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“Synthetic Applications of Carbohydrates”

Dr. Luigi Cirillo

Supervisors
Prof. Michelangelo Parrilli Dr. Emiliano Bedini

Ph.D. Coordinator Assessor
Prof. Lucio Previtera Prof. Giovanni Palumbo
Dottorato di Ricerca in Scienze Chimiche
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“Applicazioni sintetiche di carboidrati”

Dott. Luigi Cirillo

Tutori
Prof. Michelangelo Parrilli Dr. Emiliano Bedini

Coordinatore Relatore
Prof. Lucio Previtera Prof. Giovanni Palumbo
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Chapter 1 Introduction

1.1 Carbohydrates in nature and the “sugar code”

Carbohydrates have been established as the most abundant – and arguably the most structural diverse – organic molecules found in nature. In contrast with proteins and nucleic acids, whose biological relevance has a longstanding reputation, carbohydrates have been considered always as poor-information and repetitive macromolecules with only structural functions. Only recently they have been recognized to play a crucial role in biological processes and in the transfer of biological information. It’s well known in fact that oligosaccharides and glycoconjugates (glycolipids, glycoproteins and proteoglycans) dominate the landscape of the cell (forming the so called “glycocalyx”\(^1\); Fig. 1.1) and are responsible for cell-cell interaction, the recognition of virus and bacteria, binding of toxins and are involved in cell growth, cell differentiation and cell motility.

One of the main reason why carbohydrates are widely used in the transfer of biological\(^1\)

\(\text{Fig. 1.1 The glycocalyx}\)

\(^1\) It is notable that even though the glycocalyx comprises only about 8-10% of the mass of the plasma membrane, in a typical mammalian cell it forms a continuous (albeit not uniform) layer ~8 nm thick occupying roughly the same volume as the proteins and lipids of the membrane.
information is their structural properties (e.g., glycosidic linkage variations, branching and anomeric isomers, sulfation) that makes these molecules extremely complex in contrast to nucleotides and amino acids (FIG. 1.2).

![Figure 1.2: Illustration of the chemical complexity of a monosaccharide unit compared to nucleotide and amino acid.](image1)

In addition to the chemical complexity of oligosaccharides, another factor that has to be considered in the study of biological information transfer is the shape they assume in solution. In particular the shape of a glycan is determined by the conformation of the furanose/pyranose rings and the relative positioning of the ring in the chain. As far as the ring conformation is concerned, it has been largely demonstrated that the \( \text{C}_{4} \) chair conformation (\( \text{C}_{4} \) for L-sugars) is the most energetically preferred pyranose ring structure. Some exceptions can occur, as in the case of L-iduronic acid in heparan and dermatan sulfate that assumes a skew-boat form \( \text{S}_{0} \) to accommodate mechanical stress, conformational flexibility and elasticity.

![Figure 1.3: Dihedral angles \( \Phi \) and \( \Psi \) define the possible conformation of a disaccharide.](image2)

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However, the main contribution to the definition of the shape of glycans originates from the changes of the two dihedral angles (Φ and Ψ, FIG. 1.3). Since the pyranose rings linked by glycosidic bond and their exocyclic substituents are rather bulky, their size imposes topological restraints on the intramolecular movement of the oligomers. This spatial factor that limits the range of interchangeable conformations has been inferred by molecular mechanics and dynamic calculations and by experimental evidences driving from sophisticated nuclear magnetic resonance. 4

<table>
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<tr>
<th>Lectin Source and lectin</th>
<th>Abbreviations</th>
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<tr>
<td><strong>Plant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concavalin A</td>
<td>ConA</td>
<td>Manα1-OCH₃</td>
</tr>
<tr>
<td><em>Griffonia Simplicifolia</em> lectin 4</td>
<td>GS4</td>
<td>Lewis b (Leᵇ) tetrasaccharide</td>
</tr>
<tr>
<td>Ricin</td>
<td></td>
<td>Gal(β1→4)Glc</td>
</tr>
</tbody>
</table>
| Wheat germ agglutinin    | WGA           | Neu5Ac(α2→3)Gal(β1→4)Glc 
                          |              | GlcNAc(β1→4)GlcNAc |
| **Animal**               |               |           |
| Galectin-1               | MBP-A         | Gal(β1→4)Glc |
| Mannose-binding protein A|               | High mannose octasaccharide |
| **Viral**                |               |           |
| Influenza virus hemagglutinin | HA         | Neu5Ac(α2→3)Gal(β1→4)Glc |
| Polyoma virus protein    | VP1           | Neu5Ac(α2→3)Gal(β1→4)Glc |
| **Bacterial**            |               |           |
| Enterotoxin              | LT            | Gal       |
| Cholera toxin            | CT            | GM1 pentaaccharide |

Table 1.1: some lectins and the oligosaccharide ligand they bind.

The high density of information stored as sequence and shape in oligosaccharide provides therefore a “sugar code” that can be deciphered by a number of proteins called

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Lectins. These proteins occur in a wide variety of cell-cell recognition, signaling and adhesion processes and in intracellular targeting of newly biosynthesized proteins and have a molecular complementarity that allows interaction with specific oligosaccharides (Table 1.1).

Lectins that are involved in cell-cell recognition and in adhesion in a wide variety of biological processes are called selectins (these proteins are mainly situated on the plasma membrane). One of the most known and studied process in which selectins take part is the movement of T-lymphocytes (immune cells) through the capillary wall, from blood to tissue at the site of infection or inflammation. In the vicinity of the site of infections, P-selectins can interact with a specific oligosaccharide unit that is present on the surface of the T lymphocyte. This interaction slows the T-cells as they adhere to and roll along the endothelial lining of the capillary. A second interaction between integrin molecules and an adhesion protein on the cell surface, stops the T-cells and allows them to move.

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Chapter 1: Introduction

through the capillary wall into the infected tissues to initiate the immune attack. Some microbial pathogens have lectins that mediate bacterial adhesion to host cells or toxin entry into cells. Pathogenic strains of *Salmonella*, for example, produce pili that facilitate adherence to human intestinal cell mucosa, thereby causing food poisoning and infectious diarrhea (Fig. 1.5a). In most cases these pili are formed by a major subunit from which a “tip-adhesin” protrudes and mediates for host-recognition.

![Fig. 1.6: Mechanism of viral entry into host cell](image)

In *Bordetella pertussis* instead, a surface-anchored protein (afimbrial adhesin) filamentous hemagglutinin (FHA) promotes strong attachment of the bacteria to the ciliated epithelial cells of the bronchi and trachea, triggering local inflammation and tissue injury (Fig. 1.5b). Viruses also exploit surface glycans as binding epitopes. It has been largely studied the case of influenza virus that binds to sialic acids remarkably distinguishing between α2,3-linked and α2,6-linked sialosides (Fig. 1.6a). Herpes simplex virus (HSV) adheres with the surface of the host cell firstly through a low-affinity interaction of its surface glycoproteins gB and gC. Then a higher-affinity binding of viral protein gD to a member of the tumor necrosis factor–nerve growth factor (TNF/NGF)

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receptor family promotes membrane fusion (Fig. 1.6b). Studies on the mechanism of the entry of human immunodeficiency virus (HIV) seems to involve the surface glycoprotein gp120 that binds sequentially to the CD4 receptor on T cells and then to a coreceptor such as chemokine receptor CCR4. After this interaction a conformational change in gp120 is triggered and the HIV factor is capable of initiating membrane fusion. (Fig. 1.6c).

1.1.1 **Multivalency: the way carbohydrates interact**

As mentioned above, protein-carbohydrate interactions are critical in several biological processes such as bacterial and viral pathogenesis and the inflammatory response. Despite the importance of these specific recognition processes, individual protein-carbohydrate interactions often are of low affinity and worse broad specificity: proteins often bind with little discrimination to a set of related monovalent carbohydrate structures. 14 This feature is incompatible with the relevance of carbohydrates mediated processes and the way Nature has developed to obtain stable and functional complexes is the formation of multiple carbohydrate interactions that could operate in each recognition event to give the necessary functional affinity. 15 This means that multiple receptors must be arranged in such a way as to bind efficiently to multiple saccharide ligands. The requirement for the spatial arrangement of the receptors and the binding sites on the ligand to be compatible means that the specificity in multivalent binding could be achieved, not only via complementarity between individual receptor–ligand pairs, but also by controlling the spatial arrangement between individual recognition elements of multivalent ligand (FIG. 1.7). It is worth noting that although many studies have been done, there is not a complete mechanistic description of multivalent binding because of the complexity of such systems. For a divalent ligand, it seems that the free energy of binding to a multivalent receptor will be greater than the sum of the contributions of each individual site. This primarily results from the fact that once the

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ligand has attached itself to a cell by one site, it is closer to the second site and will suffer a smaller entropy loss by binding to it.\textsuperscript{16}

It has been largely shown\textsuperscript{17} that multivalency has several functional advantages in biological processes. In a polyvalent system, for example, the strength of a signal can vary greatly, depending on the number of ligand-receptor pairs that participate. This capability to generate a broad range of signal strengths might, in principle, provide a capability to generate a graded (or graduated) response to a biological signal.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1_7.png}
\caption{Specific recognition in multivalent interactions. Cells can use several strategies to bind to a multivalent ligand: (a) forming a cluster of many monovalent receptors on a small area of the cell surface, (b) using oligomeric receptors, or (c) using receptors with more than one saccharide binding type. Multivalent ligands with incompatible relative orientations (d) or spacing (e) of the saccharide units in the multivalent array will not bind tightly.}
\end{figure}

One example of this type of graded response might be the clearance of pathogens by antibody-mediated attachment to macrophages. Where a single antibody is unable to cause a macrophage to ingest a pathogen, more antibodies should strengthen the degree of polivalency and therefore increase the likelihood that the pathogen will be recognized and cleared (FIG. 1.8a).\textsuperscript{18} On the other hand, multivalency can be used also for

preventing undesired interactions (especially those that are themselves multivalent). For example (Fig. 1.8b) mucins (proteins presenting oligosaccharides terminating in sialic acid) can bind to influenza and other SA-binding viruses and thereby inhibit their attachment to target cells. In this case multivalency can be used both for tight binding to pathogen and for steric stabilization of the pathogen surface. In Fig. 1.8c is shown also an example of the so called “conformal contact” that occurs when the formation of a multivalent bound allows an intimate contact between the two cells in order to make the communication processes more rapid and efficient.

![Fig. 1.8: Examples of multivalency in Nature](image)

Since multivalency is involved in the interaction of carbohydrates structures with their receptors, multivalent compounds are synthesized and tested for several biological applications. 19 Examples include carbohydrate functionalized gold nanoparticles whose antiadhesive properties involving cancer cell lines have been reported by Penades and Wolfenden, M.L.; Cloniger, M.J. *Bioconjugate Chem.* 2006, 17, 958.
co-workers. Also Kihlberg and co-workers reported antiviral properties of a human serum albumin as scaffold with up to 19 sialyllactose motifs attached. The glycoprotein was designed to inhibit binding of an adenovirus involved in epidemic koratoconjunctivitus and was found to generate a 1000-fold potency increase in the IC$_{50}$ value.

Recently Disney and co-workers reported the synthesis of a mannose-functionalized polymer (poly (p-phenylene ethynylene) PPE, FIG.1.9) which allowed multivalent detection of Escherichia Coli bacteria. Through laser scanning confocal microscopy it was possible to detect fluorescent bacterial aggregate due to the multivalent interactions between the mannose binding bacterial pili and the functionalized polymer (FIG. 1.10). Several

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experiments with diluted solutions of mannose-binding *E. coli* were performed and it was clearly shown that fluorescently stained clusters could be observed with as little as $10^4$ bacteria.\textsuperscript{23}

The multivalent binding of the polymer was demonstrated by testing it for inhibition of ConA-induced hemagglutination of sheep erythrocytes. The concentration of mannose displayed by the PPE to inhibit hemagglutination was more than 500-fold less than the monomeric mannose derivatives, indicating that polymers can bind Con A in a multivalent manner.

1.2 Synthetic Aspects

1.2.1 Protecting Groups in Carbohydrate Chemistry

In organic chemistry protecting groups are often divided in two categories: permanent protecting groups (which will remain through all the synthetic steps and will be removed at the end of the synthesis) and temporary protecting groups (whose removal occurs generally during the synthesis for the liberation of the functionality). The protecting groups used in carbohydrate chemistry are the same as in the other areas of organic chemistry. The conditions for their introduction and removal and their stability and orthogonality are also the same. The main difference in carbohydrate chemistry is the vast number of protecting groups needed and the continuous research of regioselective protections. It is worth noting that in carbohydrate chemistry protecting groups not only protect, but they also confer other effects on the molecule: for example, they increase or decrease the reactivity and they can also participate in reactions, thus affecting the stereochemical outcomes.

The most important protecting groups in carbohydrate chemistry are obviously the hydroxyl protecting groups, including the anomeric hemiacetals ones. Amino-protecting groups (for amino-deoxy sugars) for the synthesis of aminosugars-containing oligosaccharides are also of interest.

1.2.1.1 Hydroxyl protecting groups

This kind of protecting groups are the most widely used in carbohydrate chemistry because of the large number of hydroxyl groups to protect.

For a permanent protection of the free hydroxyl groups only acetate and benzoate esters and benzyl ethers are typically used due to their stability and efficient introduction/deprotection properties needed to make them suitable for these purposes.

As opposed to the small selection of permanent protecting groups, there is a wide choice of temporary protecting groups and new protection methods are continuously being developed.

1.2.1.1.1 Esters protecting groups

The most common esters protecting groups are reported in the Fig. 1.11. Standard conditions for esterification, which can be performed also on reducing sugars, are the acyl chloride (especially for benzoates) or the anhydride (especially for acetates) in pyridine. The acetylation can be performed also in acid conditions using in situ generated HI for the activation of the acetic anhydride. 25 In addition esters (especially acetates) have a tendency to migrate both in acidic and basic conditions. 26 For example, in cis-
hydroxyls there is normally a preferred migration from the axial position to the equatorial one and in 4,6-diols the migration goes from O-4 to O-6 preferentially. Esters can be easily cleaved using base conditions such as NaOMe, KOH and NH₃, but they can be also cleaved using acid conditions (MeOH/HCl). The relative order of stability of the most common ester groups reflects both steric and electronic aspects and is as follows:

\[ t\text{-BuCO} > \text{PhCO} > \text{MeCO} > \text{ClCH₂CO} \]

It is worth noting that acetates can be also used as temporary protecting groups in presence of benzoates because they can be selectively removed both under acid conditions (MeOH/HCl) and in basic conditions (Mg(OMe)₂/MeOH). As far as the cleavage of chloroaetate esters is concerned, several mild reagents have been reported such as 2-mercaptoethylamine, thiourea or hydrazinedithiocarbonate (H₂NNHC(=S)SH).

1.2.1.1.2 Ethers protecting groups

The most common ether protecting groups are reported in Fig. 1.12. They include benzyl and p-methoxybenzyl, allyl, trityl and silyl ethers. Benzyl ethers are widely used as permanent protecting groups for their well-known stability. Their installation can be performed under strongly base conditions (benzyl bromide and sodium hydride in a nonprotic polar solvent). It has been also demonstrated that benzylation can be performed with benzyl trichloroacetimidate and a

catalytic amount of acid (generally TfOH) with good yields. Benzyl ethers are usually cleaved through catalytic hydrogenolysis employing various Pd-catalysts. An alternative is a Birch-type reduction (Na/liquid ammonia) which is surprisingly mild towards other functionalities such as phosphates and even the hemiacetals of reducing sugars.

There is a clear distinction between in the reaction conditions for the installation of the \( p \)-methoxybenzyl and allyl ethers (usually formed under strongly basic conditions – \( p \)-methoxybenzyl and allyl bromide with sodium hydride) and the trityl and silyl ethers (usually formed under weakly basic conditions – typically with trityl or silyl chloride in pyridine).

The \( p \)-methoxybenzyl group can be easily removed under oxidative conditions with DDQ \(^{32}\) or CAN \(^{33}\) in wet dichloromethane or acetonitrile, while allyl ethers are typically cleaved with a two stage deprotection: initial rearrangement of the allyl ether using strong base or metal-complex catalysts (e.g. Wilkinson’s catalyst) is followed by Lewis

\( \text{Bn} = \text{CH}_2\text{O} \)

\( \text{pMBn} = \text{CH}_2\text{O} \text{Me} \)

\( \text{All} = \text{CH}_2\text{CH} = \text{CH}_2 \)

Fig. 1.12: Commonly used ether protecting groups

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acid-catalyzed hydrolysis of the obtained vinyl-ether. Recently SmI$_2$ was reported as an efficient deallylation reagent following a radical mechanism (it is worth noting that in these conditions anomeric allyl groups are relatively stable).  

