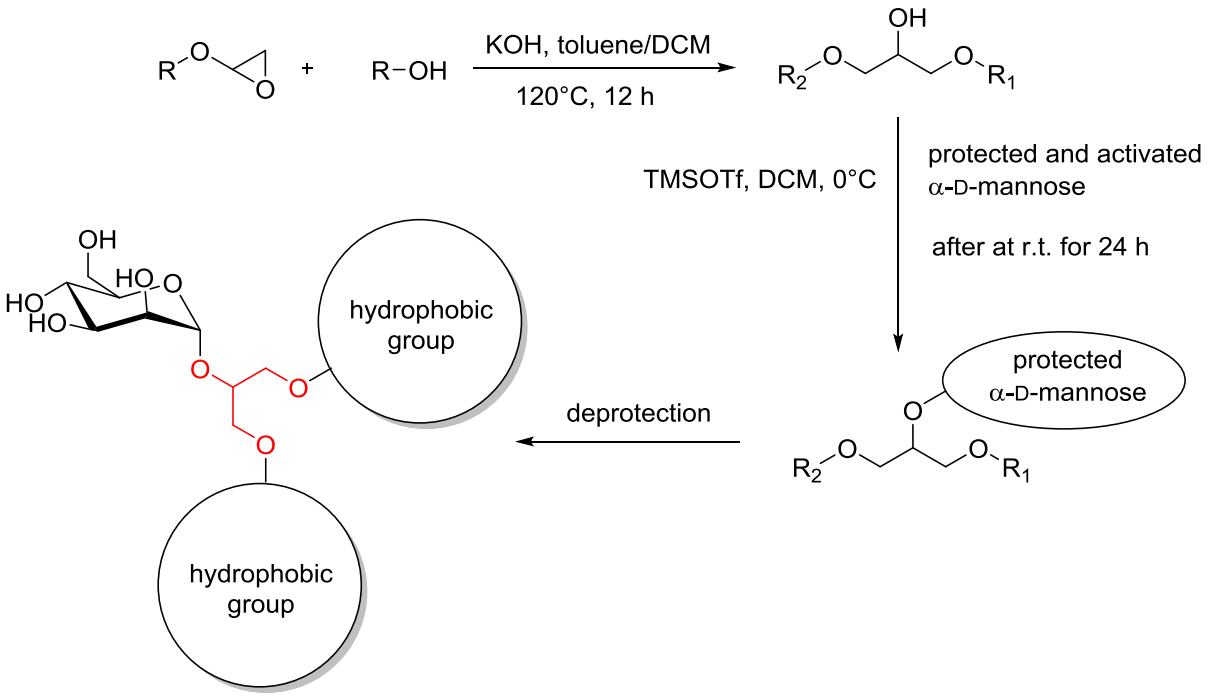


Figure 19: Scheme of the general procedure of forming glycoconjugates with the glycerol linker

The second part of experimental work will be performed at the Department of organic and industrial chemistry at the University of Milan under the supervision of Prof. Dr. Anna Bernardi. Our major aim will be the synthesis of a library of pseudo-mannosides, which will be tested for their specificity for different lectins in a microarray format by the host laboratory. Synthetic plan is depicted in **Figure 22** and **23**. The first part will be devoted to the synthesis of the pseudo-dimannoside scaffold, and the second to functionalizing the pseudo-dimannoside with different amines in an attempt to form modified mannosides.

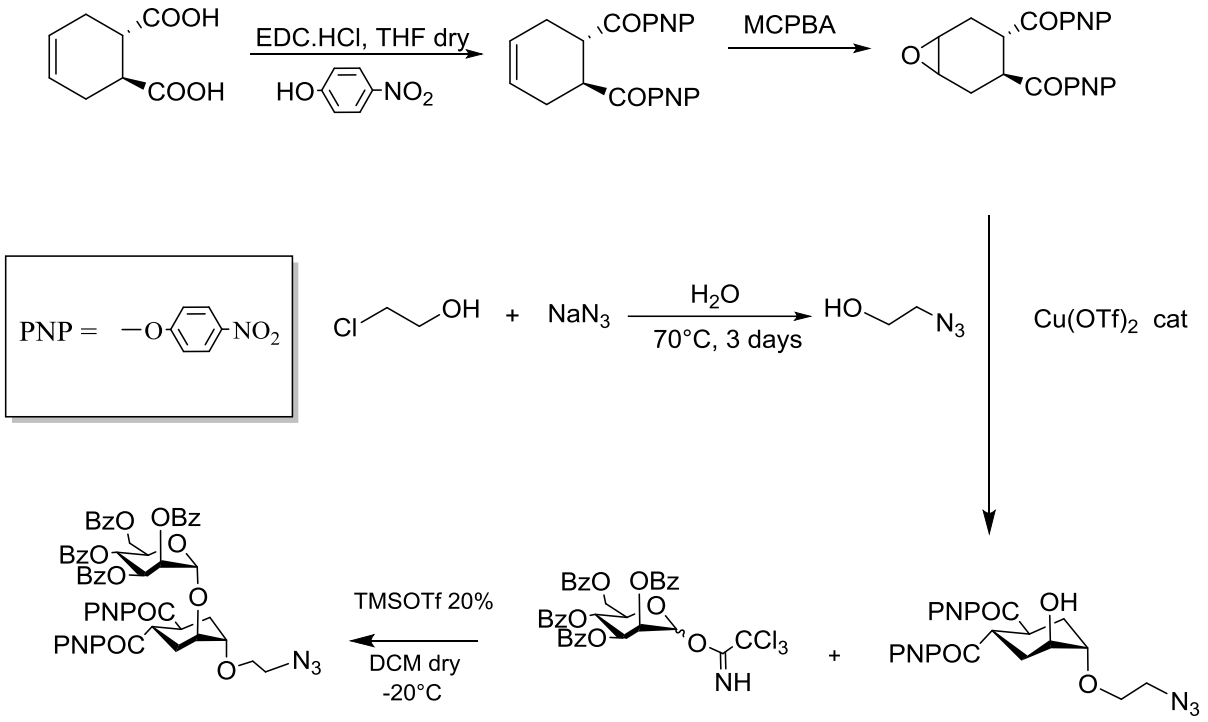


Figure 20: Synthesis of the pseudo-dimannoside scaffold

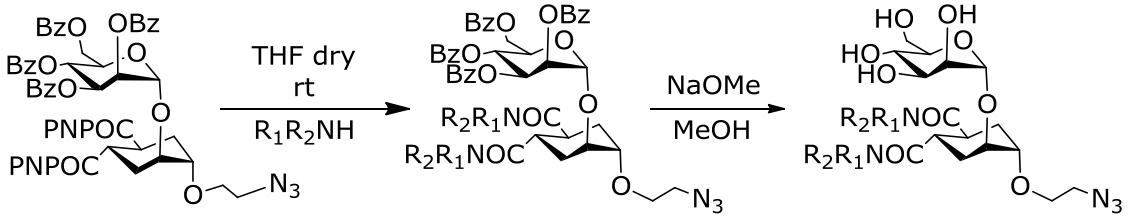
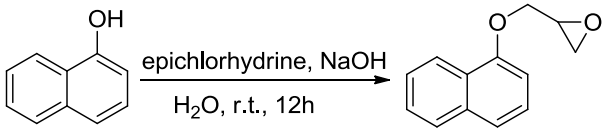


Figure 21: Functionalization of the pseudo-dimannoside with amines to obtain suitable amides.

3. EXPERIMENTAL WORK

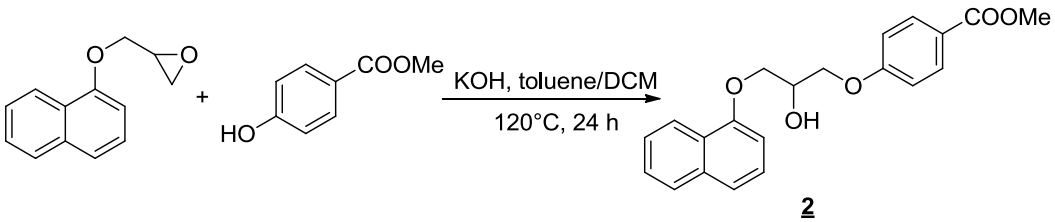
3. 1. REACTION SCHEMES

3. 1. 1. Synthesis of 2-((naphthalen-1-yloxy)methyl)oxirane (bimolecular nucleophilic substitution)



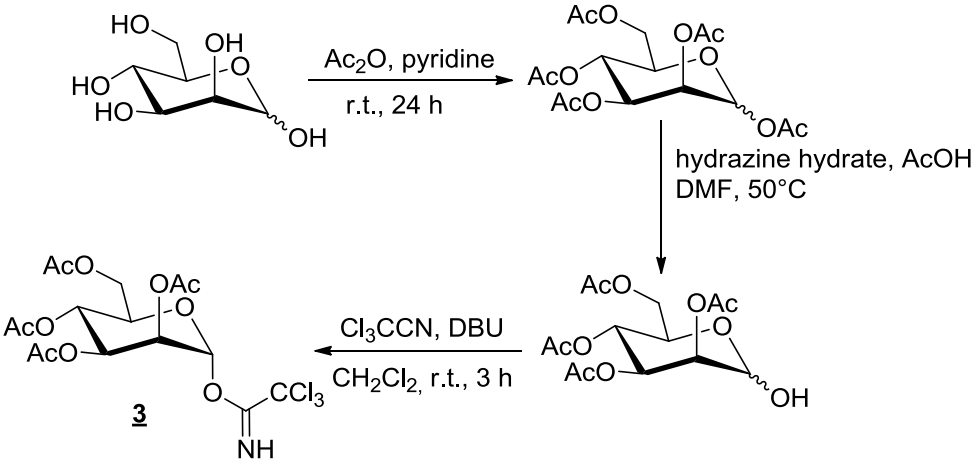
1

3. 1. 2. Synthesis of 1-(4-methoxycarbonyl)phenoxy)-3-(naphtalen-1-yloxy)propan-2-ol (nucleophilic attack of the alcohol on the activated oxirane)



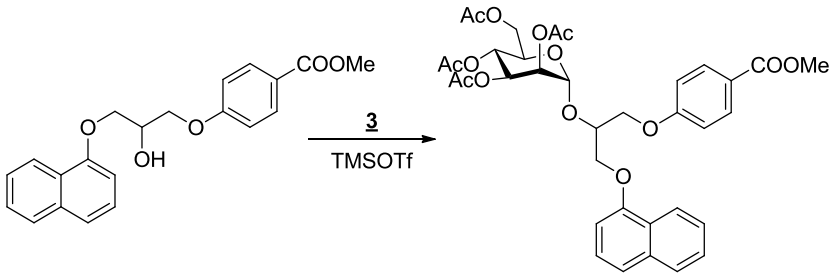
2

3. 1. 3. Synthesis of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (per-*O*-acetylation, selective removal of the acetal on the anomeric hydroxyl group, activation of the acetylated mannose to obtain alpha conformation only)



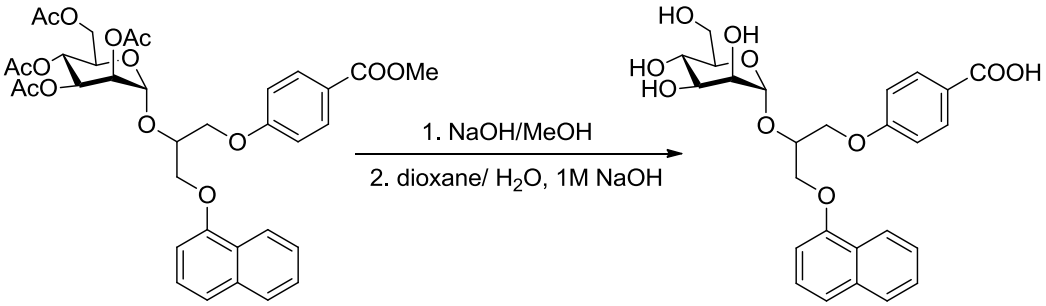
3

3. 1. 4. Synthesis of 1-(4-methoxycarbonyl)phenoxy-3-(naphtalen-1-yloxy)propan-2-yl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (glycosylation with TMSOTf)



4

3. 1. 5. Synthesis of 1-(4-methoxycarbonyl)phenoxy)-3-(naphtalen-1-yloxy)propan-2-yl α-D-mannopyranoside (deprotection with Zemplén method)

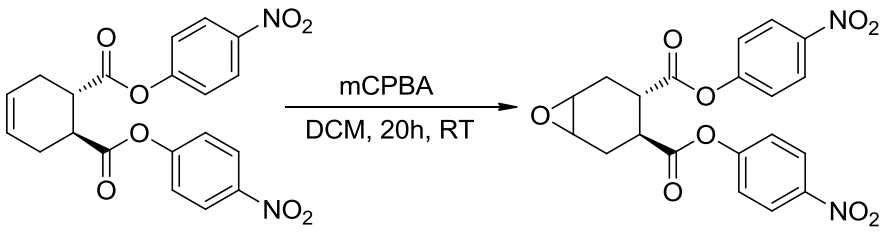


5

3. 1. 6. Synthesis of 4-cyclohexene-1,2-dicarboxylic acid bis(4-nitro)phenylester (formation of the activated ester)

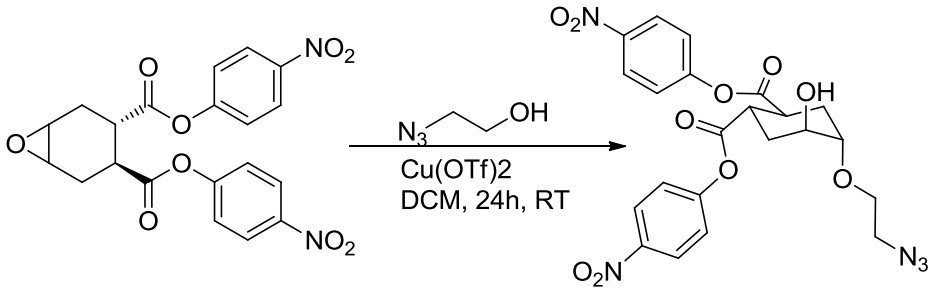
6

3. 1. 7. Synthesis of 7-oxabicyclo(4.1.0]heptane-3,4-dicarboxylic acid bis(4-nitro)phenylester (formation of the epoxide)



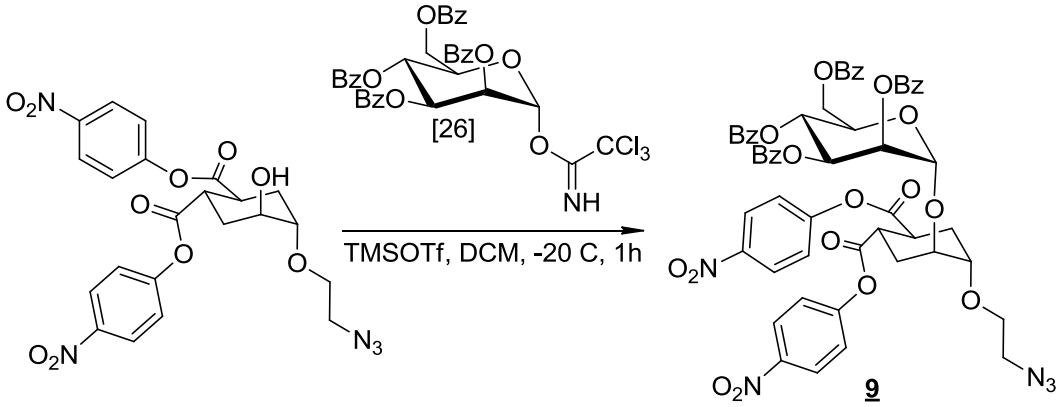
7

3. 1. 8. Sythensis of 1,2-cyclohexanedicarboxylic acid, 4-hydroxy-5-(2-azidoethoxy)-1,2-bis(4-nitro)phenyl ester (opening of the epoxide using 2-azidoethanol)