TBDMS and TBDPS are the most common silyl ethers used in carbohydrate chemistry for their acid stability. A problem of this kind of protecting group is that they, like esters, can migrate to neighboring free hydroxyl groups under mild base conditions. Silyl ethers can be easily cleaved by treatment with fluoride ion (HF/acetonitrile; TBAF/THF).

Tritol protecting groups are cleaved by acid hydrolysis. Higher is the number of mono- or di-methoxy substituents on the trityl, more labile is the group toward acid hydrolysis. Noteworthy, no trityl protecting groups are able to withstand classical glycosylation conditions.

1.2.1.1.3 Acetals and ketals protecting groups

Among the known acetals and ketals protecting groups (FiG. 1.13), in carbohydrate chemistry benzylidene and isopropylidene acetals are the most widely used ones for simultaneous protections of two hydroxyl groups. They are introduced by standard conditions (the aldehyde or the dimethoxy-acetal and some acid catalysis) and are removed by acid hydrolysis (regioselective opening of these protecting groups will be discussed afterwards). Benzylidene acetals are formed preferentially as six-membered dioxane cycles (i.e. 4,6-O-benzylidene derivatives), while isopropylidene acetals are more

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**Fig. 1.13**: Acetal and ketal protecting groups in common use.

**Fig. 1.14**: Acetal and ketal formation on methyl α-D- manno pyranoside

**Fig. 1.15**: Acetal and ketal formation on methyl α-D- galactopyranoside

**Fig. 1.16**: Formation and opening of 1,6-anhydro derivative
stable as five-membered dioxolane rings formed on \textit{cis}-diols. Examples of acetics formation and not selective deprotection are reported in Fig.1.14 and Fig.1.15 An important alternative for benzylidene acetal is the more acid labile \textit{p}-methoxybenzylidene acetal that can be cleaved selectively in the presence of the benzylidene one. In contrast to benzylidene, isopropylidene and \textit{p}-methoxybenzylidene acetics, that exhibit all the same regioselectivity for vicinal \textit{cis}-diols, the newly developed butane-2,3-diacetal (BDA) shows completely different regioselectivity providing simultaneous protection of \textit{trans}-diols. \footnote{36}

Silyl acetics are also used as a valid alternative to the acetics discussed thus far and they can be removed by treatment with fluoride ion.

\textbf{1.2.1.2 Anomeric protecting groups}

In general anomeric position is the first one to be protected during a series of protecting groups manipulations. The anomeric centre can be protected as an alkyl, benzyl or allyl glycoside by a classical Fischer reaction in which a monosaccharide is suspended in an alcohol and treated with a catalytic amount of an acid. An acyl group can be also used for the protection of the anomeric centre, it can be removed under milder basic conditions (e.g. hydrazine acetate or ammonium carbonate in DMF, \footnote{37} piperidine in THF).


More specific anomic protecting groups are the 2-trimethylsilylethyl (TMSE) \(^{38}\) and p-methoxyphenyl glycosides, \(^{39}\) both introduced by glycosylation reactions on protected derivatives. The former is cleaved by treatment with BF\(_3\)OEt\(_2\) while the second is cleaved by CAN-oxidation similarly to p-methoxylbenzyl ethers.

1,6-Anhydro bridges can be considered as a protecting group for the anomeric centre (and the 6-hydroxyl group). 1,6-Anhydro derivatives are formed by an internal displacement where the leaving group can be in either the 1- or the 6- position. \(^{40}\)

Examples are the treatment with bases of phenyl \(\beta\)-glycosides or 6-O-tosyl-derivatives with a free hemiacetals group or the treatment with Lewis acids of methyl glycosides at high temperatures (FIG. 1.16). \(^{41}\) The 1,6-anhydro-bridge is stable to most reaction conditions but very susceptible to mild acetolysis conditions to give the 1,6-diacetates.

1.2.1.3 Amino protecting groups

The most common protecting groups for amino functions in carbohydrate chemistry are represented in FIG. 1.17. It is worth noting that an important feature of the protecting groups used for the amino function at position 2 is to allow the stereoselective formation of the correct anomer during glycosylation reactions (i.e., participating groups such as amides or carbamates for 1,2-trans-linkages and nonparticipating groups for 1,2-cis-linkages). Presently there is one generally useful amine precursor that is nonparticipating that is the azido (N\(_3\)) group.


\(^{41}\) Aberg, P.; Ernst, B. Acta Chem. Scand. 1994, 48, 228.
Among the amide type protecting groups the acetyl group is usually discarded since, in glycosylation reactions, it participates forming a stable oxazoline ring instead of the desired glycoside. For this reason there is a wide interest in the development of 2-amino protecting groups with the ability to participate but not form oxazolines.  

The first developed protecting group is the phthalimido that can be incorporated treating the substrate with phthalic anhydride in basic conditions. A drawback of this method is the hard basic condition (typical hydrazine, hydroxylamine and alkyldiamine) needed for its removal, which results in low yields of the unprotected derivatives. For this reason other phthaloyl-based protecting groups such as the tetrachlorophthalimides (TCP) and dimethylmaleoildes (DMM) were developed: they are formed in similar conditions but they can be removed under milder basic conditions. Another frequently

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participating protecting group in oligosaccharide synthesis is the trichloroethylcarbamate (Troc) group. 46

The azido group can be easily introduced through a diazo-transfer reaction using triflyl azide 47 or imidazole-1-sulfonyl azide hydrochloride 48 and converted in the amino free group under hydrogenolysis conditions.

1.2.1.4 Selective Protection and Deprotection Methodologies

1.2.1.4.1 Selective Protection Methodologies

The purpose of developing selective protection for the preparation of building blocks useful for the synthesis of oligosaccharide structures has been carried out over the years but it is still an important topic in the carbohydrate chemistry. In the scheme below are represented the most commonly utilized protection methodologies:

- Different reactivity of the OH groups;
- Stannyl Activation;
- Phase-Transfer Alkylation and Acylation;
- Cu(II) Activation;
- Reductive Opening of the cyclic Acetals;
- Cyclic Orthoesters Opening.

• **Different reactivity of the OH groups:**

To simplify, the reactivity order of hydroxyl groups in saccharides is primary OH > equatorial OH > axial OH, mainly due to steric factors. 26 Thus using a bulky reagent (e.g. triphenylmethyl or tert-butyldiphenylsilyl chloride, FIG. 1.18) under various basic conditions it is possible to obtain the 6-O-monoprotected hexose derivative in high yields. This is especially useful if the anomeric position is already protected.

Additionally, primary hydroxyl groups can be selectively benzoylated using special reagents as benzoyl cyanide/triethylamine 49 or 1-acyloxy-1H-benzotriazoles. 50 Finally, regioselective acylations catalyzed by lipases are possible with the most common site of reaction at primary hydroxyls. 51

• **Stannyl activation:**

By reacting the hydroxyl groups of saccharides with alkyl tin oxide reagents, stannylene ethers and acetals are formed. The formation of such derivatives enhances the nucleophilicity of the oxygen atoms in a regioselective way and makes the consecutive

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alkylation or acylation of saccharides possible. The regioselectivity associated with stannyl activation is irrespective of which type of alkyltin derivative is used. The most common alkyl tin oxide reagent is dibutyltin oxide producing dibutyl stannylene acetals that, after treatment with an appropriate electrophile, give access to esters or silyl or alkyl ethers. The structure of the intermediate stannylene is not yet well understood.

The outcome of the reaction is not always predictable because of the selectivity depends on several parameters (the electrophile, additives, etc) but, generally speaking, dibutyl tin acetals derived from mixed primary and secondary diols are selectively alkylated at the primary positions. A tin acetal derived from a cis-1,2-cyclohexanoid diol is selectively

\[ \text{Fig. 1.19: Examples of stannyl activated regioselective protection} \]

alkylated at the equatorial position, whereas regioselectivities are generally poor for equatorial-equatorial diols. As far as the formation of esters via stannylene is concerned, the regioselectivities are generally poorer as those observed with alkylations and so the methods is not very common (FIG.1.19).

- **Phase-Transfer Alkylation and Acylation:**

In this method a two-phase system (H$_2$O/CH$_2$Cl$_2$) is used. The aqueous phase contains a base (NaOH, 5%) and the organic phase an electrophile, usually an alkyl halide. The diol to be protected is usually partitioned between the two phases. In the water phase one of the two hydroxyl groups is selectively deprotonated and with the assistance of a phase-transfer reagent (usually a tetrabutylammonium salt) the molecule is transported in the organic phase where it is selectively alkylated or acylated. Regarding regioselectivity, primary hydroxyl groups are preferentially protected and in the case of 2,3 diols the oxygen at position 2 is selectively protected because of its higher acidity due to the proximity of the ring oxygen (FIG. 1.20).

![Phase-transfer benzylations](image)

**FIG. 1.20 Examples of phase-transfer benzylations**

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Owing to the strongly basic conditions, this method is incompatible with acylations. However, it is reported a selective tosylation with high regioselectivities through phase-transfer techniques.  

- **Cu(II) Activation:**

Dianions can be easily obtained treating diols with a strong base such as NaH. Once they are formed, it is possible to complexate them by copper(II) ions. Reaction of the complexes with various electrophile agents (alkyl halides, acyl chlorides) can lead to the formation of selectively protected sugar derivatives. Using this method disubstituted products are obtained in low yields and it is worth noting that the regioselectivity is completely reversed to those observed in the phase transfer methodologies (*i.e.*, 4,6-diols give mainly 4-substitution and 2,3-diols give mainly 3-substitution, Fig. 1.21).

![Diagram of copper complexation and selective protection](image)

**Fig. 1.21: Examples of regioselective protection from copper complexes**

Using this technique both selective alkylation (allylation, benzylation) and acylation (acetylation, benzoylation and pivaloylation) are possible but often the outcomes depend on reaction conditions and on structural features.  

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- **Reductive Opening of Cyclic Acetals:**

The importance of cyclic acetals as protecting groups is strongly increased since it has been possible to open up the acetal ring regioselectively in order to obtain alkyl ethers and a free hydroxyl group.  

All the methods for the reductive opening of acetals are based on a combination of an hydride reagent with a Lewis acid (or a proton acid): firstly, LiAlH$_4$/AlCl$_3$ were used for the reductive opening of benzylidene acetals. This method yields 4-O-benzyl derivatives with high selectivity especially if there is a bulky substituent in the 3-position. In dioxolane acetals of cis-diols (e.g., 2,3-manno- or 3,4-galacto-), the selectivity depends on the configuration of the acetal: exo-phenyl derivative gives the equatorial benzyl ether while the endo-derivative gives the axial one with absolute selectivity. This methodology is not always applicable because of the incompatibility of several protecting groups with the reagents system used (Fig. 1.22). For these reasons several procedures for reductive benzylidene opening have been developed that give preferentially the 4-O-benzyl or the 6-O-benzyl derivatives (Table 1.2).

The same procedures (with slight modifications) have been used also for the regioselective opening of other types of acetal protecting groups such as p-methoxybenzylidene acetals and allyl-type acetals (Fig. 1.22).

Subsequently, there has been a continuous development of new reagents, all of which are variations of the same general theme (Lewis acid/hydride reagent), optimizing yield and selectivity for specific derivatives.

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Benzylidene acetals can also be opened under oxidative conditions to give benzoyl esters halogen derivatives (Figure 1.23a) (which are useful for the synthesis of deoxy-sugars), or simply hydroxyl benzoates (Figure 1.23b).

<table>
<thead>
<tr>
<th>Lewis Acid / Proton Acid</th>
<th>Reducing Agent</th>
<th>Solvent</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ph₃BBr</td>
<td>PhS or BH₃·THF</td>
<td>CH₂Cl₂</td>
<td>6-OH 61</td>
</tr>
<tr>
<td>Bu₂BOTf</td>
<td>BH₃·THF</td>
<td>CH₂Cl₂</td>
<td>6-OH 62</td>
</tr>
<tr>
<td>AlCl₃</td>
<td>Me₃NBH₃</td>
<td>PhCH₃ or CH₂Cl₂</td>
<td>6-OH</td>
</tr>
<tr>
<td>BF₃OEt₂</td>
<td>Me₂NHBH₃</td>
<td>CH₂Cl₂</td>
<td>4-OH 63</td>
</tr>
<tr>
<td>HCl</td>
<td>NaCNBH₃</td>
<td>THF</td>
<td>4-OH 65</td>
</tr>
<tr>
<td>CF₃COOH</td>
<td>Et₃SiH</td>
<td>CH₂Cl₂</td>
<td>6-OH 58</td>
</tr>
<tr>
<td>CF₃SO₂H</td>
<td>NaCNBH₃</td>
<td>THF</td>
<td>4-OH 61</td>
</tr>
<tr>
<td>Cu(OTf)₂</td>
<td>BH₃·THF</td>
<td>CH₂Cl₂</td>
<td>6-OH 66</td>
</tr>
</tbody>
</table>

Table 1.2: Methods currently used for regioselective opening of benzylidene acetals.

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Fig. 1.22: Examples of regioselective reductive benzylidene opening

Fig. 1.23: Examples of oxidative opening of benzylidene acetals
**Cyclic Orthoester Opening:**

Cyclic orthoesters cannot be considered as a class of protecting groups because of their extreme acid lability. They are however useful intermediates for the synthesis of building blocks for glycosylation reactions. One of the major advantages of the orthoester method is that they can be opened by mild hydrolysis to give the corresponding ester derivative exposing a free hydroxyl group. 

Usually, the opening of the orthoester ring of a vicinal *cis*-diol gives the ester on the axial protecting group. Care has to be taken in the opening process to avoid acyl migration to the unprotected hydroxyl group (this process is however slow in acid solutions and can be avoided using orthobenzoates instead of orthoacetates).

![Diagram of orthoester formation and openings](image)

**Fig. 1.24:** Examples of orthoesters formation and openings.

In 4,6-*O*-orthoesters, the opening reaction gives a mixture of 4-*O*-protected sugar and 6-*O*-protected sugar; this feature is not relevant when a 6-*O*-acetate is desired because the acetate group can easily migrate from position 4-*O* to position 6-*O*. When 2,3-...
orthoesters of thioglycosides are used, care must be taken to avoid participation of the sulfur affording the formation of 2-thiolated-O-alkyl glycosides.  

1.2.1.4.2 Selective Deprotection Methodologies

Selective deprotection methodologies are not as common as selective protection ones and several efforts are made to improve their efficiency. In literature, however are reported few standards methods that utilize this approach. For example a well-known example is represented by the selective removal of the primary benzyl ethers by acetylation (FIG. 1.25).  

![Fig. 1.25: Regioselective deprotection of a primary hydroxyl group](image)

It has also been reported a debenzylation procedure that allows the selective removal of benzyl groups in anhydro-sugars with a catalysis promoted by Lewis acids. The outcome of the deprotection was dependent on the Lewis acid and on the configuration of the sugar (FIG. 1.26).  

Also primary acetyl groups can be selectively removed utilizing lipases but the applicability of the method is too substrate-dependent to be considered of general relevance.

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Fig. 1.26: Lewis acid catalyzed debenzylation.
1.2.2 The Formation of the Glycosidic Linkage

Interglycosidic bond formation is generally achieved by condensing a fully protected glycosyl donor, which bears a potential leaving group (LG) at its anomeric centre, with a suitable glycosyl acceptor that contains often only one free hydroxyl group (FIG. 1.27). \(^72\)

![Figure 1.27: Basic glycosylation reaction mechanism](image)

The reaction is usually performed in presence of an activator called “promoter”, whose function is to assist the departure of the leaving group. The promoter is often used in catalytic amounts, although in some instances they are used in stoichiometric amounts. Other additives such as molecular sieves or any base that may act as acid scavenger are used. An aspect to be considered is without doubt the electronic feature of the protecting groups on both glycosyl acceptor and glycosyl donor. On this point is based the “armed-disarmed strategy” elaborated by Fraser-Reid and coworkers in 1988: \(^73\) It was noted that ester-type protecting groups (OAc, OBz, etc.) strongly lowered the reactivity of \(n\)-pentenyl glycosyl donors, in comparison to the effect of ether-type protecting groups. A justification of such an observation, was that electron-withdrawing groups such as esters protecting groups decrease the electron density and then the nucleophilicity of leaving group.

Recently, Demchenko and coworkers classified glycosyl donors as superarmed, armed, moderately armed and disarmed. The classification took place from the observation that perbenzylated \(S\)-benzoxazolyl glycosides were less reactive than perbenzylated glycosyl

donors with an ester protecting group at C-2 (FIG. 1.28). This difference in reactivity was explained by the assistance of the departure of the leaving group by the ester protecting group at C-2 (this effect is called “neighboring group participation” and has relevant effects on the stereochemical outcome of a glycosylation reaction, as it will be discussed in next section). The increased reactivity could be explained therefore in term of the “O-2/O-5 cooperative effect”: the stabilization for the glycosylation intermediate could be achieved through two possible sources of electron pair donation. The first comes from a lone pair on the neighboring endocyclic ring oxygen, O-5 and the second deriving from the anchimeric assistance of the protecting group at C-2. It is obvious that electron-withdrawing groups will decrease the amount of the first electron donation.

**1.2.2.1 Stereochemical Aspects of Glycoside Bond Formation**

As anticipated before, the formation of a new glycosidic linkage is one of the most challenging aspects of oligosaccharide synthesis and there are many factors that affect the outcome of a glycosylation reaction in terms of yield and α:β ratio. In relation to the

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orientation of the substituent at the neighboring carbons, the glycosidic linkages can be divided in 1,2-trans and 1,2-cis glycosidic bonds. The ease of formation of these bonds is strictly related to the strength of the anomeric effect, which is comparatively stronger in the α-manno-type than in the α-gluco-type sugars, and on the protecting group pattern on the glycosyl donor. Thus, the ease of glycoside bond formation decreases from the α-manno- by the β-gluco-, to the α-gluco- and, finally, to the β-manno-type (FIG. 1.29).

The reason why 1,2-trans glycosidic bonds are simpler to obtain is the possibility to install at the C-2 position a protecting group (a classical example is the acyl protecting group) that assists the departure of an activated leaving group forming a more stable dioxolenium ion (FIG. 1.30). Consequently the glycosyl acceptor can only attack from the back side to form the 1,2-trans glycoside. A drawback in the use of the participating protecting group is the possible formation of an orthoester that prevents the formation of a 1,2-trans glycoside. 76

Beside acyl groups, other participating groups have been used for the synthesis of 1,2-
trans glycosidic bond: among these the dialkyl phosphates protecting groups, \(^{77}\) the 2-
pyridylmethyl group \(^{78}\) and a wide number of “improved” esters groups (such as 4-
acetoxy-2,2-dimethylbutanoyl ester (ADMB), \(^{79}\) 3-(2-hydroxyphenyl)-3,3-
dimethylpropanoate (DMBPP) and 3-(2-hydroxy-4,6-dimethylphenyl)-3,3-
dimethylpropanoate groups (TMBPP) \(^{80}\) and methylsulfonylthoxycarbonyl (Msc) \(^{81}\))
that have the advantage to decrease the amount of orthoester formation.

It is worth noting that nowadays protecting groups are designed not only for the
obtainment of 1,2-\(\text{cis}\) glycosides. This is the case of the chiral auxiliary groups developed
by Boons and coworkers in 2005 for stereoselective glycosylation reactions. \(^{82}\)

\(^{82}\) Kim, J.H.; Yang, H.; Boons, G.J. Angew. Chem. Int. Ed. 2005, 44, 947. For applications of this
auxiliary group is formed by a 1-phenyl-ethyl moiety with a nucleophilic group at one end. According to the configuration of the chiral centre, the auxiliary can lead to the preferential formation of a cis- or trans-decalin system because of steric interactions (Fig. 1.31). The attack of the nucleophile on this will give the 1,2-cis glycoside or the 1,2-trans glycoside. This technique has been successfully applied for the synthesis of branched oligosaccharides \(^{83}\) and of a repeating unit of a \(\alpha\)-glucan pentasaccharide found in \(A.\) carmichaeli. \(^{84}\)

All the examples considered up to know were based on the participation of a group on the \(C-2\). Participating protecting groups at other positions than \(C-2\) also exist and are called “remote participation groups”. \(^{85}\) They are largely used and, although it is hard to say

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\(^{83}\) Boltje, T.J.; Kim, J.; Park, J.; Boons, G.J. Nat. Chem. 2010, 2, 552.


how these groups work, their application is surely helpful in the development of stereocontrolled oligosaccharide synthesis.

When there is no a participating group on the glycosyl donor, the anomic outcome of the glycosylations is markedly influenced by the nature of the solvent (the so called “solvent participation”). Ether-type solvents have a tendency to shift stereoselectivities through the formation of \(\alpha\)-glycosides because of the preferential formation of the \(\beta\)-oxonium ion intermediate (for reverse anomeric effect) that reacts to form the \(\alpha\)-glycoside. \(^{86}\) Nitrile-type solvents have a well-known tendency to form \(\beta\)-glycosides. It is not known yet which mechanism is involved in this case but it seems that acetonitrile, as polar solvent, favors an \(S_N1\) mechanism implying the formation of an oxonium ion that is solvated preferentially at the \(\alpha\)-face forming the kinetically controlled \(\alpha\)-nitrilium-nitrile complex. This complex finally gives the \(\beta\)-glycoside after the attack of the glycosyl acceptor (in this case the equilibration process to the most thermodynamically stable \(\beta\)-nitrilium-

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nitrile for reverse anomeric effect is slower than for ether-type oxonium ion). For these reasons the glycosyl acceptor attack forms the β-glycoside (Fig. 1.32). 87

1.2.2.2 Glycosyl Donors and Activation Conditions

Glycosyl donors are usually divided according to the heteroatom attached to the anomeric centre. A rough classification of different types of glycosyl donors is represented in (Fig. 1.33). The number of glycosyl donors and activation conditions is still increasing and several efforts are made to elaborate methodologies of wide applicability. 88 Here will be discussed only the most common and used glycosyl donors: glycosyl halides, thioglycosides, trichloroacetimidates and N-phenyltrifluoroacetimidates.

Fig. 1.33: Types of glycosyl donors.

1.2.2.2.1 Glycosyl halides

Glycosyl bromides and chlorides are the first class of glycosyl donors used in glycosylation reactions. Glycosyl bromide can be easily obtained by treatment of a per-

O-acetylated sugar with a solution of HBr in acetic acid. The more stable α-anomer is usually obtained in high yield. Glycosyl chloride can be obtained instead by treatment of aldosyl acetates with aluminum chloride or phosphorous pentachloride. A milder and more versatile procedure for the synthesis of glycosyl chloride is based on the Vilsmeier-Haack reagent (Me$_2$N=CHCl) formed by reaction of N,N-dimethylformamide (DMF) with oxalyl chloride [ClC(O)C(O)Cl]. In general glycosyl bromides are more reactive than glycosyl chloride. Glycosylation reactions involving glycosyl chlorides and glycosyl bromides are performed with a heavy metal salt as promoter (the procedure is known as Koenigs-Knorr reaction). Most used promoters are Ag$_2$CO$_3$ or Ag$_2$O, silver imidazolate, Hg(CN)$_2$ and HgBr$_2$, HgO and HgBr$_2$ and AgClO$_4$. It is worth noting that besides heavy metals salts, glycosyl chlorides and bromides could be activated also with Lewis acids such as SnCl$_4$, BF$_3$OEt$_2$, ZnCl$_2$, ZnCl$_2$–TrCl, Sn(OTf)$_2$, Cu(OTf)$_2$ or InCl$_3$.

**Glycosyl fluorides** are very stable compared to glycosyl chlorides and bromides and for a long time they were not considered as glycosyl donors. However, Mukaiyama and coworkers demonstrated that these compounds could be activated with AgClO$_4$/SnCl$_2$.

Several methods for the activation of glycosyl fluorides have been reported and

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Chapter 1: Introduction

now commonly used promoters are BF₃·OEt₂, Cp₂MCl₂·AgClO₄ (M=Hf, Zr), and Cp₂HfCl₂·AgOTf. Several methods are reported for the synthesis of glycosyl fluorides but the most common procedure is based on the reaction between a thioglycoside with NBS and (diethylamino)sulfur trifluoride (DAST) or the reaction of a lactol with DAST or 2-fluoro-1-methylpyridinium p-toluensulfonate.

**Glycosyl iodides** have been considered for a long time too unstable to be of synthetic utility and only recently it has been shown that they can offer some advantages respect to glycosyl bromides and chlorides in terms of time, efficiency and selectivity.

![Glycosylation reaction](image)

**Fig. 1.34:** Examples of glycoside synthesis with glycosyl iodides as donors.

Glycosylation reactions involving glycosyl iodides occurs usually with a S₂N₂ displacement of the anomeric iodide and this feature is of particular interest when there

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is not a participating protecting group at C-2 as it can be shown in Fig. 1.34a. Very recently the high selectivity of glycosyl iodides in glycosylation reactions has been also utilized for the synthesis of morphine-6-glucuronide, an important starting material for pharmacological evaluation.  

1.2.2.2 Thioglycosides

Thioglycosides are also very common in carbohydrates synthesis; the advantage of thioglycosides is that the thioether group on the anomeric position is stable under a wide range of protecting group manipulations, thus acting itself as a temporary protecting group.

Thioglycosides are often prepared treating a per-O-acetylated sugar with a thiol in presence of an activator (e.g. BF₃·OEt₂). Other procedures involve the initial formation of a S-glycosyl isothiouronium intermediate salt which is then S-alkylated by treatment with a mild base and an appropriate alkyl halide (usually the isothiouronium intermediate is formed from a glycosyl bromide or per-O-acetylated glycosyl iodide). This procedure has the advantage to avoid the use of thiols that are well-known smelly reactants.

Despite their stability, thioglycosides can be activated using mild thiophilic reagents (usually soft electrophiles) (FIG. 1.35). The sulfonium ion (B) is then a better leaving group and the oxonium ion (C) is rapidly formed.\(^\text{113}\)

The first method for the activation of thioglycosides was reported by van Boom and coworkers and is based on the use of a stoichiometric amount of N-iodocuccinimide (NIS) with a catalytic amount of triflic acid used as promoter.\(^\text{114}\) Other \textit{in situ} generating iodonium systems have been proposed (\textit{e.g.} NIS/AgOTf)\(^\text{115}\) and many variants have been developed. For example, Mukaiyama and coworkers have introduced a new activation methodology based on the use of either NIS or NBS and a catalytic amount of TrB(C\(_6\)F\(_5\))\(_4\) as promoter systems.\(^\text{116}\) Recently, also bromonium ion (generated \textit{in situ} by the reaction of N-bromosuccinimide and a catalytic amount of Bi(OTf)\(_3\)) has been employed for the activation of thioglycosides.\(^\text{117}\) Other activation methods are based nowadays on soft sulfur electrophile. Initially sulfonium or sulfenyl triflates (DMTST, MeSOTf and PhSOTf) were used but recently sulfenamide activators in combination with Lewis acids were proposed.\(^\text{118}\) Sulfinates in combination with Tf\(_2\)O have received much attention because it has been demonstrated that these sulfinyl systems are able to

\(^{113}\) Several methods are reported for the activation of thioglycosides; for a recent list of activation methods (1998-2007) see ref. 89a.


activate thioglycosides also at low temperature.\textsuperscript{119} Sulfinyl-systems mediated glycosylations have been used for the synthesis of numerous complex structures.\textsuperscript{120} An interesting example of these reactions is shown in FIG. 1.36, in which a tetrasaccharide was constructed one-pot in less than 2 h from the thioglycoside building blocks by using benzensulfinyl morpholine/Tf$_2$O as promoter in the preactivation steps.\textsuperscript{121}

\begin{center}
\includegraphics[width=\textwidth]{fig136.png}
\end{center}

\textbf{FIG. 1.36: Oligosaccharides synthesis with thioglycosides as donors.}

1.2.2.3 O-Glycosyl Imidates

O-Glycosyl imidates (and in particular trichloracetimidates and more recently also N-phenyltrifluoroacetimidates) are probably the most common glycosyl donors used in glycosylation reactions.

Glycosyl trichloroacetimidates are usually prepared by treatment with the electron-deficient trichloroacetonitrile (Cl₃CCN) that adds to the hydroxyl of lactols under basic conditions. In presence of a weak base (e.g. K₂CO₃), it is possible to isolate the β-imidate as the kinetic product because in these conditions the β-alkoxide forms preferentially. Using a strong base (DBU or NaH) alkoxide equilibration occurs with the more stable α-alkoxide predominating and giving the α-trichloroacetimidate. ¹²³

Recently also polymer-supported DBU and TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene) have been employed for the synthesis of trichloroacetimidates.

The most common activation catalysts for trichloroacetimidates are TMSOTf and BF₃·OEt₂ (using the latter promoter at very low temperature and in absence of a

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participating substituent it is possible to have a S_N2-type glycosylation reaction with inversion of configuration of the anomeric centre involved in the glycosylation). It is worth noting that the trichloroacetamide released after the activation is not basic and the acid is not consumed by the leaving group.

Several other promoters have been developed including protic and Lewis acids (such as TfOH, pyridinium p-toluensulfonate (PPTS), and ZnBr). Various metal triflates (Sm(OTf), Sn(OTf), Yb(OTf), and Cu(OTf) ) are used nowadays for the activation of trichloroacetimidates. The advantage of this reagents is that they can be easily stored without particular precautions and sometimes give better results in terms of yield and stereoselectivity: for example, AgOTf was used as catalyst and found more efficient in TMSOTf-sensitive glycosylation reactions.

More recently, silica supported perchloric acid (HClO-SiO), HB(C_F)_4, I/ET_3SiH system and acid washed molecular sieves were used as efficient promoters in various glycosylation reactions with trichloroacetimidates as glycosyl donors.

Besides the introduction of new promoters, a new technique in the glycosylation reaction is the so called inverse procedure (Fig. 1.38). According to this procedure, the donor is added to a mixture of glycosyl acceptor and promoter in order to minimize the decomposition products (such as orthoesters). In the inverse procedure it is thought that acceptor molecules aggregate around the catalyst and the intramolecular glycosylation takes place.

Glycosyl trichloroacetimidates have been largely used up to now in the synthesis of a large numbers of biological relevant molecules such as calicheamicin, eleutherobin, glycosphingolipids and vancomycin.

**Glycosyl N-phenyltrifluoroacetimidates** (PTFAI) are generally prepared treating an anomeric hemiacetal with N-phenyltrifluoroacetyl chloride with a base (K$_2$CO$_3$, DBU, DIPEA, NaH or Cs$_2$CO$_3$) in CH$_2$Cl$_2$ or THF (Fig. 1.39). This type of

\[ \text{Fig. 1.38: Yield difference between normal and inverse procedure.} \]

---

glycosyl donors can be activated by the same activators used for the activation of trichloroacetimidates but often they require more forceful conditions. This difference in reactivity was used to activate a trichloroacetimidate glycosyl donor in presence of a PTFA in a multistep procedure. 151

The great diffusion of this type of glycosyl donors can be explained in term of a greater stability respect to the trichloroacetimidates 152 and of a minimization of the by-products often encountered with trichloroacetimidate donors: in the course of a trichloroacetimidate glycosylation, trichloroacetamide liberated from the donor can give N-glycosides. The use of PTFAI donors have demonstrated to minimize the amount of by-products in the synthesis of β-mannosides 153 and in the synthesis of cell-wall

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151 Adinolfi, M.; Iadonisi, A.; Ravidà, A. Synlett 2006, 583.
152 For a recent minireview on PTFAI donors see: (b) Yu, B.; Sun, J. ChemCommun. 2010, 46, 4668.
polysaccharide fragment of *Bacillus anthracis*. PTFAI donors has been extremely useful in glycosylation reactions involving deoxysugars as, for example, in the synthesis of the O-antigen from *Xanthomonas campestris pv campestris* (Fig. 1.40). In the first glycosylation reaction also TCAI and phosphate donors were used but the yield and the stereoselectivity were not successful while the use of PTFAI with a non-participating benzylsulfonyl group at C-2 position provided the desired disaccharide in a 99% yield and an α:β ratio of 2:3.