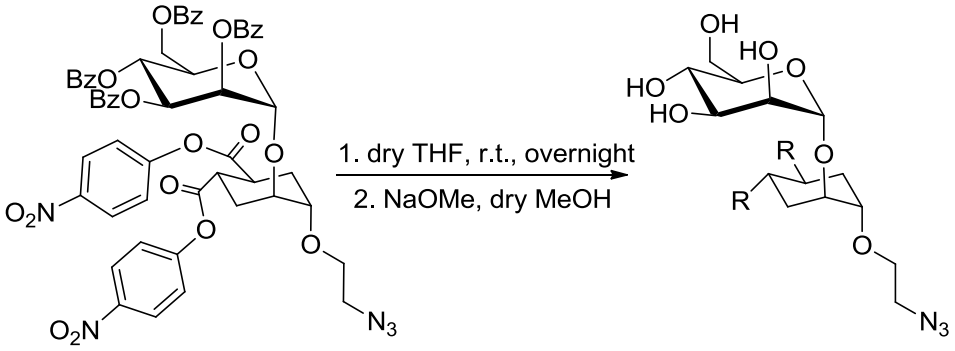
8

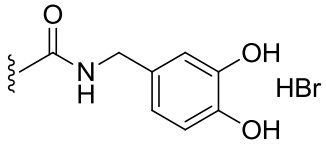
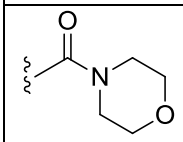
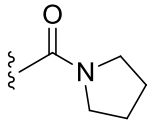
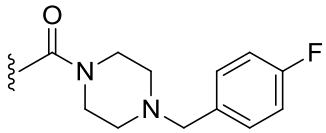
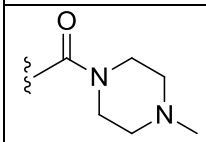
3. 1. 9. Synthesis of 1,2-cyclohexanedicarboxylic acid, 4-(2-azidoethoxy)-5-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)oxy]-, 1,2-bis(4-nitro)phenyl ester, (1S,2S,4S, 5S)(glycosylation with TMSOTf)



9

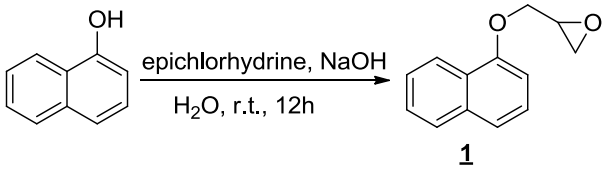
3. 1. 10. Synthesis of amide derivates and deprotection of benzoylated sugar



R	compound
	**16**
	**17**
	**18**
	**19**
	**20**

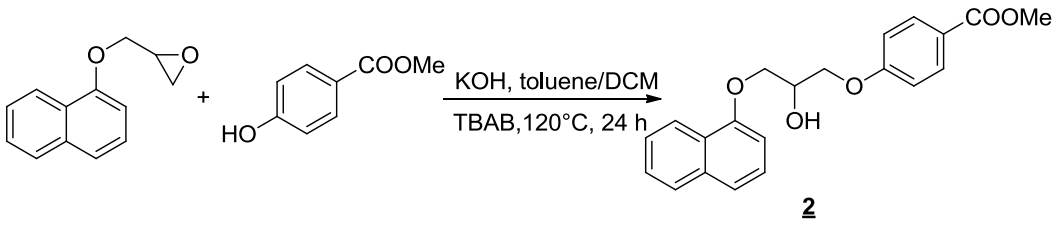
3. 2. SYNTHESIS PROCEDURES AND ANALYTICAL RESULTS

3. 2. 1. Synthesis of 1-(4-methoxycarbonyl)phenoxy)-3-(naphtalen-1-yloxy)propan-2-ol



1

1-naphthol was suspended in a solution of NaOH in water. Epichlorohydrine was added in drops while stirring and the reaction was left at room temperature for 12 hours. The reaction mixture was extracted with diethyleter, the organic phase washed successively with 1M NaOH, dried over sodium sulphate, filtered and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography using ethyl acetate/ petroleum ether (1:3) as eluent. Compound **1** was used in the next step without full characterization.



2

Potassium hydroxide (0.299 g, 5.329 mmol, 1eq) was added to a solution of methyl 4-hydroxybenzoate (0.811 g, 5.329 mmol, 1eq) in 15 mL of MeOH and stirred for 10 minutes. Then MeOH was co-evaporated three times with toluen to insure a dry mixture. The dry mixture, 2-((naphthalen-1-yloxy)methyl)oxirane (1.067 g, 5.329 mmol, 1 eq) and tetrabutylammoniumbromide (0.344 g, 1.066 mmol, 0.2 eq) were added into 20 mL of toluene and 1 mL of dichloromethane. The reaction ran for 24 h at 120°C, then left to cool to room temperature and the solvent evaporated at reduced pressure. The crude was purified by flash column chromatography using ethyl acetate with gradient of hexane from 30% to 50% as eluent.

Description: yellow oil

η: 69.6 % (1.307 g)

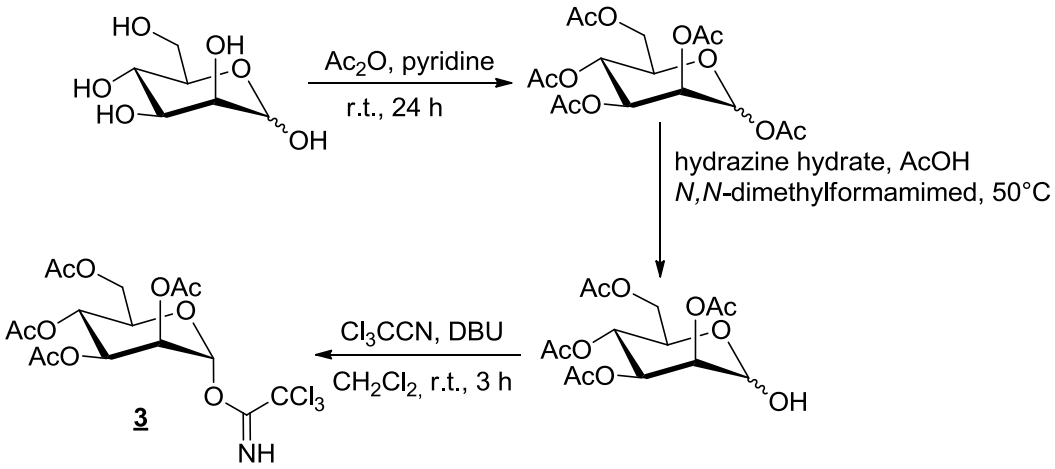
^{1}H NMR (400MHz, DMSO-d_6): δ = 3.36 (s, 4H, 2 x CH_2), 3.80 (s, 3H, CH_3), 4.51-4.63 (m, 1H, CH), 5.59 (dd, 1H, OH, *J*=4.4 Hz, *J*=6.2 Hz), 6.97-7.00 (m, 1H, Ar-H), 7.08-7.12 (m, 1H, Ar-H), 7.38-7.53 (m, 5H, Ar-H), 7.83-7.94 (m, 4H, Ar-H) ppm.

^{13}C NMR (100MHz, DMSO-d_6): δ= 51.8 (CH_3); 67.3 (CH); 69.3 (CH_2); 69.6 (CH_2); 105.2, 114.5 (3 x Ar-C); 120.0 (Ar-C-C=O); 125.2, 131.2, 134.0 (10 x Ar-C); 153.9 (Ar-C-O); 162.4 (Ar-C-O); 165.8 (C=O) ppm.

IR (KBr): ν = 3432, 2947, 2364, 1718, 1605, 1579, 1509, 1436, 1399, 1256, 1170, 1103, 1035, 846, 793, 769, 696, 571 cm^{-1}.

HRMS *m/z* for $C_{21}H_{20}O_5$ ([M+H^+]) calculated: 353.1389, found: 353.1388.

3. 2. 2. 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate



Acetic anhydride (20 mL, 0.21 mol) was added drop-wise to a stirred solution of D-mannose (4.981 g, 41.5 mmol) in anhydrous pyridine (25 mL) at 0°C under argon. The mixture was allowed to warm to room temperature and stirred overnight. Ethyl acetate (100 mL) was added and the organic phase successively washed with saturated aqueous $NaHCO_3$ solution (2 x 80 mL), 1 M HCl (2 x 80 mL) and brine (80 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent evaporated under reduced pressure. The compound was obtained as a mixture of α and β anomer.

To a solution of hydrazine hydrate (5.03 mL, 56.2 mmol) in *N*,*N*-dimethylformamide (20 mL) acetic acid was added (3.75 mL, 65.5 mmol). A solution of 1,2,3,4,6-penta-*O*-actyl-D-mannopyranose (18.277 g, 46.9 mmol) in *N*,*N*-dimethylformamide (10 mL) was added and the reaction mixture stirred at 50°C overnight. The mixture was concentrated *in vacuo*, then water (70 mL) was added and extracted with ethyl acetate (3 x 100 mL). The organic phase was washed successively with 1 M HCl (3 x 100 mL), saturated aqueous $NaHCO_3$ solution (2 x 100 mL) and brine (100 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure.

To a stirred solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose (3.600 g, 12.8 mmol) in dry dichloromethane (50 mL) were added trichloroacetonitrile (15.4 mL, 0.15 mmol) and DBU (0.534 mL, 3.58 mmol). The reaction mixture was stirred for 3 h, at room temperature. The crude product was then concentrated *in vacuo* and purified with flash column chromatography using ethyl acetate/hexane (1:3) as eluent (37).

Description: yellow oil

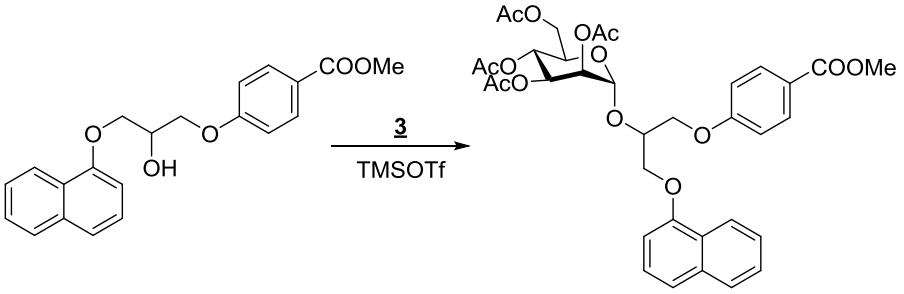
η: 27.7% (3.518 g)

^{1}H NMR (400MHz, $CDCl_3$): δ = 2.01 (s, 3H, $COCH_3$), 2.07 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.20 (s, 3H, $COCH_3$), 4.15-4.22 (m, 2H, H_5, H_6), 4.28 (dd, 1H, $H_{6'}$, $J_{5\text{-}6'}$ = 4.7 Hz, $J_{6\text{-}6'}$ = 12.0 Hz), 5.40-5.42 (m, 2H, H_3, H_4), 5.47-5.48 (m, 1H, H_2), 6.29 (d, 1H, H_1, $J_{1\text{-}2}$ = 1.8 Hz), 8.79 (s, 1H, NH) ppm.

$(\alpha]_D^{20}$ = +9.3 (c = 0.26 in methanol)

IR (NaCl): ν = 3321, 2941, 1744, 1678, 1637, 1534, 1434, 1368, 1211, 1155, 1078, 1043, 973, 938, 835, 795, 643, 599 cm^{-1}.

3. 2. 3. Synthesis of 1-(4-methoxycarbonyl)phenoxy-3-(naphtalen-1-yloxy)propan-2-yl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside



4

To a solution of **2** (0.437 g, 1.240 mmol, 1 eq) and **3** (0.611g, 1.240 mmol, 1 eq) in dry dichloromethane (10 mL) under argon, trimethylsilyl trifluoromethanesulfonate (TMSOTf) (0.225 mL, 1.240 mmol, 1 eq) was added at 0°C. After stirring at 0°C for 30 minutes the reaction was allowed to warm to room temperature and stirred overnight. Et_3N (0.25 mL) was then added, the solvent removed under reduced pressure and crude product purified by flash column chromatography using ethyl acetate with gradient of hexane from 30% to 50% eluent (37).

Description: yellow oil

η: 38.9% (0.329 g)

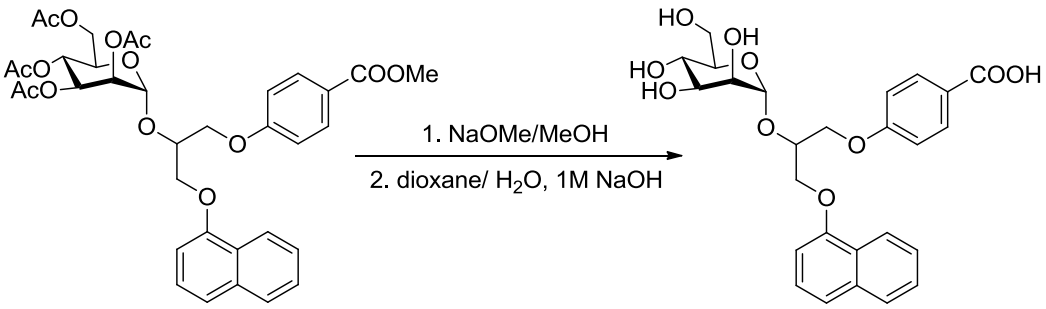
^{1}H NMR (400MHz, DMSO-d$_6$): δ = 1.99 (s, 4H, $COCH_3$), 3.34 (s, 8H, $COCH_3$), 3.80-3.82 (m, 3H, CH_3), 3.98-4.05 (m, 2H, Ar-O-CH_2-CH), 4.11-4.18 (m, 2H, Ar-O-CH_2-CH), 4.22-4.30 (m, 1H, H_5), , 4.39-4.58 (m, 3H, $H_{1'}$, H_6), 5.05-5.22 (m, 3H, H_2, H_3, H_4), 5.34* (d, 1/2H, H_1, *J*=4.86 Hz), *5.37 (d, 1-/2H, H_1, *J*=1.64 HZ), 6.98-7.18 (m, 3H, Ar-H), 8.10-8.21 (m, 1H, Ar-H), 7.80-7.98 (m, 3H, Ar-H), 7.36-7.57 (m, 4H, Ar-H) ppm. *each peak belongs to one diastereoisomer.

^{13}C NMR (100MHz, DMSO-d$_6$): δ = 14.0 (CH_3C=O); 20.3 (CH_3C=O); 20.4 (CH_3C=O); 20.5 (CH_3C=O); 51.8 (OCH_3); 59.7 (CHCH_2OAc); 65.2 (C_6); 67.0 (C_4); 68.7 (C_2); 73.6 (C_3); 73.9 (1 CH_2);96.0 (1 CH_2);96.3 (CH_2CHOCH_2);105.3 (C_1); 105.5, 114.5, 120.5, 121.4, 124.8, 126.1, 126.5, 131.2, 131.4 , 153.4 , 153.5 , 162.0 , 162.1 , 162.9(Ar-C); 165.8, 169.3 (Ar-CO); 169.5 (C=O); 169.6, 169.6, 169.8, 170.0 (OCOCH$_3$) ppm.