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1.3 **Aim of the thesis**

For the reasons set out in the previous section, several efforts have been made from the scientific community to have a better understanding of how carbohydrates interact in biological systems and which role they play.

In this thesis some synthetic aspects of carbohydrates will be treated. In particular in Chapter 2 it will be explored the mechanism and synthetic potentials of the acetolysis reaction on 6-deoxysugars.

In Chapter 3 it will be proposed a new synthetic methodology for the obtainment of peptidoglycan fragments in order to have a better understanding through biological essays of how the peptidoglycan is recognized both in plants and in mammals and how possible modifications affect its biological activity.

In Chapter 4 it is proposed a new route for the synthesis of a class of glycomometics in which the two saccharide units are linked together by an urea-linkage without using the anomeric centre. The chemistry and the self-assembling properties of gluco- and galacto-urea-derivatives will be then evaluated.

Portions of this work have been adapted from the following articles that were co-written by the author:

1) **Cirillo, L.; Parrilli, M.; Bedini, E.** “Acetolysis of 6-Deoxysugar Disaccharide Building Blocks: *exo* versus *endo* Activation”; *Eur. J. Org. Chem.* **2008**, *5704*-5714;


Chapter 2: Acetolysis of 6-deoxysugars

2.1 Introduction

Acetolysis is a widely used reaction in both analytical and synthetic carbohydrate chemistry, consisting in the cleavage of the glycosidic bond and the temporary acetylation of the hydroxyls groups thus formed and/or present before solvolysis. Acetolysis finds extensive application to the selective depolymerization of polysaccharides and provides useful information for their structural elucidation. In synthetic carbohydrate chemistry, its main applications are to convert alkyl or aryl glycosides of mono- and oligosaccharides into 1-O-acetylated derivatives, which are useful building blocks for the synthesis of other glycosyl acceptors and glycosyl donors. Owing to the importance of acetolysis reactions in glycochemistry, several studies on the mechanism of these reactions exist in literature. The first step is the activation of endocyclic or exocyclic anomeric oxygen toward glycoside cleavage promoted by acetylum ion, which is formed from \( \text{Ac}_2\text{O} \) in acidic conditions. It has been shown that endo-activation proceeds more rapidly, nonetheless the distribution of acetolysis products is usually governed not by kinetic but by thermodynamic conditions, providing 1-O-Ac-pyranoside product A after acetic acid attack on the oxocarbenium ion (Fig. 2.1). On the contrary, the activation at the endo-site first affords acyclic acetyl methyl

\[ \text{Ac}_2\text{O} + \text{glycoside} \rightarrow \text{oxocarbenium ion} \rightarrow \text{1-O-Ac-pyranoside} \]

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Chapter 2: Acetolysis of 6-deoxysugar

Acetal B, which can be activated in turn by a second acetylium ion to give acyclic diacetyl acetal D and/or 1-O-Ac-furanoside derivative E. In acidic conditions, both B and D derivatives are in equilibrium with the aldehyde form, C. In the literature there are only a few scattered examples of acetolysis of common hexose glycosides affording predominantly endo-activation mechanism products. 158

It is obvious that, in the case of 6-deoxyhexoses, acetolysis with activation at endo-site should be more easily achieved because the lack of the electron-withdrawing oxygen atom at the 6-position enhances the nucleophilicity of the endocyclic oxygen. Indeed, some examples were reported in literature on the acetolysis of 6-deoxyhexose glycosides affording B-, C-, D-, or E-like derivatives as the main products. 159

![Diagram](Image)

**Fig. 2.1:** Endo- versus exo-activation in acetolysis reactions

Investigations on the mechanism of acetolysis reactions on deoxyhexose, and above all 6-deoxyhexose, will be of great interest because of the large diffusion of such


carbohydrates in O-antigen lipopolysaccharides, glycoproteins, glycolipids, and in other glycoconjugates such as cardioglycosides, natural antibiotics and anticancer agents.

Recently, it has been reported that the outcome of acetolysis reaction with 10/10/1 (v/v/v) Ac$_2$O/AcOH/TFA on fully protected methyl glycosides disaccharides can be correlated to the arming-disarming properties of the protection groups on the 2-O-positions (Fig. 2.2): using an opportune protecting group strategy and the reported procedure, it was possible to obtain the 1-O-Ac-pyranoside derivative (A-like product) without cleaving the interglycosidic bond. The results were not dependent on the configuration of the moieties which formed the disaccharides.

![Diagram](image)

**Fig. 2.2:** Acetolysis reactions governed by arming/disarming effect.

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Chapter 2: Acetolysis of 6-deoxysugar

In the same paper it was made a screening of acetolysis conditions that would allow a kinetically controlled endo-activation mechanism. Several acetolysis conditions were tested on a model disaccharide (1, Table 2.1) and using a slight modification \(^{163}\) of the known procedure consisting in ZnCl\(_2\) in 2:1 (v/v) Ac\(_2\)O/AcOH \(^{164}\) (10 instead of 20 eq. of ZnCl\(_2\), 5\(^\circ\)C instead of room temperature) the 1-O-Ac-furanoside derivative (4) was obtained in good yield (76\%) together with a small quantity of 1-O-Ac-pyranoside (16\%) (also in this case the glycosidic linkage between the two sugar residues was not affected and acetolysis occurred only at the residue with an arming benzyl group at the 2-O position).

In next sections it will be therefore discussed the possibility to obtain kinetically controlled B-, C-, D-, E-like disaccharide products and which role the configuration of the sugar has in the outcome of the acetolysis reactions.

2.2 Results and Discussion

In order to investigate the role of sugar configuration in driving the acetolysis reaction, per-O-benzylated methyl glycosides of the most common natural 6-deoxysugars (compounds 5, 6, 7, 8 and 9, FIG. 2.3) and a set of 6-deoxysugar disaccharide methyl glycosides were synthesized through standard procedures. In particular disaccharide products were obtained by the standard glycosylation reaction of L-rhamnose thioglycoside 10 \(^{165}\) with the known glycosyl with the known glycosyl acceptors 11, \(^{166}\) 12, 13, \(^{167}\) 14, \(^{168}\) 15, and the novel acceptor 17 (obtained by phase transfer obtained by the

Table 2.1: Screening of acetolysis conditions on model compound 1: [a] Reaction conditions: A: 10/10/1 (v/v/v) Ac₂O/AcOH/TFA, 70°C; B: 10/10/1 (v/v/v) Ac₂O/AcOH/TFA, 5°C; C: 100/1 (v/v) Ac₂O/H₂SO₄, 0°C; D: ZnCl₂ (10 eq), 2:1 (v/v) Ac₂O/AcOH, 5°C. [b] Isolated yield.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Protocol</th>
<th>Product (% Yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td><img src="image" alt="Acetolysis Product 1" /> 2, 95%</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td><img src="image" alt="Acetolysis Product 2" /> No reaction</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td><img src="image" alt="Acetolysis Product 3" /> 3, 25%</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td><img src="image" alt="Acetolysis Product 4" /> 4, 76%</td>
</tr>
</tbody>
</table>


The obtained products were then subjected to acetolysis reaction by the ZnCl₂ protocol described above as well as with the 10/10/1 (v/v/v) Ac₂O/AcOH/TFA procedure at 70°C. The results are summarized in Tables 2.2-2.4, and are divided according to the configuration of the sugar moiety involved in the acetolysis reaction: as expected, the latter protocol allowed selective acetolysis through an exo activation mechanism.

![Fig. 2.3: 6-deoxymethyl glycosides used in acetolysis reaction](image)

both in monosaccharide methyl glycoside and in the monose unit of the disaccharides protected with an arming benzyl group at the 2-O position; on the contrary the outcome of the acetolysis reactions with ZnCl₂ in 2:1 (v/v) Ac₂O/AcOH was strictly dependent on the sugar configuration. In fact on rhamno-configured units, acetolysis with ZnCl₂ always afforded the products under kinetic control: per-O-benzylated rhamnose methyl glycoside 5 gave the 1-O-acetylated furanoside 29 (E-like product) as the main product (63%, entry 1 Table 2.2). In the same way, in the case of disaccharides with a rhamno-configured unit at the pseudo-reducing end, a preference was noted for an endo-type activation mechanism: compounds 20, 25 and 26 were transformed into 1-O-Ac-furanoside disaccharide derivatives (E-like products) in good yields (entries 2, 5,6; Table 2.2), whereas compounds 27 and 28 gave predominantly the
Chapter 2: Acetolysis of 6-deoxysugar

Fig. 2.4: Synthesis of a set of 6-deoxysugar disaccharide methyl glycosides. Reagents and conditions: (a) NIS, TfOH, AW-300 MS (4Å), CH₂Cl₂, -20°C, 20: 49%; 21: 83%; 22: 82%; 23: 87%; 24: 88%; 25: 40%; BnBr, KOH, TBAI, toluene, room temp. 72%; (c) i. PdCl₂, 1:1 (v/v) CH₂Cl₂/MeOH, room temp.; ii. Ac₂O, py, room temp. 75% (after two steps); (d) NaNH₂, DMF, 70°C, 64%; (e) Ac₂O, py, room temp. 99%; (f) BzCl, py, room temp. 83%.
open-chain 1-O-acetyl-1-O-methyl acetals (B-like products) 33 and 35 (entries 3, 4; Table 2.2). 1-O-Ac-pyranoside derivatives were isolated as minor products (15-25% yields). In the case of substrate 28, the formation of the B-like derivative 35 could be supposedly due to the lower electron-donating effect of the acetyl-protecting group at the 4α-O position of 28 compared with the benzyl group at the same position in compounds 20, 25 and 26. This avoids the conversion of B-like products into E-like products, whereas this transformation is rather easier in the presence of a benzyl group at the O-4α-position. The preference for compound 27 to afford the B-like derivative cannot be easily rationalized and several factors (such as possible complexation of the allyl group with Zn2+) should be taken in account.

In contrast to rhamno-configured species, all the experiments with fuco-configured species (6, 7, 23 and 24) with ZnCl2 protocol proceeded through an exo-activation mechanism giving the thermodynamically controlled 1-O-Ac-pyranoside derivatives 40 and 41 (Table 2.3).

As far as the quinovo-configured species are concerned, the behavior in the acetolysis reaction with ZnCl2 protocol was strictly dependent on the configuration of the methyl aglycon: α-configured species 8 and 21 were acetolyzed by an exo-activation mechanism, which furnished 1-O-Ac-pyranoside derivatives 45 and 48 respectively, whereas β-derivatives 9 and 22 gave open chain derivatives 42, 43, 45 and E-like derivatives 44, 47. These results suggest that the role of sugar configuration is crucial for driving the acetolysis using ZnCl2 protocol toward an exo- or endo-activation mechanism. The preference for a mechanism with activation at the endocyclic or exocyclic oxygen atom is strongly dictated by the anomeric configuration. The exocyclic oxygen is involved in n→σ* donation to the C₁-O bond only in the α-anomer. 2e The overall effect is a general
increased nucleophilicity of the endocyclic oxygen in the β-anomers, which explains the preference of β-quinovio-configured methyl glycosides to give endo-derived products with respect to the α-counterparts. In the β-fucoside case the main stereoelectronic effect is the strong through-space donation of the axially oriented O-4 substituent into the oxocarbenium ion formation, which highly enhances the first rate constant for the exo-activation involving galacto-configured alkyl glycosides with respect to gluco-configured counterparts; the result is that both fucoside anomers afford exclusively 1-O-Ac-pyranosides through an exo-activation mechanism. With the α-methyl glycosides, the role of configuration at O-2 seems to be very important too. Indeed compounds having an axial O-2 such as the rhamno-configured products afford products derived by an endo-activation, whereas α-methyl glycosides having an equatorial O-2 gave 1-O-Ac-pyranosides in high yields through an exo-activation mechanism. This could be ascribed to the destabilization effect of the axial hydroxyl group at position 2 on the incipient oxocarbenium ion for its major electron withdrawing properties. Interestingly, the analogies of the results on mono- and disaccharides suggests that a potential Zn(II)-sugar complexation has a minor effect on the competition between exo- and endo-mechanism, because the geometry of such coordination should be highly dependent on saccharide structure.

Table 2.2: Acetolysis of 6-deoxysugar thamno configured methyl glycosides: * Isolated yield; b Anomeric ratio measured by 1H NMR spectroscopy; c Anomeric ratio measured by isolation of the two anomers.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Methyl Disaccharide</th>
<th>Products (% yield) *</th>
<th>Protocol A: ZnCl₂ (10eq.), Ac₂O, 5°C</th>
<th>Protocol B: 1:1:0.1 v/v/v Ac₂O/AcOH/TFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td></td>
<td>29, 63%; 30 22% b</td>
<td>30, 62%</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td></td>
<td>31, 64%; α/β = 2:1 b</td>
<td>32, 55%</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>33, 77%; dr=6:1 b</td>
<td>34, 73%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td></td>
<td>35, 66%; dr=1.3:1 b</td>
<td>Not determined</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>36, 71% c</td>
<td>37, 74%; α/β = 2.5:1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>26</td>
<td>38, 62%</td>
<td>39, 75%; α/β = 2:1 b</td>
<td></td>
</tr>
</tbody>
</table>
Table 2.3: Acetolysis of 6-deoxysugar *fuco* configured methyl glycosides: *a* Isolated yield; *b* Anomeric ratio measured by 1H NMR spectroscopy.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Methyl Disaccharide</th>
<th>Products (% yield) <em>a</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protocol A: ZnCl₂ (10eq.), Ac₂O, 5°C</td>
<td>Protocol B: 1:1:0.1 v/v/v Ac₂O/AcOH/TFA</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>40, 72%; α/β = 5:1 <em>b</em></td>
</tr>
<tr>
<td></td>
<td>40, 83%; α/β = 3:1 <em>b</em></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>40, 99%; α/β = 4:1 <em>b</em></td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>41, 73%; α/β = 3:1 <em>b</em></td>
</tr>
<tr>
<td></td>
<td>41, 83%; α/β = 3:1 <em>b</em></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>41, 82%; α/β = 6:1 <em>b</em></td>
</tr>
<tr>
<td></td>
<td>41, 87%; α/β = 6:1 <em>b</em></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.4: Acetolysis of 6-deoxysugar *quinovo* configured methyl glycosides: *a* Isolated yield; *b* Anomeric ratio measured by 1H NMR spectroscopy.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Methyl Disaccharide</th>
<th>Products (% yield) <em>a</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protocol A: ZnCl₂ (10eq.), Ac₂O, 5°C</td>
<td>Protocol B: 1:1:0.1 v/v/v Ac₂O/AcOH/TFA</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>42, 15%; 43, 23%; 44, 24%; α/β = 2:1 <em>b</em></td>
</tr>
<tr>
<td></td>
<td>45, 59%; α/β = 3.5:1 <em>b</em></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>45, 64%; α/β = 2:1 <em>b</em></td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>46, 43%; 47, 41%; α/β = 4:1 <em>b</em></td>
</tr>
<tr>
<td></td>
<td>48, 56%; α/β = 4:5:1 <em>b</em></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>48, 96%; α/β = 6:1 <em>b</em></td>
</tr>
<tr>
<td></td>
<td>48, 96%; α/β = 6:1 <em>b</em></td>
<td></td>
</tr>
</tbody>
</table>
2.3 Conclusions

In conclusion it has been demonstrated that under Ac₂O/AcOH/TFA reaction conditions the sugar configuration has not effect on the outcome of the acetolysis reaction because these conditions promote an exo-activation mechanism regardless of the configuration of the sugar. In the case of ZnCl₂ promoted acetolysis reaction, the product distribution is indeed strongly influenced by the 6-deoxyhexose configuration which has a primary role in determining the endo- or the exo- activation mechanism.

2.4 Experimental Section

General methods: ¹H and ¹³C NMR spectra were recorded with Varian XL-200 (¹H: 200 MHz; ¹²C: 50 MHz), Varian Gemini-300 (¹H: 300 MHz; ¹³C:75 MHz), Bruker DRX-400 (¹H: 400 MHz; ¹³C:100 MHz), or Varian INOVA 500 (¹H: 500 MHz; ¹³C:125 MHz) instruments in CDCl₃ (CHCl₃ as internal standard: ¹H: CHCl₃ at δ=7.26 ppm; ¹³C: CDCl₃ at δ=77.0 ppm). Assignment of the proton chemical shifts was based on 1D HOHAHA experiments. Positive MALDI-TOF MS spectra were recorded with an Applied Biosystem Voyager DE-PRO MALDI-TOF mass spectrometer in the positive mode: compounds were dissolved in CH₃CN at concentration of 1 mg/mL and one microliter of these solutions were mixed with one microliter of a 20 mg/mL solution of 2,5-dihydroxybenzoic acid in 7:3 CH₃CN/water. Optical rotations were measured with a JASCO P-1010 polarimeter. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with Merck Silica Gel 60 F₂₅₄ as the adsorbent. The plates
were developed with a 10% H$_2$SO$_4$ ethanolic solution and by heating to 130°C. Column chromatography was performed on Merck Kiesegel 60 (63-200 mesh).

**Methyl 4-**O-**Allyl-2-**O-**benzyl-$$\alpha$$-L-rhamnopyranoside (17):** Diol 16 (301 mg, 1.38 mmol) was dissolved in toluene (15 mL) and treated with TBAI (476 mg, 1.28 mmol), BnBr (328 μL, 2.75 mmol), and then KOH (690 mg, 10.8 mmol). The mixture was stirred at room temp. for 30 min and then treated with 1:1 AcOEt/water (25 mL) and stirred overnight. After that the organic layer was collected, dried with anhydrous Na$_2$SO$_4$, filtered, and concentrated. The residue was subjected to column chromatography (7 to 10% ethyl acetate in petroleum ether) to afford 17 (310 mg, 72%) as a yellowish oil. [α]$_D$ = -9 (c = 0.8, CH$_2$Cl$_2$). 1H NMR (200 MHz, CDCl$_3$): $\delta$ = 7.34 (m, 5 H, Ar-H), 5.93 (m, 1 H, OCH$_2$CH=CH$_2$), 5.26 (dq, $J_\text{vic}=17.4$, $J_\text{long-range}=1.4$ Hz, 1 H, trans OCH$_2$CH=CHH), 5.13 (d, $J_\text{vic}=10.2$, $J_\text{long-range}=1.4$ Hz, 1 H, cis OCH$_2$CH=CHH), 4.73 (dt, $J_\text{gem}=11.6$ Hz, 1 H, OCH=CHPh), 4.68 (d, $J_1,2=1.4$ Hz, 1 H, 1-H), 4.58 (d, $J_\text{gem}=11.6$ Hz, 1 H, OCH=CHPh), 4.33 (ddt, $J_\text{gem}=12.4$, $J_\text{long-range}=1.4$ Hz, 1 H, OCHHCH=CH$_2$), 4.13 (ddt, $J_\text{gem}=12.4$, $J_\text{long-range}=1.4$ Hz, 1 H, OCHHCH=CH$_2$), 3.83 (br. m, 1 H, 3-H), 3.69 (dd, $J_2,3=3.6$, $J_2,1=1.6$ Hz, 1 H, 2-H), 3.59 (dq, $J_3,4=9.2$, $J_3,6=6.2$ Hz, 1 H, 5-H), 3.31 (s, 3 H, OCH$_3$), 3.18 (t, $J_3,5=9.2$ Hz, 1 H, 4-H), 1.31 (d, $J_{6,5}=6.2$ Hz, 3 H, 6-H) ppm. $^{13}$C NMR (CDCl$_3$, 50 MHz): $\delta$ = 137.7 (C$_{\text{ipso}}$-Bn), 135.0 (OCH$_2$CH=CH$_2$), 128.5–127.8 (C-Ar), 116.8 (OCH$_2$CH=CH$_2$), 98.0 (C-1) 81.9, 78.5, 73.8, 73.0, 71.4, 67.0 (C-2, C-3, C-4, C-5, OCH$_3$Ph, OCH$_2$CH=CH$_2$), 54.6 (OCH$_3$), 18.0 (C-6) ppm. MS (MALDITOF): calcd. for C$_{17}$H$_{24}$O$_5$ [M]$^+$ 308.16; found 330.99 [M + Na]$^+$. C$_{17}$H$_{24}$O$_5$ (308.37): calcd. C 66.21, H 7.84; found C 66.00, H 7.70.
Typical Glycosylation Procedure: A mixture of the acceptor (173 μmol) and thioglycoside donor (222 μmol) was co-evaporated three times with toluene (2 mL). The residue was dried, mixed with NIS (282 μmol), and freshly activated with AW-300 molecular sieves (4 Å) under Ar, cooled to −20 °C, and suspended in CH₂Cl₂ (3.0 mL). A 0.57 M solution of TfOH in CH₂Cl₂ (100 μL, 57 μmol) was added. After 1 h stirring at −20 °C, the mixture was diluted with CH₂Cl₂ and washed with 1:1 (v/v) 1 M NaHCO₃/10% Na₂S₂O₃. The organic layer was collected, dried with anhydrous Na₂SO₄, filtered, concentrated, and purified by chromatography (ethyl acetate in toluene).

Methyl 2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-2-O-allyl-4-O-benzyl-α-L-rhamnopyranoside (20): [α]D = −50.6 (c = 1.8, CH₂Cl₂). ¹H NMR (200 MHz, CDCl₃): δ = 7.30 (m, 5 H, Ar-1H), 5.96 (m, 1 H, OCH₂CH=CH₂), 5.39–5.26 (m, 3 H, 2β-H, 3β-H, trans OCH₂CH=CHH), 5.23 (d, Jvic = 10.4 Hz, 1 H, cis OCH₂CH=CHH), 5.04 (t, J4,3 = J4,5 = 9.5 Hz, 1 H, OCH₂CH=CH₂), 5.03 (s, 1 H, 1α-H), 4.80 (d, Jgem = 11.0 Hz, 1 H, OCH₂CH₂Ph), 4.65 (s, 1 H, 1α-H), 4.62 (d, Jgem = 11.0 Hz, 1 H, OCH₂CH₂Ph), 4.22 (dd, Jvic = 5.6 Hz, 1 H, OCH₂CH=CH₂), 4.07 (dd, Jgem = 13.0, Jvic = 5.6 Hz, OCH₂CH=CH₂), 4.04–3.97 (m, 2 H, 3α-H, 5β-H), 3.65–3.56 (m, 3 H, 2α-H, 4α-H, 5α-H), 3.32 (s, 3 H, OCH₃), 2.06, 2.05, 1.97 (3 s, 9 H, 3 COCH₃), 1.30 (d, Jα,5 = 5.7 Hz, 3 H, 6α-H), 1.21 (d, Jα,5 = 6.3 Hz, 3 H, 6β-H) ppm. ¹³C NMR (CDCl₃, 50 MHz): δ = 169.9–169.8 (3 CO), 138.1 (C_oAc-Bn), 134.9 (OCH₂CH=CH₂), 128.3–127.5 (C-Ar), 117.4 (OCH₂CH=CH₂), 99.1, 98.4 (C-1α, C-1β), 80.6, 78.3, 77.7, 75.3, 72.0, 71.1, 69.8, 69.1, 67.9, 66.8 (C-2α, C-2β, C-3α, C-3β, C-4α, C-4β, C-5α, C-5β, OCH₂Ph, OCH₂CH=CH₂), 54.6 (OCH₂CH₂), 20.8–20.7 (3 CH₃CO), 17.9, 17.4 (C-6α, C-6β) ppm. MS (MALDI-TOF): calcd. For C₃₂H₄₀O₁₂ [M]+
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Methyl 2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-O-benzyl-α-D-quinovopyranoside (21): [α]D = −17.3 (c = 1.8, CH2Cl2). 1H NMR (200 MHz, CDCl3): δ = 7.34–7.26 (m, 10 H, Ar-H), 5.38–5.27 (m, 3 H, 1A-H, 2B-H, 3B-H), 4.97 (t, J4,5 = J4,5 = 10.0 Hz, 1 H, 4B-H), 4.84 (d, Jgem = 11.2 Hz, 1 H, OCHHPh), 4.68 (d, Jgem = 12.0 Hz, 1 H, OCHHPh), 4.61 (d, Jgem = 11.2 Hz, 1 H, OCHHPh), 4.55 (d, Jgem = 12.0 Hz, 1 H, OCHHPh), 4.45 (d, J1,2 = 3.4 Hz, 1 H, 1A-H), 4.11 (t, J3,4 = J4,3 = 9.4 Hz, 1 H, 3A-H), 4.02 (dq, J5,6 = 6.2 Hz, 1 H, 5A-H), 3.76 (dq, J5,4 = 9.4 Hz, 1 H, 5B-H), 3.61 (dd, J2,3 = 9.4 Hz, 1 H, 2A-H), 3.30 (s, 3 H, OCH3), 3.09 (t, J4,3 = J4,5 = 6.4 Hz, 1 H, 6A-H), 0.88 (d, J6,5 = 6.2 Hz, 3 H, 6B-H) ppm. 13C NMR (CDCl3, 50 MHz): δ = 170.1, 169.9, 169.8 (3 CO), 137.9, 137.6 (2 Cgem-Bn), 128.4–127.2 (C-Ar), 97.6, 97.1 (C-1A, C-1B), 82.4, 81.0, 75.6, 74.9, 72.7, 70.9, 69.7, 69.2, 66.5, 66.1 (C-2A, C-2B, C-3A, C-3B, C-4A, C-4B, C-5A, C-5B, 2 OCH2Ph), 54.9 (OCH2), 20.7–20.6 (3 CH3CO), 17.8, 16.9 (C-6A, C-6B) ppm. MS (MALDI-TOF): calcd. for C33H42O12 [M]+ 630.27; found 653.11 [M + Na]+.

Methyl 2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-O-benzyl-β-D-quinovopyranoside (22): [α]D = −50.3 (c = 3.0, CH2Cl2). 1H NMR (200 MHz, CDCl3): δ = 7.33–7.26 (m, 10 H, Ar-H), 5.38 (d, J1,2 = 1.0 Hz, 1 H, 1A-H), 5.32–5.23 (m, 2 H, 2α-H, 3B-H), 4.98–4.81 (m, 3 H, 4B-H, 2 OCHHPh), 4.70 (d, Jgem = 11.4 Hz, 1 H, OCHHPh), 4.56 (d, Jgem = 11.8 Hz, 1 H, OCHHPh), 4.27 (d, J1,2 = 7.8 Hz, 1 H, 1A-H),
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4.03 (dq, J\(_{5,4}\) = 10.0, J\(_{5,6}\) = 6.2 Hz, 1 H, 5\(_b\)-H), 3.83 (t, J\(_{5,4}\) = J\(_{3,2}\) = 9.2 Hz, 1 H, 4\(_A\)-H), 3.54 (s, 3 H, OCH\(_3\)), 3.48–3.38 (m, 2 H, 2\(_A\)-H, 5\(_A\)-H), 3.15 (t, J\(_{3,2}\) = J\(_{3,4}\) = 9.2 Hz, 1 H, 3\(_A\)-H), 2.03, 1.97, 1.90 (3 s, 9 H, 3 CH\(_3\)CO), 1.37 (d, J\(_{6,5}\) = 6.0 Hz, 3 H, 6\(_\alpha\)-H), 0.87 (d, J\(_{6,5}\) = 6.2 Hz, 3 H, 6\(_\beta\)-H) ppm. \(^{13}\)C NMR (CDCl\(_3\), 50 MHz): \(\delta = 170.1, 169.8, 169.7, 138.0, 137.7, 128.2–127.3, 104.2, 97.2, 82.9, 82.0, 76.3, 75.2, 74.3, 71.1, 69.8, 69.1, 66.1, 82.9, 82.0, 76.3, 75.2, 74.3, 71.1, 70.8, 69.4, 69.1, 66.1, 82.9, 82.0, 76.3, 75.2, 74.3, 71.1, 69.8, 69.1, OCH\(_2\)Ph), 56.9 (OCH\(_3\)), 20.7–20.6 (3 CH\(_3\)CO), 17.8, 16.9 (2 OCH\(_2\)Ph), ppm. MS (MALDI-TOF): calcd. for C\(_{33}\)H\(_{42}\)O\(_{12}\) [M]\(^+\) 630.27; found 652.89 [M + Na]\(^+\). C\(_{33}\)H\(_{42}\)O\(_{12}\) (630.68): calcd. C 62.85, H 6.71; found C 62.70, H 6.67.

Methyl 2,3,4-Tri-O-acetyl-\(\alpha\)-L-rhamnopyranosyl-(1→3)-2,4-di-O-benzyl-\(\alpha\)-D-fucopyranoside (23): [\(\alpha\)]\(_D\) = −71.0 (c = 2.1, CH\(_2\)Cl\(_2\)). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta = 7.43–7.28, 5.46, 5.18, 5.08, 5.01, 4.90, 4.89, 4.79, 4.67, 4.65, 4.59, 4.44, 4.41, 4.15, 4.01, 3.98, 3.90, 3.84, 3.68, 3.54, 3.35, 2.16, 2.02, 1.12 ppm. \(^{13}\)C NMR (CDCl\(_3\), 50 MHz): \(\delta = 170.4, 170.0, 169.9, 138.3, 138.2, 129.0–127.7, 98.4, 93.9, 75.5, 74.6, 74.5, 74.4, 73.3, 71.1, 70.3, 68.9, 66.6, 65.8, 20.9, 20.7, 20.6 ppm.
(C-6\text{\textalpha}, C-6\text{\textbeta}) ppm. MS (MALDI-TOF): calcd. for C\text{33}H\text{42}O\text{12} [M]^+ 630.27; found 653.01 [M + Na]^+. C\text{33}H\text{42}O\text{12} (630.68): calcd. C 62.85, H 6.71; found C 62.82, H 6.72.

Methyl 2,3,4-Tri-\textit{O}-acetyl-\textalpha-L-rhamnopyranosyl-(1→3)-2,4-di-\textit{O}-benzyl-\textbeta-D-fucopyranoside (24): [\alpha]_D = -61.0 (c = 2.0, CH\text{2}Cl\text{2}). \textit{H} NMR (300 MHz, CDCl\text{3}): \delta = 7.42–7.27 (m, 10 H, Ar-H), 5.40 (dd, \textit{J}\textsubscript{5,4} = 9.9, \textit{J}\textsubscript{3,4} = 3.3 Hz, 1 H, 3\text{\textbeta}-H), 5.18 (dd, \textit{J}\textsubscript{2,3} = 3.3, \textit{J}\textsubscript{2,1} = 1.8 Hz, 1 H, 2\text{\textbeta}-H), 5.03 (t, \textit{J}\textsubscript{4,3} = \textit{J}\textsubscript{4,5} = 9.9 Hz, 1 H, 4\text{\textbeta}-H), 4.96 (s, 1 H, 1\text{\textbeta}-H), 4.93 (d, \textit{J}\textsubscript{gen} = 10.5 Hz, 1 H, OCH\text{HPh}), 4.91 (d, \textit{J}\textsubscript{gen} = 11.4 Hz, 1 H, OCH\text{HPh}), 4.71 (d, \textit{J}\textsubscript{gen} = 10.5 Hz, 1 H, OCH\text{HPh}), 4.60 (d, \textit{J}\textsubscript{gen} = 11.4 Hz, 1 H, OCH\text{HPh}), 4.26 (d, \textit{J}\textsubscript{1,2} = 7.2 Hz, 1 H, 1\text{\textalpha}-H), 4.16 (dq, \textit{J}\textsubscript{5,4} = 9.9, \textit{J}\textsubscript{5,6} = 6.0 Hz, 1 H, 5\text{\textalpha}-H), 3.74 (m, 2 H, 2\text{\textalpha}-\textalpha-H, 3\text{\textalpha}-H), 3.56 (s, 1 H, 4\text{\textalpha}-H), 3.55 (s, 3 H, OCH\text{3}), 3.48 (q, \textit{J}\textsubscript{5,6} = 6.3 Hz, 1 H, 5\text{\textalpha}-H), 2.15, 2.01, 2.00 (3 s, 9 H, 3 CH\text{3}CO), 1.23 (d, \textit{J}\textsubscript{6,5} = 6.3 Hz, 3 H, 6\text{\textalpha}-H), 0.96 (d, \textit{J}\textsubscript{6,5} = 6.0Hz, 3 H, 6\text{\textbeta}-H) ppm. \textup{\textup{13}}C NMR (CDCl\text{3}, 50 MHz): \delta = 170.2, 169.9, 169.7 (3 CO), 138.6, 138.2 (2 C\text{\\textalpha}\textsubscript{\text{\textalpha}}-Bn), 129.0–127.4 (C-Ar), 105.0, 94.0 (C-1\text{\textalpha}, C-1\text{\textbeta}), 77.7, 77.3, 75.3, 75.1, 75.0, 71.6, 70.4, 70.2, 69.0, 66.4 (C-2\text{\textalpha}, C-2\text{\textbeta}, C-3\text{\textalpha}, C-3\text{\textbeta}, C-4\text{\textalpha}, C-4\text{\textbeta}, C-5\text{\textalpha}, C-5\text{\textbeta}, 2 OCH\text{2}Ph), 56.9 (OCH\text{3}), 20.9–20.8 (3 CH\text{3}CO), 17.2, 17.0 (C-6\text{\textalpha}, C-6\text{\textbeta}) ppm. MS (MALDI-TOF): calcd. for C\text{33}H\text{42}O\text{12} [M]^+ 630.27; found 653.15 [M + Na]^+. C\text{33}H\text{42}O\text{12} (630.68): calcd. C 62.85, H6.71; found C 62.75, H 6.62.