IR (KBr): ν = 3448, 2954, 2364, 1752, 1606, 1581, 1509, 1438, 1371, 1241, 1171, 1137, 1104, 1046, 848, 794, 771, 736, 695, 600, 497 cm^{-1}.

HRMS *m/z* for $C_{35}H_{38}O_{14}$([M+H^+]) calculated: 683.2340, found: 683.2338.

3. 2. 4. Synthesis of 1-(4-methoxycarbonyl)phenoxy)-3-(naphtalen-1-yloxy)propan-2-yl α-D-mannopyranoside



5

The protected mannopyranoside (0.379 g, 0.555 mmol, 1 eq) was dissolved in dry methanol and sodium methoxide solution in methanol was added. The reaction mixture was stirred at room temperature for 30 minutes and then the acidic ion exchange resin Amberlite® IR120 H was added for neutralization. After stirring the mixture for 10 minutes, the resin was filtered off, washed with methanol and the solvent removed *in vacuo* (37).

The deprotected mannopyranoside (0.200 g, 0.389 mmol, 1 eq) was dissolved in a 1:1 mixture of dioxane and water (5 mL), and 1M NaOH (1.199 mL, 1.199 mmol, 3 eq) was added to the solution. The reaction mixture was left stirring overnight at room temperature. After the reaction was completed, the solvent was evaporated under reduced pressure and the reaction crude purified by automatic flash chromatography using methanol with gradient of water with 0.1% of trifluoroacetic acid from 40% to 100% as an eluent.

Description: yellow oil that solidifies in time

η: 37.5% (0.104 g)

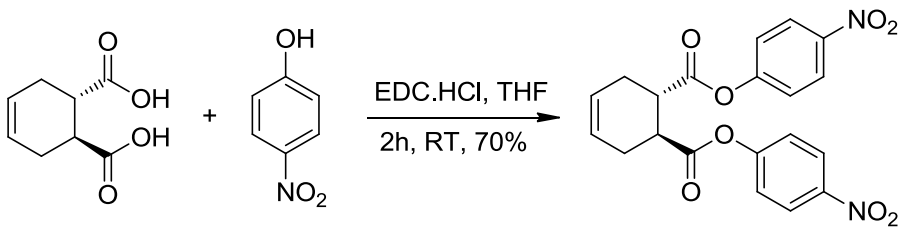
^{1}H NMR (400MHz, CD_3OD): δ = 3.91-3.62 (m, 6H, H_2, H_3, H_4, H_5, H_6), 4.76-4.64 (m, 1H, $H_{1'}$), 4.53-4.42 (m, 4H, 2 x Ar-O-CH_2-CH), 5.22* (d, 1H, H_1, *J*=1.7 Hz), *5.28 (d, 1H, H_1, *J*=1.7 Hz), 7.01-6.98 (m, 1H, Ar-H), 7.11-7.07 (m, 2H, Ar-H), 7.51-7.38 (m, 4H, $H_{3'}$, Ar-H), 7.83-7.80 (m, 1H, Ar-H), 8.02-7.98 (m, 2H, $H_{4'}$), 8.27-8.23 (m, 1H, Ar-H) ppm. *each peak belongs to one diastereoisomer.

^{13}C NMR (100MHz, CD_3OD): δ = 61.3, 61.5 (C6, C6′); 66.9, 67.0($\underline{C}H_2CHCH_2$,$\underline{C}H_2CHCH_2$′), 2x67.6 ($CH_2CH\underline{C}H_2$, $CH_2CH\underline{C}H_2$′), 2x67.8 (C4, C4′), 2x 70.9 (C3, C3′), 2x 71.0 (C2, C2′); 73.7, 73.8 (CH, CH′); 74.1, 74.2, (C5, C5′); 100.4, 100.5 (C1, C1′); 104.7, 104.8 (Ar-C, Ar-C′); 2x 114.0 (Ar-C2 in C6, Ar-C2′ in C6′); 120.2, 120.3 (Ar-C, Ar-C′); 121.4, 121.5 (Ar-C4, Ar-C4′); 123.0 (Ar-C), 2x 124.9 (Ar-C, Ar-C′); 2x 125.5 (Ar-C, Ar-C′); 2x 125.6 (Ar-C, Ar-C′); 2x 126.0 (Ar-C, Ar-C′); 127.1, 127.2 (Ar-C, Ar-C′); 2x131.5 (Ar-C3 in C5, Ar-C3′ in C5′); 134.6, 134.7 (Ar-C, Ar-C′); 154.1, 154.3 (Ar-C, Ar-C′); 162.7 (Ar-C1);168.3 (C=O) ppm.

IR (KBr): ν = 3422, 3061, 2930, 1685, 1606, 1580, 1509, 1459, 1398, 1240, 1171, 1132, 1103, 1053, 1021, 978, 847, 793, 736 cm^{-1}.

HRMS (ESI-) *m/z* for $C_{26}H_{27}O_{10}$ ($[M-H]^-$) calculated: 499.1604, found: 499.1603.

3. 2. 5. Synthesis of 4-cyclohexene-1,2-dicarboxylic acid bis(4-nitro)phenylester



6

4-cyclohexene-1,2-dicarboxylic acid (1 g, 5.877 mmol, 1 eq) was dissolved in dry THF (58.77 mL) and EDC (3.943 g, 20.57 mmol, 3.5 eq) was added under stirring. After 10 minutes p-nitrophenol (2.453 g, 17.63 mmol, 3 eq) was added and the solution was stirred at room temperature for 2 h. After the reaction was finished, the solvent was evaporated under reduced pressure and the residue dissolved in 55 mL of ethyl acetate, washed with 1M HCl (2 x 40 mL),with saturated solution of$NaHCO_3$ (2 x 40 mL), followed by washing with saturated solution of NaCl (2 x 40 mL). The organic phase was dried over sodium sulphate, filtered and the solvent evaporated under pressure.

Description: pale yellow solid

η: 63.1% (1.529 g)

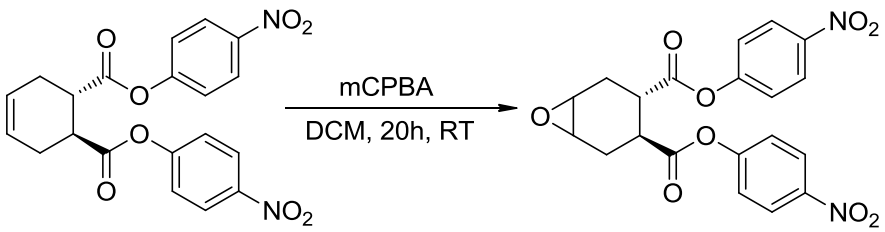
R_f: hexane/ethyl acetate = 6/4, 0.68

^{1}H NMR (400MHz, $CDCl_3$): δ = 2.37-2.51 (m, 2H, CH_2 axial), 2.74-2.80 (m, 2H,CH_2 equatorial), 3.23-3.31 (m, 2H, 2 x CH_2-C<u>H</u>-COO),5.84-5.90 (m, 2H, C<u>H</u>=C<u>H</u>), 7.27-7.31 (m, 4H, Ar-H), 8.27-8.31 (m, 4H, Ar-H) ppm.

^{13}C NMR (100MHz, $CDCl_3$): δ = 28.0 (2 x CH_2); 41.5 (2 x CH_2-<u>C</u>H-COO); 122.5 (Ar-C); 124.9 (<u>C</u>H=<u>C</u>H); 125.5 (Ar-C); 145.7 (Ar-C); 155.4 (Ar-C); 172.8 (C=O) ppm.

$(\alpha]_D^{20}$ = + 129.6 (c = 1 in chloroform)

3. 2. 6. Synthesis of 7-oxabicyclo(4.1.0]heptane-3,4-dicarboxylic acid bis(4-nitro)phenylester



7

Compound **6** (1.529 g, 3.708 mmol, 1 eq) was dissolved in dry dichloromethane, after mCPBA (0.640 g, 3.708 mmol, 1 eq) was added. The reaction was stirred under nitrogen at room temperature for 16h, after which the solvent was evaporated under reduced pressure and the reaction mixture diluted with 30 mL of ethyl acetate. The mixture was washed with 20 mL of $NaHCO_{3(aq)}$ for 3 times and with 20 mL of saturated $NaCl_{(aq)}$ for another 3 times. The organic phases were collected and dried over sodium sulphate and the solvent evaporated under reduced pressure. The crude was purified by flash column chromatography using hexane with gradient of ethyl acetate from 30% to 50% eluent.

Description: yellow oil

η: 59.7% (0.948 g)

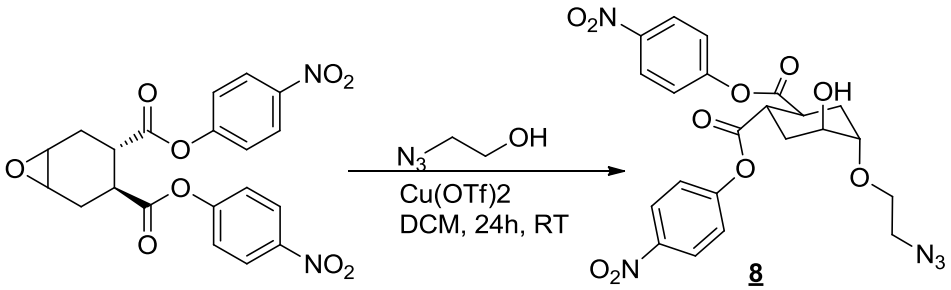
R_f: hexane/ethyl acetate = 6/4, 0.25

^{1}H NMR (400MHz, $CDCl_3$): δ = 2.15-2.22 (m, 1H, CH_2 axial), 2.36-2.42 (dd, 1H, CH-CH_2 axial), 2.57-2.64 (ddd, 1H, CH_2 equatorial), 2,74-2.78 (ddd, 1H, CH_2 equatorial), 3.03-3.10 (td, 1H, CH_2-C<u>H</u>-COO), 3.26-3.33 (td, 1H, CH_2-C<u>H</u>-COO), 3.35 (t, 1H, C<u>H</u>(O)CH, *J*=4.0 Hz), 3.41-3.42 (m, 1H, CH(O)C<u>H</u>), 7.24-7.28 (m, 4H, Ar-H), 8.25-8. 29 (m, 4H, Ar-H) ppm.

^{13}C NMR (100MHz, $CDCl_3$): δ = 26.3, 26.8 (2 x CH_2); 38.1, 40.1 (2 x CH_2-<u>C</u>H-COO); 50.3, 51.8 (<u>C</u>H(O)<u>C</u>H); 122.5, 122.6 (Ar-C); 125.5 (Ar-C); 149.9 (Ar-C); 155.3, 155.4 (Ar-C); 171.5, 172.6 (C=O) ppm.

$(\alpha]_D^{20}$ = +82.2 (c = 1.1 in chloroform)

3. 2. 7. Sythensis of 1,2-cyclohexanedicarboxylic acid, 4-hydroxy-5-(2-azidoethoxy)- 1,2-bis(4-nitro)phenyl ester



8

The epoxide (0.942 g, 2.200 mmol, 1 eq) was added to a solution of azidoethanol (11.108 g, 127.565 mmol, 58 eq) in dichloromethane. $Cu(OTf)_2$ (0.159 g, 0.440 mmol, 0.2 eq) was then added under stirring and the reaction ran under nitrogen for 16 hours. After completion the solvent was dried under reduced pressure and the crude product was purified by flash column chromatography using hexane and ethyl acetate with gradient from 20% to 80% of ethyl acetate.

Description: pale yellow oil

η: 92.1% (1.044 g)

R_f: hexane/ethyl acetate = 6/4; 0.15

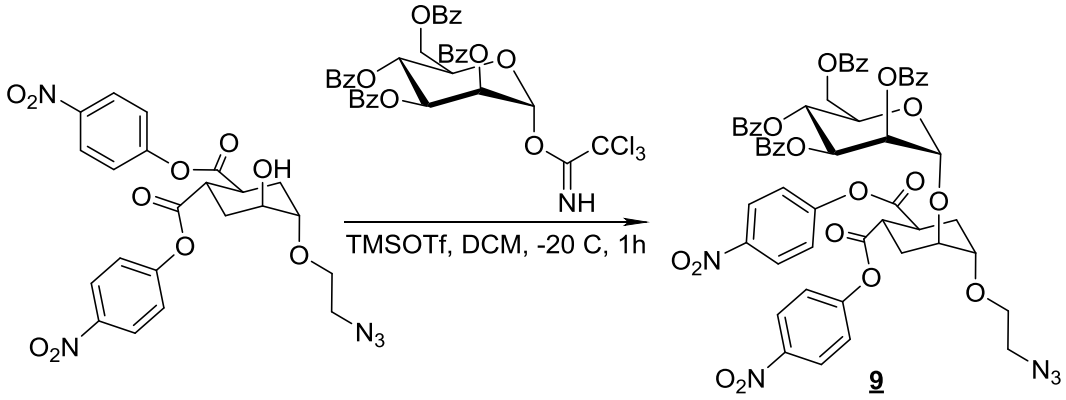
^{1}H NMR (400MHz, $CDCl_3$): δ = 2.14-2.39 (m, 4H, D_3, D_6), 3.34-3.49 (m, 4H, D_4, D_5, CH_2-N_3), 3.68-3.74 (m, 2H, D_1, O-C<u>H</u>$_2$), 3.85-3.90 (ddd, 1H, O-C<u>H</u>$_2$, *J*=3.4 Hz, *J*=6.0 Hz, *J*=9.8 Hz), 4.09-4.19 (m, 1H, D_2), 7.23-7.28 (m, 4H, Ar-H), 8.24-8.27 (m, 4H, Ar-H) ppm.

^{13}C NMR (100MHz, $CDCl_3$): δ = 27.0, 30.5 (C_{D3}, C_{D6}); 39.1, 39.4 (C_{D4}, C_{D5}); 51.1 (CH_2-N_3); 66.3 (C_{D2}); 68.7 (O-CH_2); 76.5 (C_{D1}), 122.6, 122.6 (Ar-C); 125.5 (Ar-C); 145.7, 145.8 (Ar-C-NO_2); 155.5 (Ar-<u>C</u>-O-C=O); 172.6, 172.7 (C=O) ppm.

$(\alpha]_D^{20}$ = +33.8 (c = 1.1 in chloroform)

MS (ESI) calculated for $[C_{22}H_{21}N_3O_{10}Na]^+ = [(M - N_2) + Na]^+$: 512.4; found: 512.0.

3. 2. 8. Synthesis of 1,2-cyclohexanedicarboxylic acid, 4-(2-azidoethoxy)-5-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)oxy] 1,2-bis(4-nitro)phenyl ester, (1S,2S,4S,5S)



9

A mixture of the activated mannose (1.121 g, 1.513 mmol, 1.2 eq) and **8** (0.650 g, 1.261 mmol, 1 eq) was co-evaporated with toluene 3 times. Powdered and activated acid washed 4A molecular sieves were added to the mixture and kept under vacuum for a few hours. The mixture, together with molecular sieves, was then dissolved in dry dichloromethane (12.6 mL). After the mixture was cooled to -20°C, TMSOTf (45.6 μL, 0.252 mmol, 0.2 eq) added and the reaction was stirred under nitrogen at -20°C for 1 hour. After the reaction was finished Et_3N was added and the mixture left under stirring and at -20°C for 15 minutes, then the mixture was left to settle to room temperature. The crude was filtered over theCelite® pad and washed successively with dichloromethane. The solvent was evaporated under reduced pressure and the crude purified by flash column chromatography using hexane with gradient of ethyl acetate from 10% to 80% eluent.

Description: pale yellow oil

η: 65.2% (0.899 g)

R_f: toluene/ethyl acetate = 6/4; 0.45

^{1}H NMR (400MHz, $CDCl_3$): δ = 2.14-2.22 (m, 2H, D_{3ax}, D_{6ax}), 2.47 (d, 2H, D_{3eq}, D_{6eq}), 3.31 (m, 2H, CH_2-N_3), 3.33-3.44 (m, 2H, D_4, D_5), 3.56-3.59 (m, 1H, O-C$\underline{H}_2$), 3.70-3.74 (m, 1H,O-C$\underline{H}_2$), 3.88 (s, 1H, D_1), 4.22 (s, 1H, D_2), 4.44-4.47 (m,1H, H_5), 4.54 (dd, 1H, H_{6a}, J_{6a-5}=5.1Hz, J_{6a-6b}=12.0 Hz), 4.74 (dd, 1H, H_{6b}, J_{6b-5}=2.3 Hz, J_{6a-6b}=12.1 Hz), 5.35 (s, 1H, H_1),

5.77 (s, 1H, H_2), 5.92 (dd, 1H, H_3, J_{3-4}=10.1 Hz, J_{3-2}=2.9 Hz), 6.09 (t, 1H, H_4, J_{4-3}=J_{4-5}=10.0 Hz), 7.26-7.46 (m, 12H, H_{Bz}, Ar-H), 7.51-7.64 (m, 3H, H_{Bz}) 7.82-7.84 (m, 3H, H_{Bz}), 7.95-8.00 (m, 2H, H_{Bz}), 8.02-8.13 (m, 4H, H_{Bz}), 8.25-8.30 (m, 4H, Ar-H) ppm.

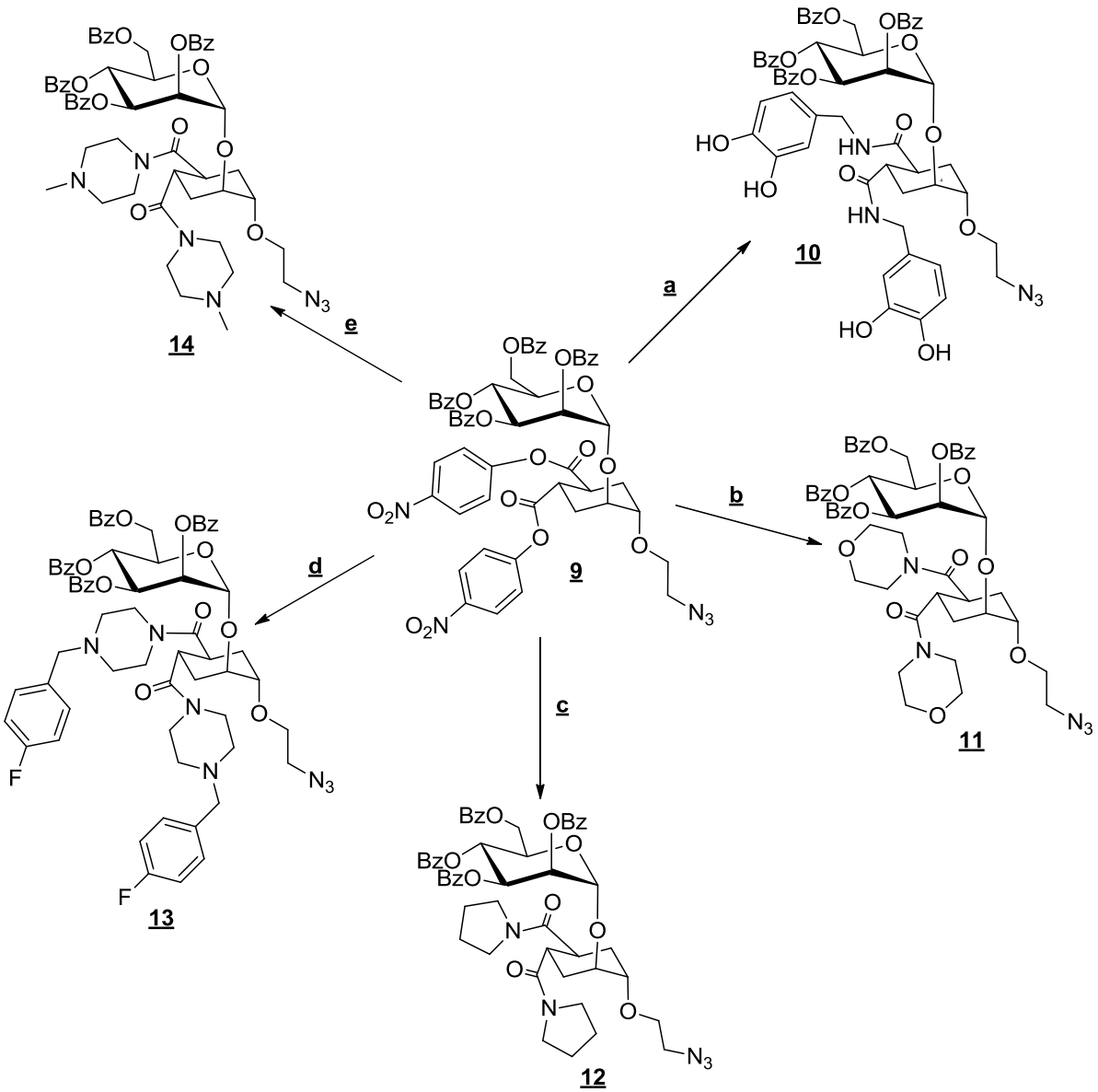
^{13}C NMR (100MHz, $CDCl_3$): δ = 27.0 (C_{D6}); 27.8 (C_{D3}); 39.2, 39.4 (C_{D4}, C_{D5}); 51.0 (CH_2-N_3); 63.5 (C_6); 67.4 (C_4); 68.7 (O-CH_2); 69.9 (C_3); 70.1 (C_5); 70.9 (C_2); 74.9 (C_{D2}); 96.8 (C_1); 122.6, 122.7 (Ar-C); 125.4, 125.5 (Ar-C); 128.4, 128.9, 128.7, 128.9 (C_{Bz}); 129.0, 129.1, 129.2, 129.3 ($Cquat_{Bz}$); 3x129.9, 2x130.1 (C_{Bz}); 133.5, 133.6, 133.8, 133.9 (C_{Bz}); 145.8 (Ar-C-NO_2); 155.4, 155.5 (Ar-C-COO); 165.7, 165.8, 166.3 (CO_{Bz}); 172.2, 172.3 (C=O) ppm.

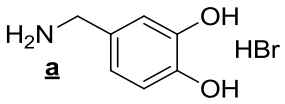
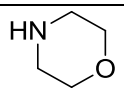
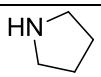
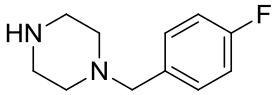
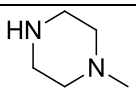
$(\alpha]_D^{20}$ = -18.0 (c = 0.5 in chloroform)

MS (ESI) calculated for $[C_{56}H_{47}N_5O_{19}Na]^+$=$[(M - N_2) + Na]^+$: 1117.0; found 1116.1.

3. 2. 9. General procedure for the synthesis of amides

Compound **9** (60 mg, 0.055 mmol, 1 Eq) was dissolved in dry tetrahydrofurane (c = 0.1 M) after which different reagents (**a** – **e**) were added to the solution. The reaction ran over night at 25°C. The solvent was evaporated under reduced pressure and the crude residue put under vacuum for an hour. The residue was dissolved in dichloromethane, applied to a samplet for the automatic chromatography and left to dry over night after which it was purified by flash column chromatography.



	reagent	amount	n (mmol]	Eq	No°
1	 **a**	36.20 mg	0.165	3	**10**
2	 **b**	14.3 µL	0.165	3	**11**
3	 **c**	13.5µL	0.165	3	**12**
4	 **d**	32 mg	0.165	3	**13**
5	 **e**	18 µL	0.165	3	**14**

1: 45.8 µL of Et_3N (0.329 mmol, 6 eq) was added to the solution because the reaction does not run if basic conditions are not ensured; the crude product was purified by flash column chromatography using chloroform with gradient of methanol from 0% to 16% as an eluent.

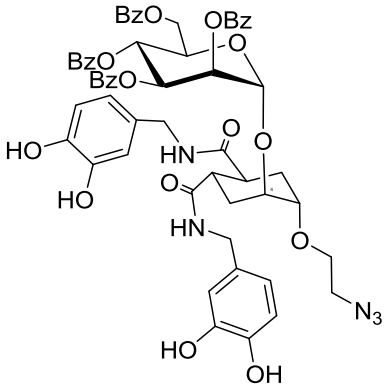
2: The crude product was purified by flash column chromatography using chloroform with gradient of methanol from 0% to 5% as an eluent.

3: The crude product was purified by flash column chromatography using chloroform with gradient of methanol from 0% to 8% as an eluent.

4: The crude product was purified by flash column chromatography using chloroform with gradient of methanol from 1% to 10% as an eluent.

5: The crude product was purified by flash column chromatography using chloroform with gradient of methanol from 2% to 20% as an eluent.

3. 2. 10.: α(1,2)pseudomannobioside tetrabenzoylated bis-3,4-dihydroxybenzyl amide



10

Description: white solid

η: 32% (18.7 mg)

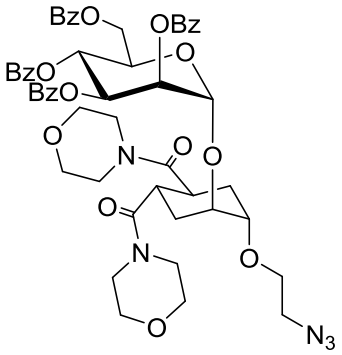
R_f: hexane/ethyl acetate = 6/4 + treated with molibden and ninhydrine solution (for monitoring the starting material) and chloroform/methanol/acetic acid = 9.2/0.8/0.1 + treated with molibden and ninhydrine solution; 0.61

^{1}H NMR (400MHz, $CDCl_3$): δ = 1.97-2.29 (m, 4H, D3, D6), 3.00 (t, 1H, D_4/D_5, *J*=11.8 Hz), 3.15-3.30 (m, 3H, CH_2-N_3, D_4/D_5), 3.51 (dd, 1H, O-C$\underline{H}_2$, *J*=4.6 Hz, *J*=28.9 Hz),3.75 (s, 1H, D_1/D_2),4.12 (dd, 5H, Ar-H, D_1/D_2, *J*=15.4 Hz, *J*=30.7 Hz),4.49 (t, 2H, H_{6a}, H_5, *J*=9.0 Hz),4.73 (d, 1H, H_{6b}, *J*=10.1 Hz),5.27 (s, 1H, H_1),5.77 (s, 1H, H_2),5.96 (d, 1H, H_3, *J*=10.0 Hz),6.12 (t, 1H, H_4, *J*=9.9 Hz),6.54 (s, 1H, NH),6.63 (dd, 4H, H_{Bz}, J=7.7 Hz, J=16.0 Hz), 7.19-7.43 (m, 14H, H_{Bz}, OH, NH),7.57 (dd, 2H, H_{Bz}, *J*=7.1 Hz, *J*=14.2 Hz),7.71 (d, 2H, Ar-H), 7.93-8.01 (m, 4H, Ar-H),8.06 (d, 2H, H_{Bz}, *J*=7.8 Hz) ppm.

^{13}C NMR (100MHz, $CDCl_3$): δ = 28.1, 28.8 (C_{D3}, C_{D6}); 41.0, 41.1 (C_{D4}, C_{D5}); 43.5, 43.6 (2 x $\underline{C}H_2$-NH); 50.7 (CH_2-N_3); 63.1 (C_6); 66.7 (O-CH_2); 68.2 (C_4); 69.9 (C_3); 70.4 (C_2); 71.4 (C_{D2}); 74.9 (C_5); 75.3 (C_{D1}); 96.4 (C_1); 129.5, 129.7, 2x129.8, 2x129.9 (C_{Bz}); 2x130.0 (Ar-C_1); 133.3, 2x133.6, 133.9 (C_{Bz}); 143.6, 143.7, 144.1, 144.2 (Ar-C-OH); 165.7, 166.2, 166.3 (CO_{Bz});175.0 (C=O) ppm.

MS (ESI) calculated for $[C_{58}H_{55}N_5O_{17}Na]^+$=$[(M - N_2) + Na]^+$: 1117.09; found: 1117.7.

3. 2. 11.: α(1,2)pseudomannobioside tetrabenzoylated bis-morpholine amide



11

Description: colourless oil

η: 66.9% (35.0 mg)

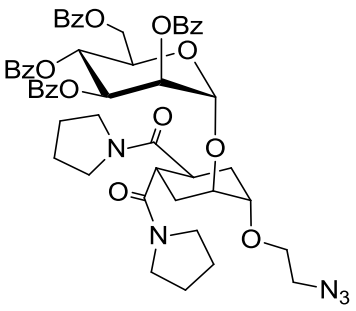
R_f: hexane/ethyl actete = 6/4 + treated with molibden solution; 0.46 (to stain the starting material); chloroform/methanol = 10/0.1 + treated with molibden solution; 0.18 (to stain the product)

^{1}H NMR (400MHz, $CDCl_3$): δ = 1.92-2.16 (m, 4H, D_3, D_6), 3.10 (d, 1H, O-CH_2/CH_2-N_3, *J*=13.3 Hz), 3.25-3.30 (m, 1H, O-CH_2/CH_2-N_3), 3.43-3.79 (m, 21H, D_1/D_2, D_4, D_5, O-CH_2/CH_2-N_3, 2xN-C$\underline{H_2}$-C$\underline{H_2}$-O), 4.09 (s, 1H, D_1/D_2), 4.42-4.52 (m, 2H, H_{6a}, H_5), 4.68 (d, 1H, H_{6b}, *J*=11.0 Hz), 5.30 (s, 1H, H_1), 5.56 (s, 1H, H_2), 5.89 (dd, 1H, H_3, *J*=2.7 Hz, *J*=10.2 Hz), 6.09 (t, 1H, H_4, *J*=10.1 Hz), 7.28 (dd, 1H, H_{Bz}, *J*= 3.8 Hz, *J*= 14.0 Hz), 7.42 (dt, 8H, H_{Bz}, *J*= 7.6 Hz, *J*= 22.7 Hz), 7.57 (ddd, 3H, H_{Bz}, *J*= 7.3 Hz, *J*= 14.7 Hz, *J*= 29.5 Hz), 7.85 (d, 2H, H_{Bz}, *J*= 7.8 Hz), 8.01 – 8.08 (m, 6H, H_{Bz}) ppm.

^{13}C NMR (100MHz, $CDCl_3$): δ = 25.7 (C_{D3},C_{D6}); 36.1, 36.2 (C_{D4},C_{D5}); 42.3,46.2, 46.5 (N-CH_2-CH_2-O); 50.7 (CH_2-N_3); 63.2 (C_6); 66.9, 67.0 (O-$\underline{C}H_2$-$\underline{C}H_2$-N); 67.2 (O-CH_2); 68.0 (C4);68.8 (C3); 69.4 (C_2); 69.9 (C_{D2}); 71.4 (C_5); 72.6, 75.6 (C_{D1}); 96.2 (C_1); 128.3, 128.4, 128.5, 2x128.7, 128.8, 129.0; 129.6, 129.7, 129.8 (C_{Bz}); 133.3, 133.4, 133.6, 133.8 (C_{Bz});163.1, 165.5, 165.8, 166.1 (CO_{Bz}); 173.3, 173.6 (CO) ppm.