Methyl 2,3,4-Tri-\textit{O}-acetyl-\textalpha-L-rhamnopyranosyl-(1→3)-4-\textit{O}-allyl-2-\textit{O}-benzyl-\textalpha-L-rhamnopyranoside (27): [\alpha]_D = -40.1 (c = 2.0, CH\text{2}Cl\text{2}). \textit{H} NMR (300 MHz, CDCl\text{3}): \delta = 7.41–7.28 (m, 5 H, Ar-H), 5.87 (m, 1 H, OCH\text{2}CH=CH\text{2}), 5.34 (m, 2 H, 1\text{\textalpha}-H, 3\text{\textbeta}-H), 5.24 (d, \textit{J}\textsubscript{\textalpha} = 16.8 Hz, 1 H, OCH\text{2}CH=CH\text{H}), 5.10 (d, \textit{J}\textsubscript{\textalpha} = 9.9 Hz, 1 H, OCH\text{2}CH=CH\text{H}), 5.01 (m, 2 H, 2\text{\textalpha}-H, 4\text{\textbeta}-H), 4.75 (d, \textit{J}\textsubscript{\textalpha} = 12.3 Hz, 1 H, OCH\text{HPh}),
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4.66 (br. s, 1 H, 1-A-H), 4.64 (d, J_{gw} = 12.3 Hz, 1 H, OCH2Ph), 4.25 (dd, J_{gw} = 12.2, J_{sc} = 5.7 Hz, 1 H, OCH2HCH=CH2), 4.10 (dd, J_{gw} = 12.4, J_{sc} = 5.7 Hz, 1 H, OCH2HCH=CH2), 3.94 (dd, J_3,4 = 9.3, J_3,2 = 3.3 Hz, 1 H, 3-A-H), 3.80 (dq, J_5,4 = 9.6, J_5,6 = 6.3 Hz, 1 H, 5-A-H), 3.65 (dd, J_2,3 = 3.0, J_2,1 = 1.8 Hz, 1 H, 2-A-H), 3.58 (dq, J_5,4 = 9.6, J_5,6 = 6.3 Hz, 1 H, 5-A-H), 3.46 (t, J_4,3 = J_4,5 = 9.6 Hz, 1 H, 4-A-H), 3.30 (s, 3 H, OCH3), 2.10, 2.02, 1.97 (3 s, 9 H, 3 CH3CO), 1.29 (d, J_6,5 = 6.3 Hz, 3 H, 6-A-H), 1.09 (d, J_6,5 = 6.3 Hz, 3 H, 6-B-H) ppm.

13C NMR (CDCl3, 75 MHz): δ = 169.7 (3 CO), 138.0 (Cipso-Bn), 134.6 (OCH2CH=CH2), 128.3–127.5 (C-Ar), 116.7 (OCH2CH=CH2), 99.2, 98.3 (C-1_A, C-1_B), 80.4, 78.3, 77.7, 74.1, 72.4, 71.1, 69.8, 69.0, 68.0, 66.8 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, OCH2Ph, OCH2CH=CH2), 54.6 (OCH3), 20.7–20.6 (3 CH3CO), 17.8, 17.4 (C-6_A, C-6_B) ppm. MS (MALDI-TOF): calcd. For C29H40O12 [M]+ 580.25; found 603.18 [M + Na]+. C29H40O12 (580.62): calcd. C 59.99, H 6.94; found C 59.75, H 6.82.

Methyl 2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-4-O-acetyl-2-O-benzyl-α-L-rhamnopyranoside (28): Compound 27 (30.4 mg, 52.2 μmol) was dissolved in 1:1 (v/v) CH2Cl2/MeOH (0.6 mL) and then treated with PdCl2 (3.7 mg, 20.9 μmol). The mixture was vigorously stirred at room temp. for 2 h and then filtered through a Celite pad, diluted with CH2Cl2, and washed with water. The organic phase was collected, dried with anhydrous Na2SO4, filtered, and concentrated. The residue was dissolved in 1:1 (v/v) Ac2O/py (1.0 mL) and stirred at room temp. overnight. The solution was then concentrated. The residue was diluted with CH2Cl2 and the resulting solution washed with 1 M HCl. The organic phase was collected, dried with anhydrous Na2SO4, filtered, and concentrated. The residue was purified by chromatography (10 to 50% ethyl acetate
in petroleum ether) to give 28 (22.9 mg, 75%) as a yellowish oil. [α]_D = −34.7 (ε = 1.1, CH₂Cl₂). ¹H NMR (200 MHz, CDCl₃): δ = 7.37 (m, 5 H, Ar-H), 5.30 (dd, J₃,₄ = 10.0, J₃,₂ = 3.2 Hz, 1 H, 2ₐ- H), 5.02 (t, J₄,₃ = 10.0 Hz, 1 H, 4ₐ- H), 4.89 (d, J₁,₂ = 1.6 Hz, 1 H, 1ₐ- H), 4.74–4.68 (m, 3 H, 1ₐ-H, OCH₂Ph), 4.00 (dd, J₂,₃ = 9.8 Hz, J₂,₁ = 3.2 Hz, 1 H, 3ₐ- H), 3.82 (dq, J₅,₄ = 9.8, J₅,₆ = 6.2 Hz, 1 H, 5ₐ- H), 3.70 (m, 2 H, 2ₐ- H, 5ₐ- H), 3.33 (s, 3 H, OCH₃), 2.13, 2.12, 2.03, 1.99 (4 s, 12 H, 4 CH₃CO), 1.20 (d, J₆,₅ = 6.2 Hz, 3 H, 6ₐ- H), 1.07 (d, J₆,₅ = 6.2 Hz, 3 H, 6ₐ- H) ppm. ¹³C NMR (CDCl₃, 50 MHz): δ = 170.2, 170.1, 170.0, 169.6 (4 CO), 138.0 (Cₐp-Bn), 128.5–127.6 (C-Ar), 98.6, 98.5 (C-1ₐ, C-1₁), 76.9, 72.7, 72.6, 71.2, 71.1, 70.2, 68.6, 66.9, 66.8 (C-2ₐ, C-2₄₁, C-3ₐ, C-3₁, C-4ₐ, C-4₁, C-5ₐ, OCH₂Ph), 54.9 (OCH₃), 20.9–20.7 (4 CH₃CO), 17.6, 17.4 (C-6ₐ, C-6₁) ppm. MS (MALDI-TOF): calcd. for C₂₈H₄₃O₁₃ [M⁺] 582.23; found 605.09 [M + Na⁺]. C₂₈H₃₈O₁₃ (582.59): calcd. C 57.72, H 6.57; found C 57.57, H 6.62.

**Methyl 3-O-Allyl-4-O-benzyl-α-D-rhamnopyranosyl-(1→3)-2,4-di-O-benzyl-α-D-rhamnopyranoside (19):** Compound 18 (250 mg, 317 µmol) was dissolved in dry DMF (5.0 mL) under Ar and then NaNH₂ (542 mg, 13.9 mmol) was added. The mixture was stirred at 70 °C for 48 h and then diluted with MeOH. AcOH was added dropwise until the pH was neutral. The solution was concentrated and the residue was dissolved in CH₂Cl₂ and washed with 1 M NaHCO₃ and brine. The organic layer was collected, dried with anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by chromatography (8 to 16% ethyl acetate in toluene) to afford 19 (129 mg, 64%) as a yellowish oil. [α]_D = +26.5 (ε = 2.0, CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃): δ = 7.37–7.19 (m, 15 H, Ar-H), 5.91 (m, 1 H, OCH₂CH=CH₂), 5.26 (dd, Jₙ₂ = 17.2, Jₖ₂ = 1.6 Hz, 1 H,
trans OCH₂CH=CHH), 5.16–5.13 (m, 2 H, cis OCH₂CH=CHH, 1β-H), 4.87 (d, J₂₋₁β = 11.0 Hz, 1 H, OCH₂Ph), 4.64 (s, 2 H, OCH₂Ph), 4.63 (d, J₁₋₁α = 11.0 Hz, 1 H, OCH₂Ph), 4.62 (d, J₁₋₁β = 11.6 Hz, 1 H, OCH₂Ph), 4.07 (dd, J₄₋₃α = 11.8, J₄₋₃β = 6.6 Hz, 1 H, OCH₂HCH=CH₂), 4.05 (dd, J₄₋₃α = 11.8, J₄₋₃β = 6.6 Hz, 1 H, OCH₂HCH=CH₂), 4.00 (d, J₂₋₁₁β = 1.3 Hz, 1 H, 2β-H), 3.80 (m, 1 H, 5β-H), 3.75–3.61 (m, 3 H, 3α-H, 3β-H, 2α-H), 3.66 (m, 1 H, 5α-H), 3.58 (t, J₄₋₃α = J₄₋₃β = 9.3 Hz, 1 H, 4β-H), 3.52 (t, J₄₋₃α = J₄₋₃β = 9.3 Hz, 1 H, 4α-H), 3.31 (s, 3 H, OCH₃), 1.32 (d, J₆₋₅α = 5.8 Hz, 3 H, 6β-H), 1.25 (d, J₆₋₅β = 6.0 Hz, 3 H, 6α-H) ppm. ¹³C NMR (CDCl₃, 50 MHz): δ = 138.7, 138.2, 138.1 (3 C ipso-Bn), 134.6 (OCH₂CH=CH₂), 128.4–127.8 (C-Ar), 117.2 (OCH₂CH=CH₂), 100.8, 98.6 (C-1α, C-1β), 81.1, 79.9, 79.5, 77.3, 76.7, 75.3, 75.1, 72.7, 70.9, 69.0, 67.9, 67.8 (C-2α, C-2β, C-3α, C-3β, C-4α, C-4β, C-5α, C-5β, 3 OCH₂Ph, OCH₂CH=CH₂), 54.9 (OCH₂), 18.2 (C-6α, C-6β) ppm. MS (MALDI-TOF): calcd. for C₃₇H₄₆O₉ [M⁺] 634.31; found 657.33 [M + Na⁺]. C₃₇H₄₆O₉ (634.76): calcd. C 70.01, H 7.30; found C 70.18, H 7.45.

**Methyl 2-O-Acetyl-3-O-allyl-4-O-benzyl-α-D-rhamnopyranosyl-(1→3)-2,4-di-O-benzyl-α-D-rhamnopyranoside (25):** Alcohol 19 (67.0 mg, 106 μmol) was dissolved in 1:1 Ac₂O/pyridine (1.5 mL). The solution was stirred overnight at room temp. and then co-evaporated four times with toluene. The residue was dissolved in CH₂Cl₂ and washed with water. The organic layer was collected, dried with anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by chromatography (6% ethyl acetate in toluene) to give 25 (71.0 mg, 99%) as a colorless oil. [α]D = +28.2 (c = 0.9, CH₂Cl₂). ¹H NMR (300 MHz, CDCl₃): δ = 7.41–7.26 (m, 15 H, Ar-H), 5.87 (m, 1 H, OCH₂CH=CH₂), 5.44 (br. s, 1 H, 2β-H), 5.25 (d, J₁₋₁α = 17.4 Hz, 1 H, trans OCH₂CH=CHH), 5.13–5.10 (m, 2 H,
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αis OCH₂CH=CHH, 1α-H), 4.94 (d, J_{gw} = 11.0 Hz, 1 H, OCH₂HPh), 4.83 (d, J_{gw} = 11.0 Hz, 1 H, OCH₂HPh), 4.72 (s, 2 H, OCH₂Ph), 4.66–4.61 (m, 3 H, 2 OCH₂HPh, 1β-H), 4.16–4.06 (m, 2 H, OCH₂HCH=CH₂, 3β-H), 3.98 (dd, J_{gw} = 12.0, J_{αβ} = 5.4 Hz, 1 H, OCH₂HCH=CH₂), 3.88 (dd, J_{αβ} = 3.3, J_{βγ} = 9.3 Hz, 1 H, 3β-H), 3.85 (m, 1 H, 5β-H), 3.71 (br. s, 1 H, 2α-H), 3.65 (t, J_{αγ} = J_{4γ} = 9.0 Hz, 1 H, 4β-H), 3.41 (m, 1 H, 5α-H), 3.36 (t, J_{αγ} = J_{4γ} = 9.6 Hz, 1 H, 4α-H), 3.31 (s, 3 H, OCH₃), 2.09 (s, 3 H, COCH₃), 1.32 (d, J_{βγ} = 5.4 Hz, 3 H, 6α-H), 1.29 (d, J_{βγ} = 5.7 Hz, 3 H, 6β-H) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ

Methyl 3-O-Allyl-2-O-benzoyl-4-O-benzyl-x-D-rhamnopyranosyl- (1→3)-2,4-di-O-benzyl-x-D-rhamnopyranoside (26): Alcohol 25 (86.3 mg, 136 µmol) was dissolved in pyridine (1.0 mL), treated with BzCl (40 µL, 345 µmol), and stirred overnight at room temp. The mixture was diluted with CH₂Cl₂ and washed with 1 M HCl and water. The organic layer was collected, dried with anhydrous Na₂SO₄, filtered, concentrated, and purified by chromatography (6 to 9% ethyl acetate in toluene) to give 25 (83.0 mg, 83%) as a colorless oil. [α]₀²⁵ = −0.8 (c = 0.9, CH₂Cl₂). ¹H NMR (200 MHz, CDCl₃): δ = 8.06–7.24 (m, 20 H, Ar-H), 5.88 (m, 1 H, OCH₂CH=CH₂), 5.71 (dd, J₂₃ = 1.6, J₂₃ = 3.0 Hz, 1 H, 2γ-H), 5.24 (dd, Jαβ = 15.6, J_{gw} = 1.8 Hz, 1 H, trans OCH₂CH=CHH), 5.17 (br. s, 1 H, 1α-H), 5.09 (dd, Jαβ = 10.2, J_{gw} = 1.8 Hz, 1 H, αis

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OCH₂CH=CHH₂, 4.98 (d, J₇-₈ = 11.0 Hz, 1 H, OCHHHPh), 4.92 (d, J₅-₆ = 11.0 Hz, 1 H, OCHHHPh), 4.77 (s, 2 H, OCHHHPh), 4.67–4.62 (m, 3 H, 2 OCHHHPh, 1₂-H), 4.22 (dd, J₉-₁₀ = 11.5, J₁₁-₁₂ = 5.2 Hz, 1 H, OC₂H₂), 4.16–3.88 (m, 4 H, OC₂H₂, 3₂A-H, 5₂A-H, 5₂B-H), 3.71–3.67 (m, 2 H, 3₂B-H, 4₂A-H), 3.54 (t, J₁₂-₁₃ = 9.2 Hz, 1 H, 4₂B-H), 3.33 (s, 3 H, OCH₃), 1.36–1.30 (m, 6 H, 6₂A-H, 6₂B-H) ppm. ¹³C NMR (CDCl₃, 50 MHz): δ = 165.4 (CO), 138.7, 138.1, 138.0 (3-Cipso-Bn), 134.7 (OCH₂CH=CH₂), 133.0 (Cpol-Bz), 129.8–127.5 (C-Ar), 117.0 (OCH₂CH=CH₂), 99.2, 98.6 (C-1₂, C-1₁), 80.9, 80.0, 77.7, 77.5, 75.3, 75.1, 72.8, 70.5, 69.6, 69.5, 68.3, 67.9 (C-2₂, C-2₁), 3₂A, C-3₂B, C-4₂B, C-4₂₁, C-5₂A, C-5₂B, 3 OCH₂Ph, OCH₂CH=CH₂), 54.6 (OCH₃), 18.2, 17.9 (C-6₂, C-6₁) ppm. MS (MALDI-TOF): calcd. for C₄₄H₅₀O₁₀ [M]+ 738.34; found 761.39 [M + Na]+. C₄₄H₅₀O₁₀ (738.86): calcd. C 71.52, H 6.82; found C 71.45, H 6.77.