MS (ESI) calculated for $[C_{52}H_{55}N_5O_{15}Na]^+$=$[(M - N_2) + Na]^+$: 1012.4; found = 1012.6.

3. 2. 12.: α(1,2)pseudomannobioside tetrabenzoylated bis-pyrrolydine amide



12

Description: pale yellow solid

η: 54.2%(28.5 mg)

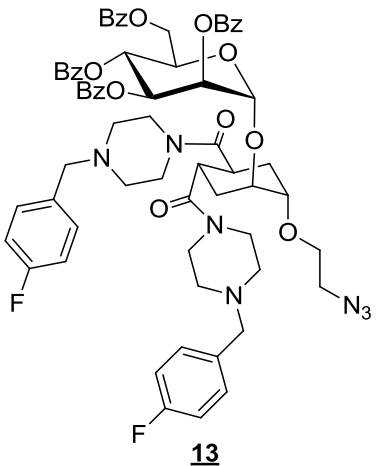
R_f: chloroform/methanol = 10/0.5 + treated with molibden solution; 0.59

^{1}H NMR (400MHz, $CDCl_3$): δ = 1.78-1.93 (m, 8H, 4xN-CH_2-CH_2), 1.96-2.04 (m, 4H, D_3, D_6), 3.17 (d, 1H, O-CH_2/CH_2-N_3, *J*= 4.3 Hz, *J*= 13.1 Hz), 3.25-3.48 (m, 8H,4xN-CH_2-CH_2), 3.52-3.61 (m, 3H, O-CH_2/CH_2-N_3), 3.79 (dd, 2H, D_1/D_2, D_4/D_5, *J*= 6.6 Hz, *J*= 16.8 Hz), 3.92 (dd, 1H, D_4/D_5, *J*= 6.8 Hz, *J*= 16.6 Hz), 4.09 (s, 1H, D_1/D_2), 4.50 (dd, 2H, H_5, H_{6a}, *J*= 7.2 Hz, J= 18.9 Hz), 4.68 (d, 1H, H_{6b}, *J*= 10.9 Hz), 5.31 (s, 1H, H_1), 5.65 (s, 1H, H_2), 5.92 (dd, 1H, H_3, *J*= 3.0 Hz, *J*= 10.2 Hz), 6.10 (t, 1H, H_4, *J*= 9.8 Hz), 7.26-7.29 (m, 4H, H_{Bz}), 7.36-7.45 (m, 6H, H_{Bz}), 7.50-7.63 (m, 3H, H_{Bz}), 7.84 (d, 2H, H_{Bz}, *J*= 7.9 Hz), 8.00-8.08 (m, 5H, H_{Bz}) ppm.

^{13}C NMR (100MHz, $CDCl_3$): δ = 24.5, 26.1, 26.2 (N-CH_2-CH_2); 27.5, 28.5 (C_{D3}, C_{D6}); 38.5, 38.7 (C_{D4}, C_{D5}); 45.9, 46.5, 46.8 (N-CH_2-CH_2);50.9 (CH_2-N_3); 63.3 (C_6); 67.1 (O-CH_2); 68.6 (C_4); 69.6, 69.8 (C_3); 71.4 (C_2); 73.7 (C_{D2}); 75.8 (C_5); 76.8 (C_{D1}); 96.7 (C_1); 126.2, 128.5, 128.6, 128.8, 129.2, 129.8, 130.0, 130.1, 133.3, 133.7, 133.7 (C_{Bz}); 165.4, 165.7, 165.8, 166.2 (CO_{Bz}); 173.1, 173.5 (CO) ppm.

MS (ESI) calculated for $[C_{52}H_{55}N_5O_{13}Na]^{+:}$ 980.4; found: 980.6.

3. 2. 13.: α(1,2)pseudomannobioside tetrabenzoylated bis-4-(4-fluorobenzyl)piperazine amide



13

Description: pale yellow oil

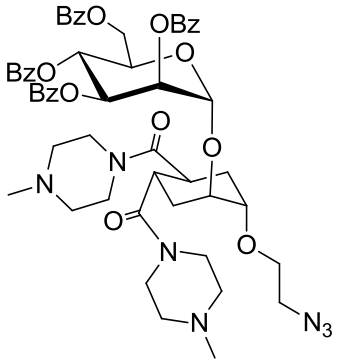
η: 55.5% (37.0 mg)

R_f: chloroform/methanol = 10/0.5 + treated with molibden solution; 0.45

^{1}H NMR (400MHz, $CDCl_3$): δ =1.90-2.07 (m, 4H, D_3, D_6), 2.36-2.72 (m, 6H, N-CH_2-CH_2-N), 3.08-3.13 (m, 1H, O-CH_2/CH_2-N_3), 3.22-3.28 (m, 1H, O-CH_2/CH_2-N_3), 3.45-3.53 (m, 10H, D_4, D_5, O-CH_2/CH_2-N_3, N-CH_2-CH_2-N, 2xN-CH_2-Ar), 3.54-3.66 (m, 6H, N-CH_2-CH_2-N), 3.72-3.77 (m, 3H, D_1/D_2, N-CH_2-CH_2-N), 4.07 (s, 1H, D_1/D_2), 4.42-4.51 (m, 2H, H_5, H_{6a}), 4.66 (t, 1H, H_{6b}, *J*=10.1 Hz), 5.28 (s, 1H, H_1), 5.54 (s, 1H, H_2), 5.87 (dd, 1H, H_3, *J*=3.0 Hz, *J*=10.2 Hz), 6.08 (t, 1H, H_4, *J*=10.1 Hz), 6.90-7.03 (m, 4H, Ar-H), 7.25-7.33 (m, 4H, Ar-H), 7.37 – 7.44 (m, 8H, H_{Bz}), 7.51-7.63 (m, 4H, H_{Bz}), 7.77 (d, 2H, H_{Bz}, *J*=7.9 Hz), 7.96 (d, 2H, H_{Bz}, *J*=7.8 Hz), 8.02 (t, 4H, H_{Bz}, *J*=7.3 Hz) ppm.

^{13}C NMR (100MHz, $CDCl_3$): δ = 27.7, 29.2 (C_{D3}, C_{D6}), 36.1 (C_{D4},C_{D5}), 41.8, 45.6, 45.9 (N-CH_2-CH_2-N), 50.7 (O-CH_2/CH_2-N_3), 52.8, 53.1, 53.2, 53.4 (N-CH_2-CH_2-N), 62.0 (O-CH_2/CH_2-N_3), 63.1 (C_6), 66.9 (C_4), 68.7 (N-CH_2-Ar), 69.3, 69.8 (C_3, C_5), 71.4 (C_2), 72.9 (C_{D1}/C_{D2}), 75.7 (C_{D1}/C_{D2}), 96.3 (C_1), 114.9, 115.0, 115.1, 115.2 (Ar-C), 128.3, 128.7, 128.8, 128.9 (Ar-C), 129.7, 129.8, 129.9 (C_{Bz}), 130.5, 130.6 (C_{Bz}), 133.3, 133.6, 133.7 (C_{Bz}), 172.8, 173.0 (CO) ppm.

3. 2. 14.: α(1,2)pseudomannobioside tetrabenzoylated bis-4-methylpiperazine amide



14

Description: clear solid

η: 70.8% (39.0 mg)

R_f: chloroform/methanol = 9/1 + treated with molibden solution; 0.28

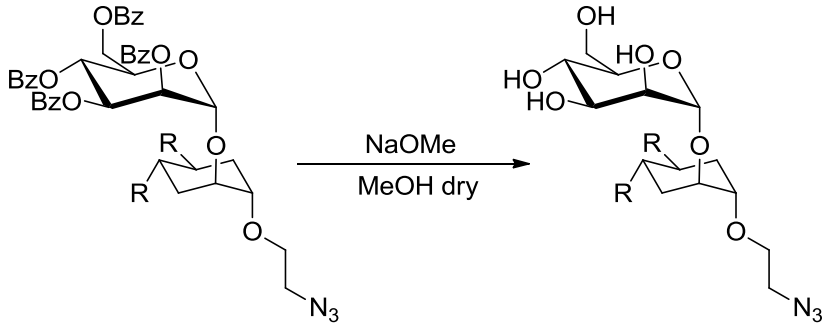
^{1}H NMR (400MHz, $CDCl_3$): δ = 1.92 – 1.96 (m, 4H, D_3, D_6), 2.30 – 2.36 (m, 10H, N-CH_2-CH_2-N, 2xCH_3), 2.40 – 2.54 (m, 4H, N-CH_2-CH_2-N), 2.65 – 2.69 (m, 1H, N-CH_2-CH_2-N), 3.11 (ddd, 1H, O-CH_2/CH_2-N_3, *J*=3.1 Hz), 3.27 (ddd, 1H, O-CH_2/CH_2-N_3, *J*=3.3 Hz), 3.43 – 3.58 (m, 4H, D_4, D_5, O-CH_2/CH_2-N_3), 3.62 – 3.68 (m, 4H, N-CH_2-CH_2-N), 3.73 – 3.82 (m, 3H, D_1/D_2, N-CH_2-CH_2-N), 4.07 (dd, 1H, D_1/D_2), 4.40 – 4.45 (m, 1H, H_5), 4.50 (dd, 1H, H_{6a}, *J*=5.4 Hz, *J*=12.0 Hz), 4.67 (dd, 1H, H_{6b}, *J*=2.5 Hz, *J*=12.0 Hz), 5.29 (d, 1H, H_1, *J*=1.3 Hz), 5.59 (dd, 1H, H_2, *J*=1.7 Hz, *J*=3.1 Hz), 5.89 (dd, 1H, H_3, *J*=3.3 Hz, *J*=10.2 Hz), 6.08 (t, 1H, H_4, *J*=10.1 Hz), 7.25 – 7.29 (m, 3H, H_{Bz}), 7.36-7.45 (m, 6H, H_{Bz}), 7.50 – 7.62 (m, 3H, H_{Bz}), 7.85 (d, 2H, H_{Bz}, J=7.3 Hz), 8.00 – 8.07 (m, 6H, H_{Bz}) ppm.

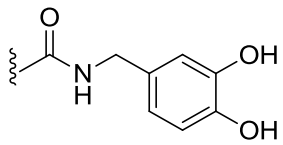
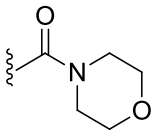
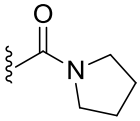
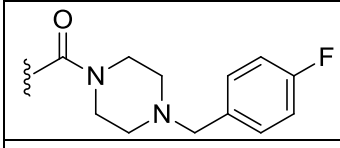
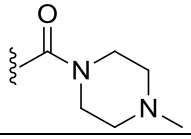
^{13}C NMR (100MHz, $CDCl_3$): δ = 27.9, 29.4 (C_{D3}, C_{D6}); 36.2, 36.2 (C_{D4}, C_{D5}); 41.8, 45.6, 45.9 (N-CH_2-$\underline{C}H_2$-N), 46.1 (CH_3); 50.8 (CH_2-N_3);55.0, 55.5, 55.6 (N-$\underline{C}H_2$-CH_2-N); 63.0, 63.3 (C_6); 67.1 (O-CH_2); 68.8 (C_4); 69.5 (C_3); 69.9 (C_2); 71.4 (C_{D2}); 73.2 (C_5); 75.8 (C_{D1}); 96.6 (C_1); 128.4, 128.6, 128.8, 128.9, 129.1, 129.2 (C_{Bz}); 129.8, 129.8, 129.9, 130.0 (C_{Bz}); 133.3, 133.7, 133.8 (C_{Bz}); 165.3, 165.6, 165.8, 166.1 (CO_{Bz}); 172.9, 173.1 (CO) ppm.

MS (ESI) calculated for $[C_{54}H_{61}N_7O_{13}H]^+$ = 1015.4 ; found = 1016.8.

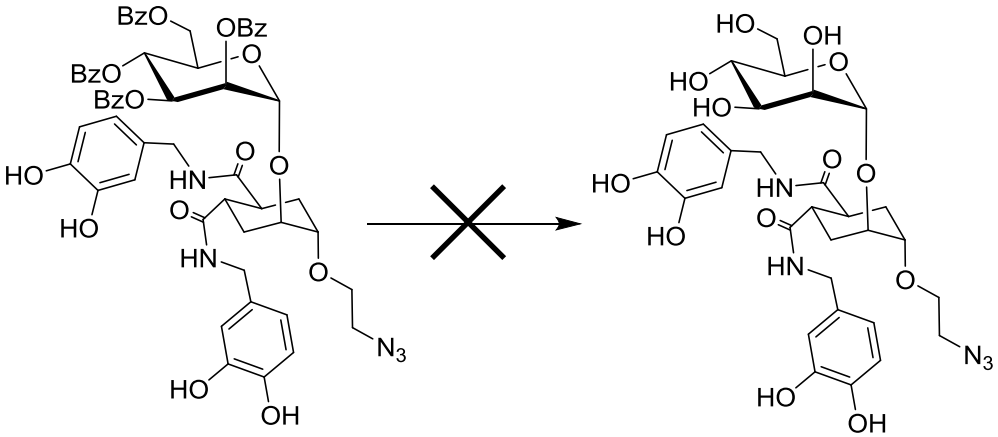
3. 2. 15. General procedure of perbenzoylated mannose deprotection (Zemplén method)

Compounds **10**, **11**, **12**, **13** and **14** were dissolved in freshly distilled MeOH under nitrogen and stirring. NaOMe was weighed in a separate flask, put under nitrogen and dissolved in freshly distilled MeOH to get a solution. Solution of NaOMe was then transmitted into the flask with compounds. The concentration of compounds in the mixture was 0.02 M and the concentration of NaOMe 0.1 M. After 1 hour, twice the amount of MeOH was added and then resins Amberlite® IR120 H for neutralization. The mixture was left under stirring for 15 minutes, then filtered and washed successively with MeOH. The solvent was evaporated under reduced pressure.



R	Amount	n (mmol]	Eq	Amount of 0,1 MNaOMe	final
	18.7 mg	0.0171	1	85 µL	**15**
	35 mg	0.0354	1	180 µL	**16**
	28.5 mg	0.0298	1	200 µL	**17**
	37.0 mg	0.0304	1	200 µL	**18**
	39.0 mg	0.0384	1	300 µL	**19**

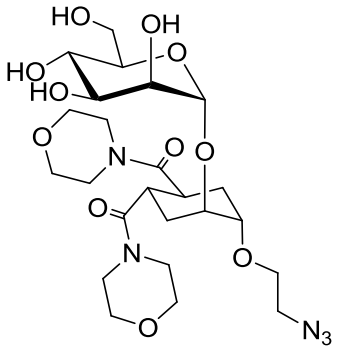
3. 2. 16.: α(1,2)pseudomannobioside bis-1,3-(3,4-dihydroxybenzyl) amide



10 **15**

After the evaporation of the solvent under pressure 1H-NMR spectrum of the residue was recorded and no peaks were detected. It was assumed that the compound was lost during the last step after adding resin Amberlite® IR120 H to neutralize the mixture. Because of the polar character of the compound it is presumed it bound very strongly to the resin and could not be washed off.

3. 2. 17.: α(1,2)pseudomannobioside bis-1,3-morpholine amide



16

Description: colourless oil

η: 49.2% (10.0 mg)

R_f: chloroform/methanol = 9.9/0.1; 0.0

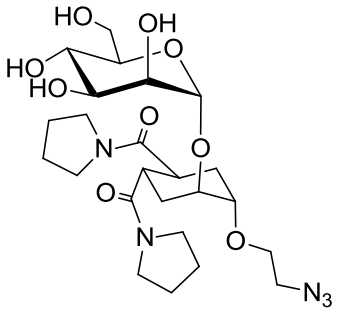
^{1}H NMR (400MHz, $CDCl_3$): δ = 1.82-1.98 (m, 4H, D_3, D_6), 3.35-3.76 (m, 26H, H_3, H_4, H_5, H_6, O-CH_2, CH_2-N_3, 2xN-CH_2-CH_2-O, D_4, D_5), 3.79-3.82 (m, 1H, D_1/D_2), 3.86 (s, 1H, H_2), 4.10 (s, 1H, D_2), 4.95 (s, 1H, H_1) ppm.

^{13}C NMR (100MHz, $CDCl_3$): δ = 28.7, 29.9 (C_{D3}, C_{D6}); 37.4, 37.5(C_{D4}, C_{D5}); 43.5 (N-$\underline{C}H_2$-CH_2-O); 47.4 (CH_2-N_3); 63.1 (C_6); 67.7 (N-CH_2-$\underline{C}H_2$-O); 68.7 (C_4); 69.9 (O-CH_2); 72.3, 72.4, 72.5 (C_{D2}, C_2, C_3); 75.8 (C_{D1}); 76.5 (C_5); 100.8 (C_1); 175.4, 175.5, 175.7, 175.7 (CO) ppm.

$(\alpha]_D^{20}$ = +16 (conc = 0.50, $CHCl_3$)

MS (ESI) calculated for $[C_{24}H_{39}N_5O_{11}Na]^+$ = 596.3 ; found = 596.7.

3. 2. 18.: α(1,2)pseudomannobioside bis-1,3-pyrrolydine amide



17

The compound was not pure after the evaporation of the solvent, so it was also purified by reverse phase flash column chromatography using water with gradient methanol from 0% to 100% as eluent.

Description: colourless oil

η: 75.1% (12.1 mg)

R_f: chloroform/methanol/acetic acid = 9/1/0.1; 0.1

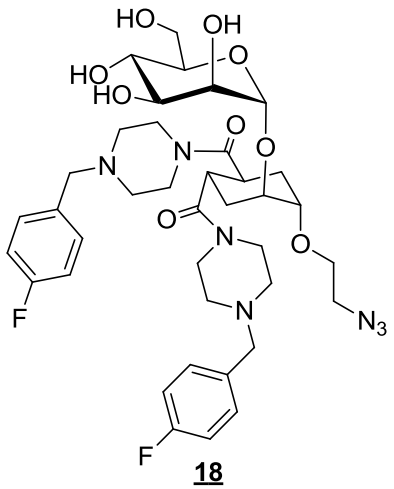
^{1}H NMR (400MHz, $CDCl_3$): δ = 1.79-1.98 (m, 12H, D_3, D_6, N-CH_2-CH_2), 3.09-3.23 (m, 2H, D_4, D_5), 3.27-3.39 (m, 10H, O-CH_2/CH_2-N_3, N-CH_2-CH_2), 3.43-3.57 (m, 4H, H_4, H_5, H_6), 3.64-3.75 (m, 4H, D_1/D_2, H_3, O-CH_2/CH_2-N_3), 3.84-3.85 (m, 1H, H_2), 4.07 (d, 1H, D_1/D_2, *J*= 2.2 Hz), 4.95 (d, 1H, H_1, *J*= 1.5 Hz) ppm.

^{13}C NMR (100MHz, $CDCl_3$): δ = 25.2, 25.3 (C_{D3}, C_{D6}); 26.9, 27.0, 27.8, 29.1(N-CH_2-CH_2-CH_2-CH_2); 39.8, 39.9 (C_{D4},C_{D5}); 47.0, 47.7, 47.8 (N-CH_2-CH_2-CH_2-CH_2); 52.1 (CH_2-N_3); 63.1 (C_6); 68.7 (C_4); 69.7 (O-CH_2); 72.3 (C3); 72.5, 72.6 (C_{D2}, C_2); 75.7 (C_{D1}); 76.6 (C_5); 100.4 (C_1); 175.4, 175.7 (CO) ppm.

$(\alpha]_D^{20}$ = +22.6 (conc = 0.65, MeOH)

MS (ESI) calculated for $[C_{24}H_{39}N_5O_9Na]^+$ = 564.3; found = 564.6.

3. 2. 19.: α(1,2)pseudomannobioside bis-1,3-(4-(4-fluorobenzyl)piperazine) amide



18

Description: clear solid

η: 92.5% (22.6 mg)

R_f: chloroform/methanol/acetic acid = 7.5/2.5/0.1; 0.0

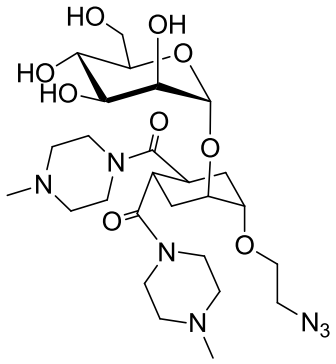
^{1}H NMR (400MHz, $CDCl_3$): δ = 1.83-1.92 (m, 4H, D_3, D_6), 2.52-2.66 (m, 8H, N-CH_2-CH_2-N), 3.34-3.38 (m, 9H, H_3, D_4, D_5, O-CH_2/CH_2-N_3, 2xN-CH_2-Ar), 3.56-3.59 (m, 2H, H_5, H_{6a}), 3.61-3.74 (m, 11H, H_4, O-CH_2/CH_2-N_3, N-CH_2-CH_2-N), 3.76-3.78 (m, 1H, D_1/D_2), 3.81 (dd, 1H, H_2, *J*=3.4 Hz, *J*=6.6 Hz), 3.85-3.88 (m, 1H, H_{6b}), 4.08-4.09 (m, 1H, D_1/D_2), 4.93 (s, 1H, H_1), 7.09 (t, 4H, Ar-H, *J*=8.3 Hz), 7.40 (d, 4H, Ar-H, *J*=5.6 Hz) ppm.

^{13}C NMR (100MHz, $CDCl_3$): δ = 28.7, 30.0 (C_{D3}, C_{D6}); 37.5, 37.6 (C_{D4}, C_{D5}); 42.5, 46.4, 46.5 (N-$\underline{C}H_2$-CH_2-N-CH_2-Ar); 52.1 (CH_2-N_3);53.4, 53.6, 53.8, 53.9 (N-CH_2-$\underline{C}H_2$-N-CH_2-Ar); 62.5 (N-CH_2-CH_2-N-$\underline{C}H_2$-Ar); 63.1 (C_6); 68.7 (C_4); 69.6 (O-CH_2); 72.3, 72.4, 72.5 (C_{D2}, C_2, C_3); 75.8 (C_{D1}); 76.5 (C_5); 100.7 (C_1); 116.0, 116.2 (Ar-C); 129.6, 130.5 (Ar-C); 132.4, 132.5, 132.6 (Ar-C); 133.6, 133.9 (Ar-$\underline{C}$-CH_2-N); 134.2 (Ar-C); 162.6, 165.0 (Ar-C-F); 175.2, 175.5 (CO) ppm.

$(\alpha]_D^{20}$ = -2.1 (conc = 0.45, MeOH)

MS (ESI) calculated for $[C_{38}H_{51}F_2N_7O_{10}H]^+$ = 788.5 ; found = 788.7.

3. 2. 20.: α(1,2)pseudomannobioside bis-1,3-(4-methylpiperazine) amide



19

Description: clear solid

η: 89.9% (20.7 mg)

R_f: dichloromethan/methanol/acetic acid = 8/2/0.1 + treated with molibden solution; 0.27

^{1}H NMR (400MHz, $CDCl_3$): δ = 1.82-2.00 (m, 4H, D_3, D_6), 2.58 (d, 6H, 2xCH_3, *J*= 8.6 Hz), 2.74-2.87 (m, 4H, N-CH_2-CH_2-N), 3.35-3.46 (m, 4H, D_4, D_5, O-CH_2), 3.55-3.92 (m, 18H, D_1/D_2, H_2,H_3, H_4, H_5, H_6, CH_2-N_3, N-CH_2-CH_2-N), 4.11 (d, 1H, D_1/D_2, *J*= 2.2 Hz), 4.96 (s, 1H, H_1) ppm.

^{13}C NMR (100MHz, $CDCl_3$): δ = 28.8, 30.0 (C_{D3}, C_{D6}); 37.6, 37.7 (C_{D4}, C_{D5}); 41.5, 41.6 (N-$\underline{C}H_2$-CH_2-N-CH_3); 45.0, 45.1 (N-CH_2-CH_2-N-$\underline{C}H_3$); 45.4, 45.5 (N-$\underline{C}H_2$-CH_2-N-CH_3);52.1 (CH_2-N_3); 54.9, 55.3 (N-CH_2-$\underline{C}H_2$-N-CH_3); 63.1 (C6); 68.7 (C4); 69.9 (O-CH2); 72.4, 72.5, 72.6 (C_2, C_{D2}, C_3); 75.8 (C_{D1}); 76.4 (C_5); 100.9 (C_1); 175.4, 175.6 (CO) ppm.

$(\alpha]_D^{20}$ = +10.8 (conc = 0.41, MeOH)

MS (ESI) calculated for $[C_{26}H_{45}N_7O_9H]^+$ = 600.3 ; found = 600.7.

4. MATERIALS AND METHODS

- **reagents and solvents**

Experimental part was performed using chemicals and solvents manufactured by Sigma-Aldrich, Acros, Alfa Aesar, Fluka and Merck. Anhydrous solvents were prepared by distillation and drying over molecular sieves.

- **chromatographic methods**

Thin layer chromatography (TLC): reactions were monitored using thin layer chromatography (TLC) for which plates with a 0.20 mm layer made by Merck – Merck DC Fertigplatten Kieselgel 60 GF_{254} with added fluorescent indicator was used. For developing the chromatograms different mobile phases were used – as indicated by each compound. Compounds were detected with an UV light (λ=254 nm) and staining reagents – ninhydrine, molibden solution, $FeCl_3$, 2,4–dinitrophenylhydrazine.

Column »flash« chromatography: Column chromatography was used as a technique of separating and purifying compounds. For all compounds the same size of silicagel particles were used (0.04-0.063 nm), while mobile phase differed from compound to compound – as indicated by each compound.

Isolera One Biotage: Isolera was also used for purifying compounds, intermediate and final. Silica gel with C-18 lipophilic bonds represented the stationary phase and mobile phase differed from compound to compound – as indicated by each compound.

- **spectroscopic methods**

Nuclear magnetic resonance (NMR): NMR spectra were recorded on a Bruker AVANCE 400 MHz instrument at 298K. Chemical shifts (δ) are reported in ppm upfield from TMS as internal standard, whereas coupling constants (J) are stated in Hz. The ^{1}H and ^{13}C – NMR resonances of compounds were assigned by the meaning of COSY and HSQC experiments. Computer programme MastReNova 8.1.2 – 11880 from MASTRELAB RESEARCH S.L. was used to assign the spectra.

Mass spectroscopy: In Slovenia mass spectra were recorded on VG-Analytical Autospec Q spectrometer with ESI method of ionisation at the Centre of mass

spectroscopy, Institut of Jožef Stefan, Ljubljana. In Milan mass spectra were recorded on ThermoFischer LCQ apparatus (ESI ionization), Waters Micromass Q-Tof (ESI ionization- HRMS) or Bruker Daltonics Microflex MALDI-TOF apparatus.

- **other**

Optical rotation: Specific optical rotation values were measured using a Perkin-Elmer 241 apparatus at 589 nm in a 1 mL cell.

Nomenclature and compound drawing: Structural formulae of compounds were drawn and their IUPAC nomination obtained with ChemDraw Ultra 12.0.2.1076. SciFinder Scholar enabled finding compounds and synthetic procedures in various databases.

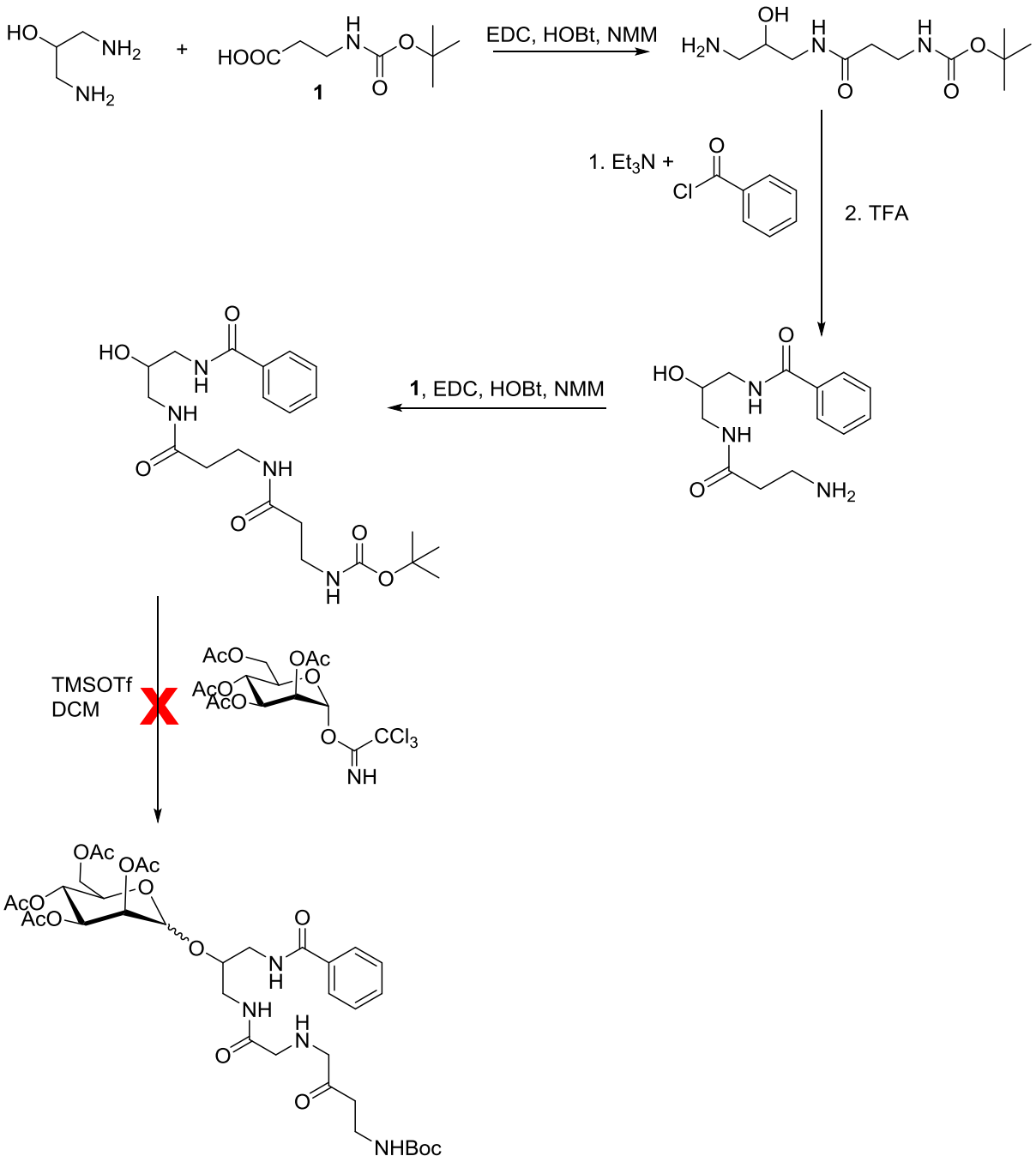
5. DISCUSSION

5. 1. Synthetic procedures

Experimental work was divided into two parts, one synthesized in Slovenia at the Department of pharmaceutical chemistry at the Faculty of pharmacy at the University of Ljubljana under the supervision of Assoc. Prof. Marko Anderluh, and the other in Italy at the Department of organic and industrial chemistry at the University of Milan under the supervision of Prof. Anna Bernardi.