**Typical Acetolysis Procedure (ZnCl₂ Protocol):** Methyl glycoside (66 μmol) was dissolved in a 2:1 (v/v) Ac₂O/AcOH mixture (1.0 mL), cooled to 5 °C, and then treated with freshly fused ZnCl₂ (660 μmol). The solution was stirred at 5 °C until TLC (ethyl acetate in toluene) showed the disappearance of the starting material. The solution was then diluted with CH₂Cl₂ and washed with 1 M NaHCO₃ and then collected, dried with anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by chromatography (ethyl acetate in toluene).

**Typical Acetolysis Procedure (TFA Protocol):** Methyl glycoside (66 μmol) was dissolved in a 1:1:0.1 (v/v/v) Ac₂O/AcOH/TFA mixture (2.1 mL). The solution was stirred at 70 °C until TLC (ethyl acetate in toluene) showed disappearance of the starting
material. The reaction was quenched by cooling to room temp. The mixture was diluted with CH₂Cl₂ and washed with 1 M NaHCO₃. The organic layer was collected, dried with anhydrous Na₂SO₄, filtered, and concentrated. The residue was purified by chromatography (ethyl acetate in toluene).

1,5-di-\textit{O}-acetyl-2,3-di-\textit{O}-benzyl-\textit{z}-L-rhamnofuranose (29): [\text{z}]_D -11.8 (c 1.6; CH₂Cl₂); \textit{H} NMR (400 MHz, CDCl₃): \( \delta \) 7.35-7.30 (m, 10H, H-At), 6.29 (d, 1H, \( J_{1,2} \) 2.3 Hz, H-1), 5.27 (quintet, 1H, \( J_{6\beta\text{gem}} = J_{5,4} \) 6.0 Hz, H-5), 4.71 (d, 1H, \( J_{\text{gem}} \) 1.6 Hz, OCHHPh), 4.68 (d, 1H, \( J_{\text{gem}} \) 11.6 Hz, OCHHPh), 4.63 (d, 1H, \( J_{\text{gem}} \) 11.6 Hz, OCHHPh), 4.50 (d, 1H, \( J_{\text{gem}} \) 11.6 Hz, OCHHPh), 4.23 (m, 2H, H-3, H-4), 4.02 (dd, 1H, \( J_{2,3} \) 4.4 Hz, \( J_{2,3} = 2.3 \) Hz, H-2), 2.07, 1.92 (2s, 6H, 2 CH₂CO), 1.32 (d, 3H, \( J_{6,\text{gem}} \) 6.4 Hz, H-6); \textit{C} NMR (100 MHz, CDCl₃) \( \delta \) 170.0, 169.8 (2 CO), 137.7, 137.4 (2 \textit{C}_\textit{cis}-\textit{Bn}), 128.5-127.5 (C-Ar), 99.3 (C-1), 81.7, 81.6, 76.8, 73.2, 72.6, 69.2 (C-2, C-3, C-4, C-5, 2 OCH₂Ph), 21.2 (2 CH₂CO), 16.1 (C-6); MALDI TOF-MS: cale découvrir C₂₄H₂₈O₇ (m/z), 428.18; found, 450.99 [M+Na]⁺.


**Acetyl 2,3,4-Tri-\textit{O}-acetyl-\textit{z}-L-rhamnopyranosyl-(1→3)-5-\textit{O}-acetyl-2-\textit{O}-allyl-\textit{z}-L-rhamnofuranoside (31):** \textit{H} NMR (400 MHz, CDCl₃): \( \delta \) 6.27 (d, \( J_{1,2} \) 4.5 Hz, 1 H\(_{\beta}\), 1\(_{\alpha,\beta}\)-H), 6.19 (d, \( J_{1,2} = 2.9 \) Hz, 1 H\(_{\alpha}\), 1\(_{\alpha,\beta}\)-H), 5.87 (m, 1 H\(_{\beta}\) + 1 H\(_{\alpha}\), OCH\(_2\text{CH}=\text{CH}_2\)), 5.39 (dd, \( J_{3,4} = 9.9, J_{\beta,\alpha} = 3.6 \) Hz, 1 H\(_{\alpha}\), 3\(_{\beta,\alpha}\)-H), 5.32 (dd, \( J_{\beta,\alpha} = 17.2, J_{\text{gem}} = 1.5 \) Hz, 1 H\(_{\alpha}\) + 1 H\(_{\beta}\), \textit{trans} OCH\(_2\text{CH}=\text{CH}_2\)), 5.26-5.18 (m, 3H\(_{\alpha}\) + 2H\(_{\beta}\), \textit{cis} OCH\(_2\text{CH}=\text{CH}_2\)), 2\(_{\beta,\alpha}\)-H, 2\(_{\beta,\beta}\)-H, 3\(_{\beta,\alpha}\)-H, 5.18-5.01 (m, 2H\(_{\alpha}\) + 2H\(_{\beta}\), 4\(_{\beta,\alpha}\)-H, 4\(_{\beta,\beta}\)-H, 5\(_{\alpha,\alpha}\)-H, 5\(_{\alpha,\beta}\)-H), 4.78 (d, \( J_{1,2} = 1.5 \) Hz, 1 H\(_{\alpha}\), 1\(_{\beta,\alpha}\)-H), 4.74 (d, \( J_{1,2} = 1.7 \) Hz, 1 H\(_{\beta}\), 1\(_{\beta,\beta}\)-H), 4.46 (d\(_{q}\), \( J_{5,4} = 9.9, J_{5,6} = 6.2 \) Hz, 1 H\(_{\beta}\), 5\(_{\beta,\beta}\)-H), 4.35 (t, \( J_{3,4} = 4.9 \) Hz, 1 H\(_{\alpha}\), 3\(_{\alpha,\alpha}\)-H), 4.26-4.19 (m, 2H\(_{\alpha}\) + 1H\(_{\beta}\), 3\(_{\beta,\beta}\)-H, 4\(_{\beta,\alpha}\)-H,
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5\(_{B-a}\)-H), 4.13–4.03 (m, 2 H\(_a\) + 2 H\(_{p}\), OCH\(_2\)CH=CH\(_2\)), 3.99 (dd, \(J_{2,3} = 5.9, J_{1,2} = 4.5\) Hz, 1 H\(_{p}\), 2\(_{A-p}\)-H)), 3.94 (dd, \(J_{1,2} = 4.6, J_{2,1} = 2.9\) Hz, 1 H\(_a\), 2\(_{A-a}\)-H), 3.91 (t, \(J_{4,5} = J_{4,6} = 5.9\) Hz, 1 H\(_{p}\), 4\(_{A-p}\)-H), 2.18 (s, 3 H\(_p\), CH\(_3\)CO), 2.13 (2 s, 3 H\(_a\) + 3 H\(_{p}\), CH\(_2\)CO), 2.08 (s, 3 H\(_a\), CH\(_3\)CO), 2.04 (2 s, 6 H\(_a\), CH\(_3\)CO), 2.03 (s, 3 H\(_p\), CH\(_3\)CO), 2.01 (s, 3 H\(_p\), CH\(_3\)CO), 1.99 (s, 3 H\(_p\), CH\(_3\)CO), 1.98 (s, 3 H\(_a\), CH\(_3\)CO), 1.35 (d, \(J_{6,5} = 6.2\) Hz, 3 H\(_{p}\), 6\(_{A-p}\)-H), 1.31 (d, \(J_{6,5} = 6.3\) Hz, 3 H\(_a\), 6\(_{A-a}\)-H), 1.17 (d, \(J_{6,5} = 6.2\) Hz, 3 H\(_a\), 6\(_{B-a}\)-H), 1.16 (d, \(J_{6,5} = 6.2\) Hz, 3 H\(_p\), 6\(_{B-p}\)-H) ppm. \(^{13}\)C NMR (CDCl\(_3\), 50 MHz): \(\delta = 170.1–169.6\) (5 CO\(_a\) + 5 CO\(_p\)), 133.8 (\(\alpha\) OCH\(_2\)CH=CH\(_2\)), 133.6 (\(\beta\) OCH\(_2\)CH=CH\(_2\)), 118.4 (\(\alpha\) OCH\(_2\)CH=CH\(_2\)), 117.7 (\(\beta\) OCH\(_2\)CH=CH\(_2\)), 99.5, 98.4 (C-1\(_{A-a}\), C-1\(_{B-a}\)), 98.8, 93.0 (C-1\(_{A-p}\), C-1\(_{B-p}\)), 81.7, 81.0, 78.6, 71.7, 71.0, 69.9, 68.8, 68.7, 67.0 (\(\alpha\) C-2\(_a\), C-2\(_b\), C-3\(_\alpha\), C-3\(_\beta\), C-4\(_\alpha\), C-4\(_\beta\), C-5\(_\alpha\), C-5\(_\beta\), OCH\(_2\)CH=CH\(_2\)), 82.8, 78.2, 72.4, 72.1, 71.5, 70.6, 68.8, 68.7, 66.7 (\(\beta\) C-2\(_\alpha\), C-2\(_\beta\), C-3\(_\alpha\), C-3\(_\beta\), C-4\(_\alpha\), C-4\(_\beta\), C-5\(_\alpha\), C-5\(_\beta\), C-6\(_\alpha\), C-6\(_\beta\), OCH\(_2\)CH=CH\(_2\)), 21.3–20.7 (5 CH\(_3\)CO\(_a\) + 5 CH\(_3\)CO\(_p\)), 17.6, 16.3 (C-6\(_{A-a}\), C-6\(_{B-a}\)), 17.5, 16.7 (C-6\(_{A-p}\), C-6\(_{B-p}\)) ppm. MS (MALDI-TOF): calcd. for C\(_{22}\)H\(_{28}\)O\(_{14}\) [M]\(^+\) 560.21; found 582.90 [M + Na]\(^+\). C\(_{22}\)H\(_{30}\)O\(_{14}\) (560.55): calcd. C 53.57, H 6.47; found C 53.70, H 6.40.

Acetyl 2,3,4-Tri-\(\alpha\)-acetyl-\(\alpha\)-L-rhamnopyranosyl-(1→3)-2-\(\alpha\)-allyl-4-\(\alpha\)-benzyl-\(\alpha\)-L-rhamnopyranoside (32): [\(\alpha\)]\(_D\) = –39.2 (c = 1.4, CH\(_2\)Cl\(_2\)). \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta = 7.32\) (m, 5 H, Ar-H), 6.09 (d, \(J_{1,2} = 2.1\) Hz, 1 H, 1\(_\alpha\)-H), 5.98 (m, 1 H, OCH\(_2\)CH=CH\(_2\)), 5.40–5.34 (m, 3 H, 2\(_\alpha\)-H, 3\(_\beta\)-H, trans OCH\(_2\)CH=CHH), 5.26 (dd, \(J_{\alpha,\alpha} = 10.5, J_{\gamma,\gamma} = 1.2\) Hz, 1 H, \(\alpha\)-\(\alpha\) OCH\(_2\)CH=CHH), 5.08 (t, \(J_{3,4} = J_{4,5} = 9.9\) Hz, 1 H, 4\(_\alpha\)-H), 5.05 (br. s, 1 H, 1\(_\beta\)-H), 4.84 (d, \(J_{\gamma,\gamma} = 11.1\) Hz, 1 H, OCHHPh), 4.64 (d, \(J_{\gamma,\gamma} = 11.1\) Hz, 1 H, OCHHPh), 4.27 (dd, \(J_{\gamma,\gamma} = 12.9, J_{\alpha,\alpha} = 5.4\) Hz, 1 H, OCHHCH=CH\(_2\)), 4.12–3.98 (m, 3 H, 3\(_\beta\)-H, 5\(_\alpha\)-H, OCHHCH=CH\(_2\)), 3.77 (dq, \(J_{5,6} = 9.6, J_{5,6} = 6.3\) Hz, 1 H, 5\(_\alpha\)-H), 3.67 (dd, \(J_{2,3} = 3.0, J_{2,1} = 1.2\) Hz, 1 H, 2\(_\alpha\)-H), 2.87 (s, 3 H, OCH\(_2\)) ppm. 

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= 2.1 Hz, 1 H, 2α-H), 3.63 (t, J_{4,3} = J_{4,5} = 9.6 Hz, 1 H, 4α-H), 2.09, 2.07, 2.06, 1.99 (4 s, 12 H, 4 CH₂CO), 1.31 (d, J_{6,5} = 6.3 Hz, 3 H, 6α-H), 1.20 (d, J_{6,5} = 6.3 Hz, 3 H, 6β-H) ppm.

¹³C NMR (CDCl₃, 50 MHz): δ = 170.0, 169.9, 169.8, 169.1 (4 CO), 137.8 (C_{paa}-Bn), 134.3 (OCH₂CH=CH₂), 128.4–127.8 (C-Ar), 117.9 (OCH₂CH=CH₂), 99.4 (C-1_b), 91.2 (C-1_a), 79.7, 78.5, 76.7, 75.6, 71.8, 70.9, 70.6, 69.8, 69.0, 66.9 (C-2_a, C-2_b, C-3_a, C-3_b, C- 4_a, C-4_b, C-5_a, C-5_b, OCH₂Ph, OCH₂CH=CH₂), 21.0–20.8 (4 CH₃CO), 18.0, 17.5 (C-6_a, C-6_b) ppm. MS (MALDI-TOF): calcd. for C_{30}H_{40}O_{13} [M⁺] 608.25; found 630.74 [M + Na⁺]. C_{30}H_{40}O_{13} (608.63): calcd. C 59.20, H 6.62; found C 59.11, H 6.70.

2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-1,5-di-O-acetyl-4-O-allyl-2-O-benzyl-1-O-methyl-D-rhamnose Acetal (33): ¹H NMR (500 MHz, CDCl₃) (major diastereoisomer): δ = 7.32 (m, 5 H, Ar-H), 5.99 (d, J_{1,2} = 7.0 Hz, 1 H, 1α-H), 5.8 (m, 1 H, OCH₂CH=CH₂), 5.35 (dd, J_{2,3} = 3.0, J_{2,1} = 1.5 Hz, 1 H, 2α-H), 5.24 (dd, J_{3,4} = 10.2, J_{3,2} = 3.0 Hz, 1 H, 3α-H), 5.19 (d, J_{6,5} = 17.5 Hz, 1 H, trans OCH₂CH=CH₂), 5.08 (m, 2 H, 1β-H, 4β-H), 5.04 (d, J_{6,5} = 10.5 Hz, 1 H, trans OCH₂CH=CH₂), 4.81 (dq, J_{5,6} = 6.6, J_{5,4} = 1.8 Hz, 1 H, 5α-H), 4.62 (s, 2 H, OCH₂Ph), 4.15 (m, 3 H, OCH₂CH=CH₂), 3.80 (m, 2 H, 3α-H, 4α-H), 3.52 (m, 4 H, 2β-H, OCH₂), 2.12 (s, 6 H, 2 CH₃CO), 2.07 (s, 3 H, CH₃CO), 2.02 (s, 3 H, CH₃CO), 1.97 (s, 3 H, CH₃CO), 1.04 (d, J_{6,5} = 6.2 Hz, 3 H, 6β-H), 1.02 (d, J_{6,5} = 6.6 Hz, 3 H, 5α-H) ppm. ¹³C NMR (CDCl₃, 75 MHz) (major diastereoisomer): δ = 170.5, 170.2, 169.9, 169.8, 169.7 (5 CO), 137.5 (C_{paa}-Bn), 134.8 (OCH₂CH=CH₂), 128.5–127.9 (C-Ar), 116.5 (OCH₂CH=CH₂), 98.7, 98.0 (C-1_a, C-1_b), 80.6, 78.8, 77.7, 73.7, 73.5, 71.0, 70.8, 69.8, 69.6, 67.1 (C-2_a, C-2_b, C-3_a, C-3_b, C-4_a, C- 4_b, C-5_a, C-5_b, OCH₂Ph, OCH₂CH=CH₂), 57.7 (OCH₂), 21.3, 21.2, 20.9, 20.8, 20.7 (5 CH₃CO), 17.4, 13.7 (C-6_a, C-6_b) ppm. MS (MALDI-TOF): calcd. for C_{30}H_{40}O_{13} [M⁺]
682.28; found 704.73 [M + Na]^+. C_{33}H_{46}O_{15} (682.71): calcd. C 58.06, H 6.79; found C 57.88, H 6.65.