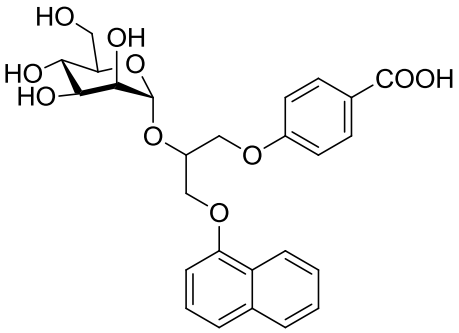
The synthetic plan of the first part was to synthesise monovalent glycomimetics, which would later be attached to a dendron core.

In **Scheme 1** we can see the synthetic route of the compound with the 1,3-aminopropan-2-ol linker, which was not completed successfully. First problems we encountered were at the beginning stages of the synthesis, where we figured out that the 1,3-aminopropan-2-ol and the Boc-β-alanine had to be bound first, because the mono-substitution of the benzoyl chloride with 1,3-aminopropan-2-ol produced a cyclic amidine and further reactions could not be performed. The deprotection of *t*-butyl carbamate from the β-alanine was also problematic since the deprotection itself was successful, but the trifluoroacetic acid was very difficult to remove. The major problem was the glycosylation step, which was not successful at all. This could probably be corrected by lowering the temperature at which the reaction ran and also ensuring that the dichloromethane used was totally dry, so it would be distilled right before setting the reaction.



Sheme 1: Synthetic route of the compound with the 1,3-aminopropan-2-ol

This route was later dropped and a glycerol linker used instead of 1,3-aminopropan-2-ol.



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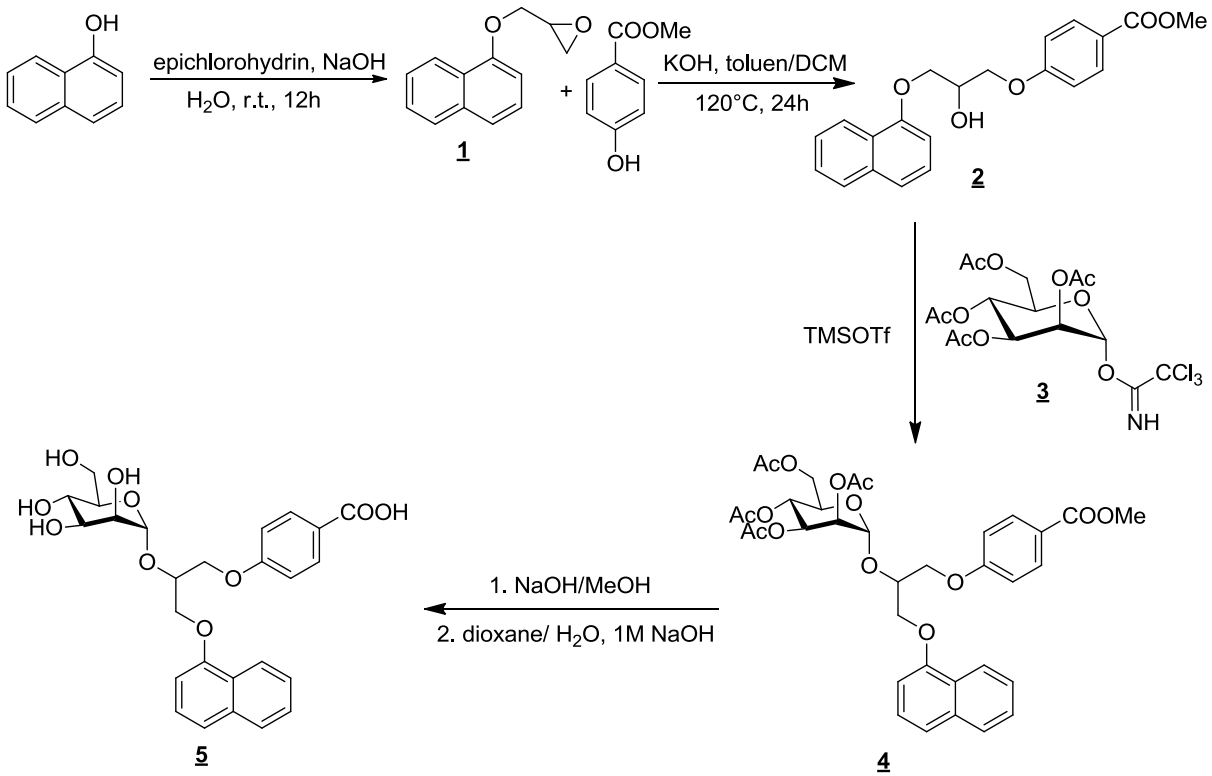
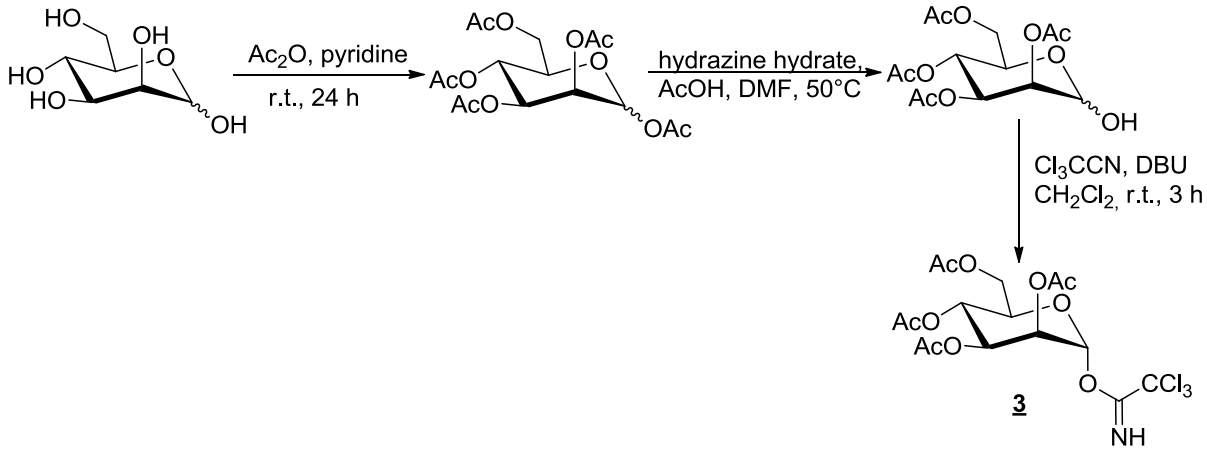
On the other hand, compound **5** was synthesised successfully through the course of 5 consecutive reactions, which are depicted in **Scheme 2**. The synthesis started with a bimolecular nucleophilic substitution in which 1-naphthol reacted with epichlorohydrin in the presence of a base to form a suitable oxirane (**1**). The next step was the nucleophilic attack of the alcohol (methyl 4-hydroxybenzoate) on the activated oxirane which produced intermediate **2**. Using the trichloroacetimidate method of glycosylation compound **2** was then reacted with mannoside trichloroacetimidate (**3**) to form compound **4**, which was later deprotected from acetate protecting groups, using the Zemplén method, and methyl protecting group to obtain the final compound (**5**).



Scheme 2: Synthesis of compound 5

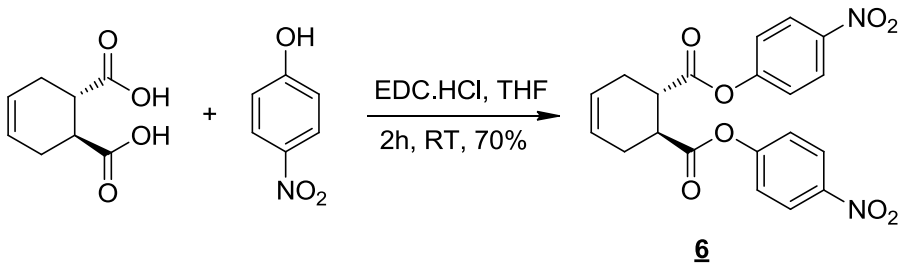
The mannoside trichloroacetimidate (**3**) was synthesised from D-mannose (**Scheme 3**): the first step was per-*O*-acetylation to protect the hydroxyl groups of D-mannose, second step was selective deprotection of the acetal on the anomeric hydroxyl group and finally the acetylated D-mannose was activated by using trichloroacetonitrile to obtain the final product (**3**). By adding DBU (1,8-diazabicycloundec-7-ene) to the reaction mixture the α anomeric configuration was ensured.



Scheme 3: Synthesis of the α-D-mannoside trichloroacetimidate

In Milan we successfully synthesised four final compounds (**16** - **19**), compound **15** was lost during the final step when methanol and resin Amberlite® IR120 H were added for neutralization. This was probably due to the fact that compound **15** was very polar because of four hydroxyl groups present on the two aromatic rings in addition to four hydroxyl groups on the sugar. Because of high polarity, the compound bound to the resin and could not be washed off, so consequently it was lost.

Synthesis of compounds **16** – **19** started with the formation of the activated ester (**6**) by reaction between cyclohex-4-ene-1,2-dicarboxylic acid and 4-nitrophenol in the presence of EDC, the course of which is depicted in **Scheme 4**.



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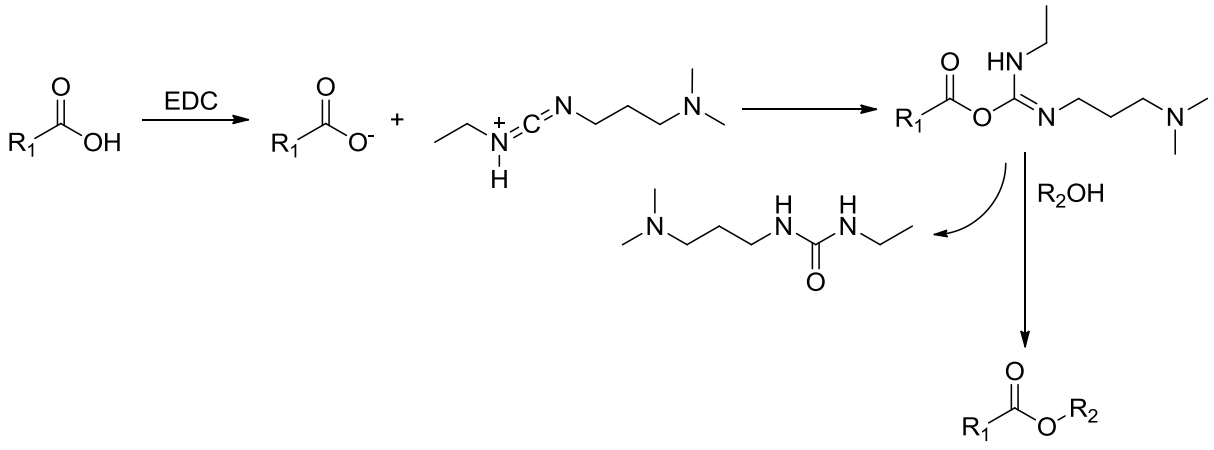
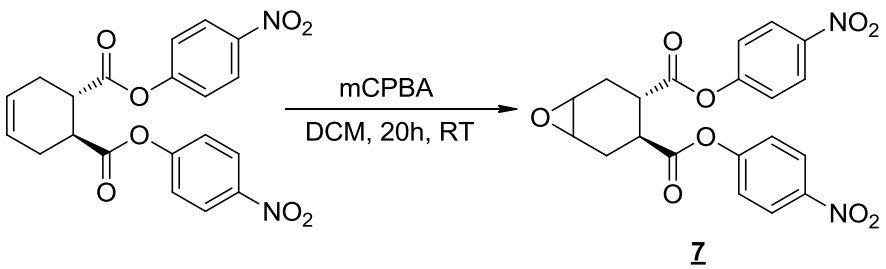
Scheme 4: Synthesis of the activated ester

EDC or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide is a carbodiimide that facilitates formation of the ester by activating the carboxylic acid. This reaction is a nucleophilic substitution with elimination of *N,N'*-disubstituted urea and the mechanism of the reaction is depicted in **Scheme 5**.



Scheme 5: Mechanism of the amide bond formation using EDC as a coupling reagent

Compound **7** was formed by oxidation of compound **6** by using *m*-chloroperoxybenzoic acid (**Scheme 6**).



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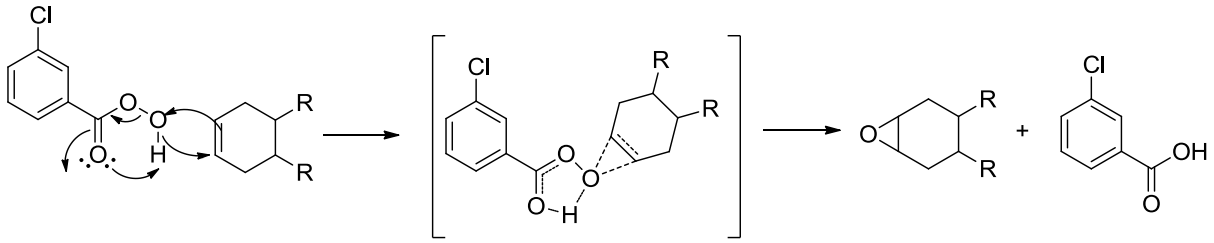
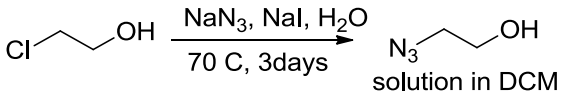
Scheme 6: Formation of the epoxide

The mechanism of the epoxidation reaction with mCPBA is depicted in **Scheme 7**.



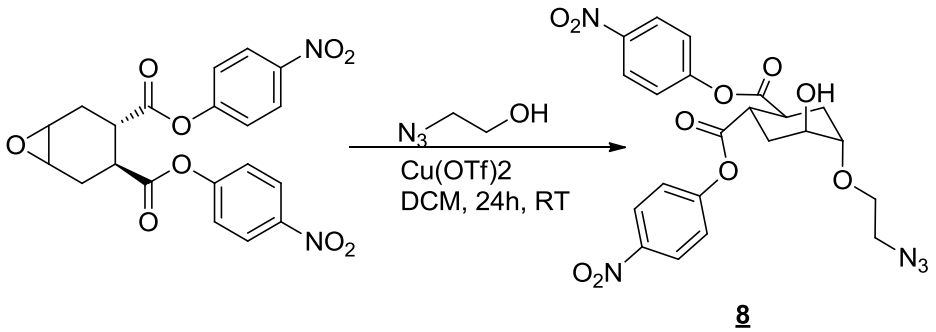
Scheme 7: Mechanism of the epoxidation with mCPBA

The resulting epoxide was a good starting compound for the nucleophilic substitution with 2-azidoethanol that followed. 2-azidoethanol was prepared by treating 2-chloroethanol with sodium azide in water (**Scheme 8**) and the resulting reaction mixture was extracted with dichloromethane.



Scheme 8: Formation of 2-azidoethanol

The product was not dried due to volatility and possible explosive properties, but simply added to the following reaction mixture (**Scheme 9**) to obtain compound **8**, which had both the activated ester and the azide installed.



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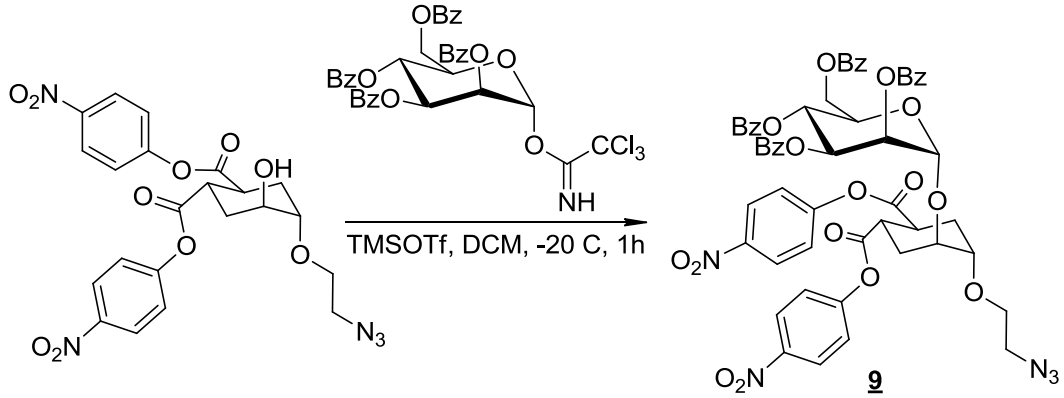
Scheme 9: Synthesis of compound 8

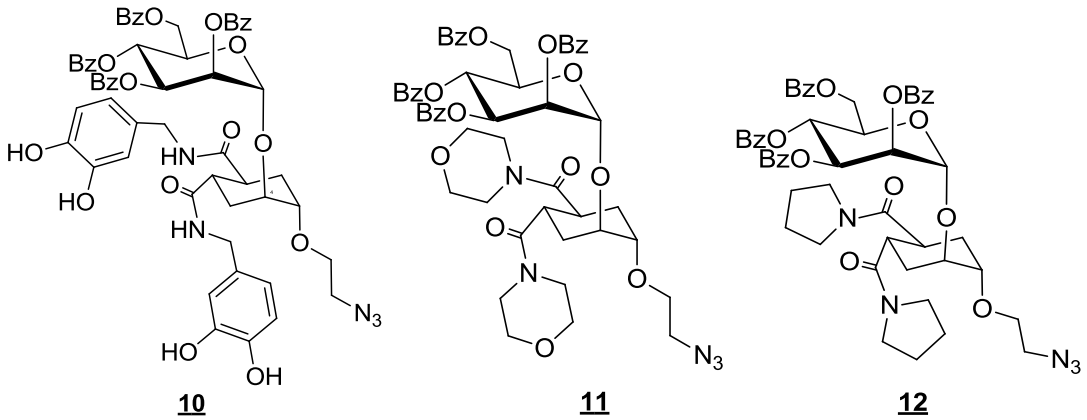
This was a very useful and time saving methodology for further work. Otherwise an alternative method with nucleophilic substitution of Cl atom with NaN_3 would have to be performed on each final derivative individually.

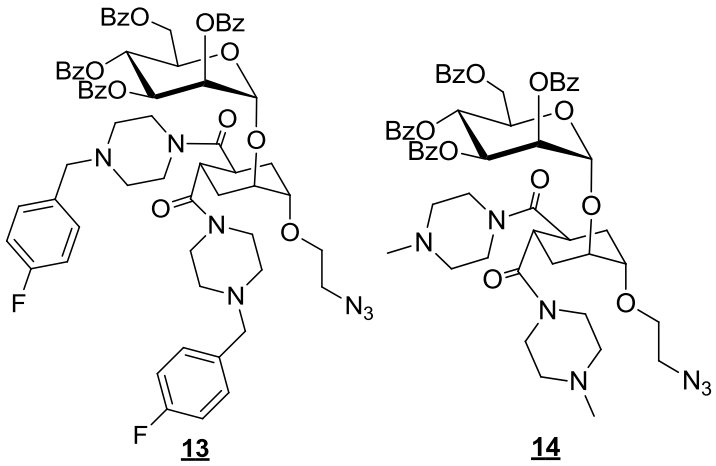
The glycosylation step was again conducted with the trichloroacetimidate method where compound **8** was the glycosyl acceptor and the mannoside trichloroacetimidate the glycosyl donor. During the glycosylation reaction, which is depicted in **Scheme 10**, the donor was activated with trimethylsilyl trifluoromethanesulfonate (a Lewis acid catalyst) and formed compound **9** after successfully reacting with the acceptor.



Scheme 10: The glycosylation reaction

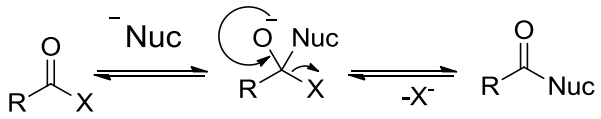
Compound **9** was then used for the formation of different types of amides, compounds **10** – **14**, which are depicted in **Scheme 11**, where a general procedure for all compounds was used.





Scheme 11: Compounds 10 – 14

The reaction is called a condensation reaction, a reaction where two functional groups combine and form a bigger molecule with the loss of a small molecule, in our case p-nitrophenol. The two compounds react through an addition mechanism and an elimination reaction and the mechanism of the reaction is depicted in **Scheme 12**.

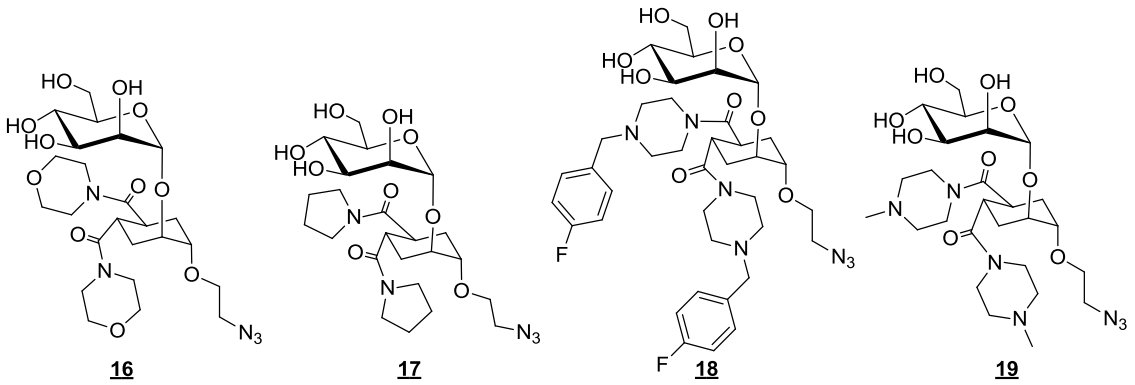


Scheme 12: Mechanism of the condensation reaction

Carbonyl carbon is attacked by the nucleophile to form a tetrahedral intermediate which then collapses, ejects the leaving group and renews the carbonyl bond.

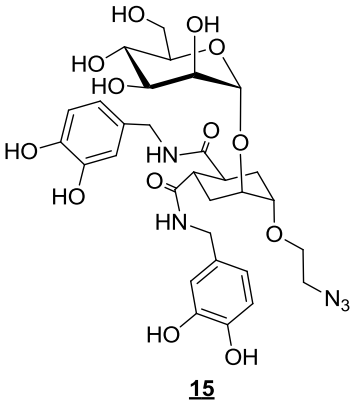
Compound **10** was very hard to monitor during the reaction due to its very polar character and many different TLC conditions were tested before finding the optimal conditions. With other compounds the reaction as well as its monitoring ran smoothly and the products obtained were quite pure.

In the end, deprotections of benzoylated compounds **10** – **14** were performed using the Zemplén method and compounds **16** – **19** were obtained, which are depicted in **Scheme 13**.



Scheme 13: Compounds 16 – 19

Compound **15** was lost during the last step of the deprotection process, that is, after adding ion-exchange resins to the mixture. Due to its highly polar nature, as depicted in **Scheme 14**, it has four additional hydroxyl groups besides the hydroxyl groups on the sugar, so we assume that it bound to the resin and could not be washed off.



Scheme 14: Structure of compound 15

5. 2. Biological testing

The compound synthesised in Ljubljana was tested for binding affinity at the Institute of Molecular Pharmacy at the University of Basel in Switzerland by Beat Ernst's group. The assay used for testing is called fluorescence polarization (**Figure 22**), the principle of which is measuring the polarization difference between a free fluorophore ligand and when the ligand is bound to a large protein. Basically, a fluorescent tracer was attached to the reference ligand and the difference between the free, partially bound and fully bound state was measured. The binding affinity of our compound was determined by competitive binding with the fluorescent ligand and then calculated using these measurements, since fluorescent polarization gives us straightforward readout of the binding degree (38, 39). Results were given in IC_{50} which is the half maximal inhibitory concentration, that is, the concentration needed to inhibit a certain process by half. It is used to measure the potency of antagonists which is directly proportional to the effectiveness of a substance to inhibit a specific biochemical or biological function. When a compound has good potency it means that it will induce a large response at low concentrations. In our case, IC_{50} is defined as the concentration of the assayed compound that causes 50% inhibition of the reference fluorescent ligand binding to FimH.

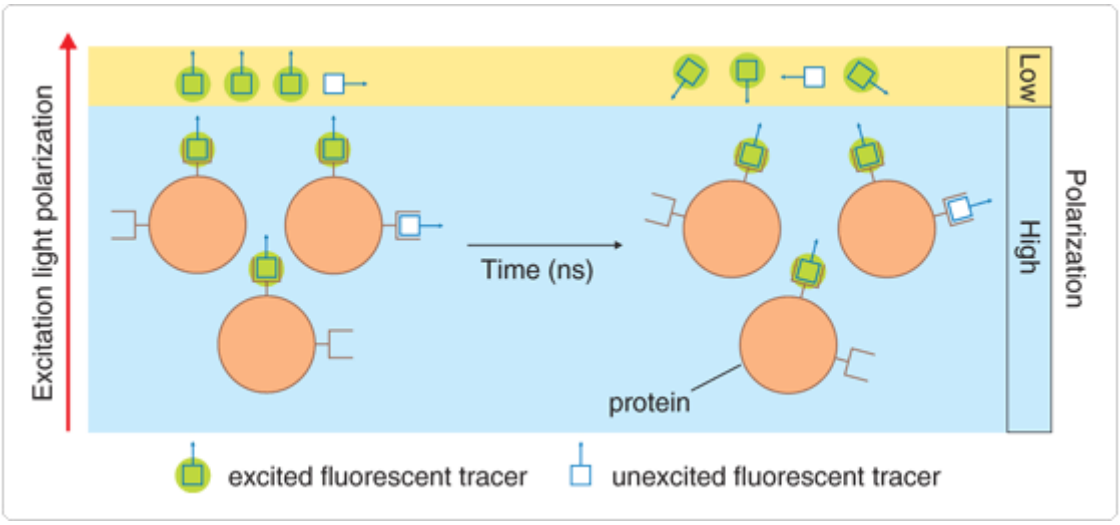


Figure 22: Representation of the fluorescence polarization

(Adjusted from 40)

According to the results, the IC_{50} value for compound **5** was 346 nM which tells us that the compound has a high affinity for the lectin. RIP (relative inhibitory potency) value for the compound is 0.17, when compared to the most potent ligand on FimH that is heptyl α-D-mannopyranoside. We can attribute this high affinity to a well-defined and narrow binding pocket that forms a broad network of hydrogen bonds. Also other saccharides, such as glucose, which do not have the hydroxyl group at position 2 in an axial position, bind to FimH with insignificant affinity (41). Therefore, the monosaccharide is the core which selectively anchors the ligand into the FimH binding site and the aglycon part is responsible for increasing binding affinity (11). Along with compound **5** other biphenyl α-D-mannopyranosides were tested, one was a group of symmetrical biphenyls and the other, in which compound **5** was included, asymmetrical biphenyls. Both groups had high affinities which were to be expected since studies of the binding site disclosed that aromatic moieties probably occupy the "tyrosine gate" and in addition also the lipophilic area opposite the tyrosines, formed by Phe142 and Ile13. With the asymmetrical group solubility was improved while maintaining the same level of affinity. Also, it is assumed that the carboxylic acid moiety forms hydrogen bonds within the binding site (26).

The compound was also tested for DC-SIGN but did not show any promising affinity for DC-SIGN, which is a proof of compound's selective binding, despite the fact that binding affinity to DC-SIGN is improved with synthesising glycomimetics (42). This might be due to the fact that it lacks the pseudo-1,2-mannobioside structure that uniquely binds to the receptor (43). Having only one mannose sugar in the structure, it seems that the aromatic moiety (37) doesn't increase the binding affinity enough to make a difference.

Compounds **16**-**19** have not been tested yet but will be in the future by a novel assay in which a clickable polymer will be synthesised and coated over a Si/SiO_2 wafer in order to detect saccharide/protein interactions. The compounds will be tested for specificity on different lectins.

6. CONCLUSIONS

Adherence of bacteria to host cells is the first step in any infectious process. Most commonly it is achieved by lectins, which can be present on bacterial or host surface that recognize complementary carbohydrates on another cells surface. It is this particular recognition that is an interesting target for future anti-infective drugs, since the action of the drug would take place in the very first step of infection. FimH and DC-SIGN are among the most promising targets of such therapy, and furthermore, both bind mannose saccharides. With the compound synthesised in Ljubljana we were trying to form a "T-shaped" structure which would accommodate lipophilic pockets surrounding the FimH mannose binding site. This was indeed later proven to be very affective given the results of biological testing. In Milan the plan was to synthesise a library of pseudo-mannosides that will later be tested for specificity for different lectins and we managed to synthesise 4 final compounds, which will be tested in the future. Given that the structure involves a pseudo-mannoside which was proven to be an affective replacement for bi-mannosides in the past, they are expected to give good results. The lipophilic moieties bound through the amide bond are expected to only contribute to the binding.

Benefits of anti-adhesion therapy are reason enough in itself to continue researching the field. Compounds with anti-adhesion effects usually mimic structures on the surface of the host or structures on bacterial surface, so they are more resistant to physiological degradation. Also they do not cause resistance since their effect is not bactericidal. The host merely comes into contact with non-functional bacteria which makes it much easier to gain immunity. The main downside to anti-adhesion therapy approach is problem of achieving avidity high enough to compete with the number of adhesion molecules present on bacterial surface. This problem may be solved by introducing monovalent glycoconjugates to scaffolds, such as dendrimers or nanoparticles. This way the same range of inhibition would be achieved but with lower concentrations.

Anti-adhesion therapy is a very promising branch in synthetic development. Its many positive features like fighting infection at the very beginning, the possibility of having a drug that would be an antibiotic as well as an antiviral drug, less chance of developing resistance, are the reasons why they are excellent complementary compounds for improving anti-infection treatment and reducing antibiotic use in the future.

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