Acetyl 2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-4-O-allyl-2-O-benzyl-α-L-rhamnopyranoside (34): [α]D = −32 (c = 0.9, CH₂Cl₂). ¹H NMR (200 MHz, CDCl₃): δ = 7.39 (m, 5 H, Ar-H), 6.15 (d, J₁₂ = 2.0 Hz, 1 H, ₁₁-H), 5.89 (m, 1 H, OCH₂CH=CH₂), 5.34–5.03 (m, 6 H, ₁₁-H, ₂₁-H, ₃₁-H, ₄₁-H, OCH₂CH=CH₂), 4.82 (d, J₆₈₂ = 12.0 Hz, 1 H, OCH₂HPh), 4.62 (dd, J₆₈₂ = 12.0 Hz, 1 H, OCH₂HPh), 4.30 (s, 12 H, ₃CH₃CO), 1.31 (d, J₆₅ = 6.2 Hz, 3 H, ₆₁-H), 1.07 (d, J₆₅ = 6.2 Hz, 3 H, ₆₁-H) ppm. ¹³C NMR (CDCl₃, 75 MHz): δ = 169.8–169.7 (4 CO), 137.5 (C-₁₁), 128.5–127.7 (C-Ar), 117.2 (OCH₂CH=CH₂), 99.5 (C-₁₈), 91.2 (C-₁₉), 79.7, 78.4, 77.2, 74.4, 72.3, 71.0, 70.9, 69.9, 69.0, 66.9 (C-₂₁, C-₂₂, C-₁₈, C-₁₉, C-₁ₐ, C-₁ₗ, C-₁₉, OCH₂Ph, OCH₂CH=CH₂), 20.8–20.7 (4 CH₃CO), 18.0, 17.5 (C-₆₁, C-₆₂) ppm. MS (MALDI-TOF): calcd. For C₃₀H₄₀O₁₃ [M]⁺ 608.25; found 631.11 [M + Na]^+. C₃₀H₄₀O₁₃ (608.63): calcd. C 59.20, H 6.62; found C 59.02, H 6.54.

2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-1,4,5-tri-O-acetyl-2-O-benzyl-1-O-methyl-D-rhamnose Acetal (35): ¹H NMR (500 MHz, CDCl₃): δ = 7.41–7.33 (m, 5 H', 5H", Ar-H), 6.00 (d, J₁₂ = 5.0 Hz, 1 H", ₁₁-H), 5.98 (d, J₁₂ = 6.0 Hz, 1 H', ₁₁-H), 5.45 (m, 1 H' + 1 H", ₄₁-H, ₄₁-H), 5.25 (m, 1 H' + 1 H", ₃₁-H, ₃₁-H), 5.15 (dd, J₂₃ = 3.0, J₂₃ = 1.8 Hz, 1 H"), 5.13 (dd, J₂₃ = 3.0, J₂₃ = 1.8 Hz, 1 H''), 2.08 (s, 12 H, ₃CH₃CO), 1.31 (d, J₆₅ = 6.2 Hz, 3 H, ₆₁-H), 1.07 (d, J₆₅ = 6.2 Hz, 3 H, ₆₁-H) ppm.
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\[ J_{\alpha,5} = 10.0 \text{ Hz}, 1 \text{ H' + 1 H''}, 4_{\text{w}-\text{H}}, 4_{\text{b}'-\text{H}} \], 4.96 (m, 1 H' + 1 H'', 5_{\alpha'} \text{ H}, 5_{\alpha''} \text{ H}), 4.91 (d, \quad J_{1,2} = 1.8 \text{ Hz}, 1 \text{ H'}, 1_{\text{w}-\text{H}}), 4.86 (d, J_{1,2} = 1.8 \text{ Hz}, 1 \text{ H''}, 1_{\text{b}'-\text{H}}), 4.78 (d, J_{\text{gw}} = 12.0 \text{ Hz}, 1 \text{ H'}, OCHHPh'), 4.69–4.61 (m, 2 H' + 1 H'', OCHHPh', OCHHPh''), 4.08 (dq, J_{5,4} = 10.0, J_{5,6} = 6.2 \text{ Hz}, 1 \text{ H'} + 1 \text{ H''}, 5_{\text{w}-\text{H}}, 5_{\text{b}'-\text{H}}), 3.93 (dd, J_{\text{d},1} = 8.0, J_{\text{d},2} = 1.8 \text{ Hz}, 1 \text{ H'}), 3_{\alpha'} \text{ H}), 3.89 (dd, J_{\text{d},1} = 8.0, J_{\text{d},2} = 3.0 \text{ Hz}, 1 \text{ H''}, 3_{\alpha'} \text{ H}), 3.66 (dd, J_{\text{d},1} = 5.0, J_{\text{d},2} = 3.0 \text{ Hz}, 1 \text{ H''}, 2_{\alpha'} \text{ H}), 3.57 (dd, J_{\text{d},1} = 6.0, J_{\text{d},2} = 1.8 \text{ Hz}, 1 \text{ H'}, 2_{\alpha'} \text{ H}), 3.53 (s, 3 \text{ H'}, OCH_3'), 3.52 (s, 3 \text{ H''}, OCH_3''), 2.17 (s, 3 \text{ H''}, CH_3CO''), 2.14 (s, 3 \text{ H'}, CH_3CO'), 2.13 (s, 3 \text{ H''}, CH_3CO''), 2.11 (s, 3 \text{ H'}, CH_3CO'), 2.07 (s, 3 \text{ H'}, CH_3CO'), 2.06 (s, 3 \text{ H'} + 3 \text{ H''}, CH_3CO', CH_3CO''), 2.05 (s, 3 \text{ H''}, 3 \text{ CH}_2\text{CO}''), 1.97 (s, 6 \text{ H'} + 6 \text{ H''}, 2 \text{ CH}_2\text{CO}', 2 \text{ CH}_2\text{CO''}), 1.11 (d, J_{6,5} = 6.2 \text{ Hz}, 3 \text{ H''}, 6_{\text{w}-\text{H}}), 1.05 (d, J_{6,5} = 6.2 \text{ Hz}, 3 \text{ H'}, 6_{\text{w}-\text{H}}), 1.03 (d, J_{6,5} = 6.6 \text{ Hz}, 3 \text{ H'}, 6_{\alpha''} \text{ H}), 1.00 (d, J_{6,5} = 6.6 \text{ Hz}, 3 \text{ H'}, 6_{\alpha'} \text{ H}) \text{ ppm}. 1^3\text{C NMR (CDCl}_3, 75 \text{ MHz}): \delta = 170.4–169.7 (5 \text{ CO'}, 5 \text{ CO''}), 137.6 (C_{\text{gw}}-\text{Bn}', C_{\text{gw}}-\text{Bn}''), 128.5–127.8 (C-\text{Ar}), 98.8, 97.1 (C-1_{\alpha'}, C-1_{\beta'}), 98.5, 97.7 (C-1_{\alpha'}, C-1_{\beta'}), 79.1, 76.8, 73.6, 73.1, 71.0, 70.1, 68.9, 68.8, 67.2 (C-2_{\alpha'}, C-2_{\beta'}, C-3_{\alpha'}, C-3_{\beta'}, C-4_{\alpha'}, C-4_{\beta'}, C-5_{\alpha'}, C-5_{\beta'}, OCH_3'), 79.1, 77.2, 73.9, 72.9, 71.1, 70.0, 68.9, 68.8, 67.2 (C-2_{\alpha'}, C-2_{\beta'}, C-3_{\alpha'}, C-3_{\beta'}, C-4_{\alpha'}, C-4_{\beta'}, C-5_{\alpha'}, C-5_{\beta'}, OCH_3''), 79.1, 77.2, 73.9, 72.9, 71.1, 70.0, 68.9, 68.8, 67.2 (C-2_{\alpha'}, C-2_{\beta'}, C-3_{\alpha'}, C-3_{\beta'}, C-4_{\alpha'}, C-4_{\beta'}, C-5_{\alpha'}, C-5_{\beta'}, OCH_3''), 79.1, 77.2, 73.9, 72.9, 71.1, 70.0, 68.9, 68.8, 67.2 (C-2_{\alpha'}, C-2_{\beta'}, C-3_{\alpha'}, C-3_{\beta'}, C-4_{\alpha'}, C-4_{\beta'}, C-5_{\alpha'}, C-5_{\beta'}, OCH_3''), 79.1, 77.2, 73.9, 72.9, 71.1, 70.0, 68.9, 68.8, 67.2 (C-2_{\alpha'}, C-2_{\beta'}, C-3_{\alpha'}, C-3_{\beta'}, C-4_{\alpha'}, C-4_{\beta'}, C-5_{\alpha'}, C-5_{\beta'}, OCH_3''), 57.9 (OCH_3'), 53.4 (OCH_3''), 21.2–20.7 (5 \text{ CH}_2\text{CO'}, 5 \text{ CH}_2\text{CO''}), 19.2, 14.5 (C-6_{\alpha'}, C-6_{\beta'}), 17.3, 14.4 (C-6_{\alpha'}, C-6_{\beta'}) \text{ ppm. MS (MALDI-TOF): calcd. for C}_{32}\text{H}_{44}\text{O}_{16} [M]^+ 684.26; found 706.80 [M + Na]^+. C}_{32}\text{H}_{44}\text{O}_{16} (684.68): calcd. C 56.13, H 6.48; found C 56.00, H 6.32.

Acetyl 3-O-Allyl-2-O-acetyl-4-O-benzyl-\alpha-D-rhamnopyranosyl-(1→3)-5-O-acetyl-2-O-benzyl-D-rhamnofuranoside (36): \textit{H NMR (400 MHz, CDCl}_3) (\alpha anomer): \delta 7.34 (m, 10 H, Ar-H), 6.20 (d, J_{1,2} = 2.7 \text{ Hz}, 1 \text{ H}, 1_{\alpha'-\text{H}}), 5.92 (m, 1 \text{ H}, OCH_2CH=CH_2), 5.30 (dd, J_{\text{gw}} = 18.0, J_{\text{gw}} = 1.8 \text{ Hz}, 1 \text{ H}, trans OCH_2CH=CHH), 5.19–5.05 (m, 3 \text{ H}, 2_{\beta'}-\text{H}, 2_{\text{w}-\text{H}}).
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5\(_\alpha\)-H, \(\alpha\)s OCH\(_2\)CH=CHH), 4.90 (d, \(J_{\text{gem}} = 11.1\) Hz, 1 H, OCHHPh), 4.78 (d, \(J_{1,2} = 1.5\) Hz, 1 H, 1\(_\beta\)-H), 4.66–4.56 (m, 3 H, 3 OCHHPh), 4.39 (t, \(J_{3,4} = J_{1,2} = 4.8\) Hz, 1 H, 3\(_\alpha\)-H), 4.22–4.01 (m, 4 H, 4\(_\alpha\)-H, 5\(_\alpha\)-H, OCH\(_2\)CH=CH\(_2\)), 3.94 (dd, \(J_{2,3} = 4.8\) Hz, 1 H, 4\(_\beta\)-H), 3.84 (dd, \(J_{3,4} = 9.2, J_{3,2} = 3.0\) Hz, 1 H, 3\(_\alpha\)-H), 3.38 (t, \(J_{4,3} = J_{4,5} = 4.8\) Hz, 1 H, 4\(_\beta\)-H), 2.14, 2.06, 2.05 (3s, 9 H, 3 CH\(_3\)CO), 1.29 (d, \(J_{6,5} = 6.3\) Hz, 3 H, 6\(_\alpha\)-H), 1.18 (d, \(J_{6,5} = 6.3\) Hz, 3 H, 6\(_\beta\)-H) ppm. \(^{13}\)C NMR (CDCl\(_3\), 75 MHz) (\(\alpha\) anomer): \(\delta = 170.0, 169.8, 169.6\) (3 CO), 138.7, 137.1 (2 C\(_{\text{gem}}\)-Bn), 134.5 (OCH\(_2\)CH=CH\(_2\)), 128.5–127.4 (C-Ar), 117.3 (OCH\(_2\)CH=CH\(_2\)), 99.6, 98.4 (C-1\(_\alpha\), C-1\(_\beta\)), 81.4, 80.8, 79.8, 77.3, 76.7, 75.0, 72.6, 70.8, 69.4, 68.6, 68.5 (C-2\(_\alpha\), C-2\(_\beta\), C-3\(_\alpha\), C-3\(_\beta\), C-4\(_\alpha\), C-4\(_\beta\), C-5\(_\alpha\), C-5\(_\beta\)), 2OCH\(_2\)Ph, OCH\(_2\)CH=CH\(_2\)), 21.3, 21.1, 21.0 (3 CH\(_3\)CO), 18.0, 16.4 (C-6\(_\alpha\), C-6\(_\beta\)) ppm. MS (MALDI-TOF): calcd. for C\(_{35}\)H\(_{44}\)O\(_{12}\) [M]\(^+\) 656.28; found 679.21 [M + Na]\(^+\). C\(_{35}\)H\(_{44}\)O\(_{12}\) (656.72): calcd. C 64.01, H 6.75; found C 64.23, H 6.66.

**Acetyl 2-O-Acetyl-3-O-allyl-4-O-benzyl-\(\alpha\)-D-rhamnopyranosyl-(1\(\rightarrow\)3)-2,4-di-O-benzyl-\(\alpha\)-D-rhamnopyranoside (37):** \([\alpha]_D = +69.6\) (c = 1.0, CH\(_2\)Cl\(_2\)). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 7.38–7.26\) (m, 15 H, Ar-H), 6.13 (d, \(J_{1,2} = 2.0\) Hz, 1 H, 1\(_\alpha\)-H), 5.86 (m, 1 H, OCH\(_2\)CH=CH\(_2\)), 5.43 (dd, \(J_{2,1} = 1.8, J_{2,3} = 3.4\) Hz, 1 H, 2\(_\beta\)-H), 5.25 (dd, \(J_{\text{gem}} = 17.0, J_{\text{gem}} = 1.6\) Hz, 1 H, \(\alpha\)s OCH\(_2\)CH=CHH), 5.08 (d, \(J_{1,2} = 1.6\) Hz, 1 H, 1\(_\beta\)-H), 4.93 (d, \(J_{\text{gem}} = 11.0\) Hz, 1 H, OCHHPh), 4.84 (d, \(J_{\text{gem}} = 11.0\) Hz, 1 H, OCHHPh), 4.76 (d, \(J_{\text{gem}} = 11.8\) Hz, 1 H, OCHHPh), 4.67 (d, \(J_{\text{gem}} = 11.8\) Hz, 1 H, OCHHPh), 4.63 (d, \(J_{\text{gem}} = 10.8\) Hz, 1 H, OCHHPh), 4.61 (d, \(J_{\text{gem}} = 11.0\) Hz, 1 H, OCHHPh), 4.09 (dd, \(J_{\text{gem}} = 12.8, J_{\text{gem}} = 5.5\) Hz, 1 H, OCHHCH=CH\(_2\)), 4.07 (dd, \(J_{3,2} = 3.2, J_{3,4} = 9.4\) Hz, 1 H, 3\(_\alpha\) H), 4.03 (dd, \(J_{\text{gem}} = 12.8, J_{\text{gem}} = 5.5\) Hz, 1 H, OCHHCH=CH\(_2\)), 3.85 (dd, \(J_{3,2} = 3.4, J_{3,4} = 9.3\) Hz, 1 H, 3\(_\beta\)-H), 3.78
Acetyl 3-O-Allyl-2-O-benzoyl-4-O-benzyl-α-D-rhamnopyranosyl-(1→3)-5-O-acetyl-2-O-benzyl-α-D-rhamnofuranoside (38a): [α]D = +5.9 (c = 0.8, CHCl3). 1H NMR (500 MHz, CDCl3): δ = 8.07–7.26 (m, 15 H, Ar-H), 6.22 (d, J1,2 = 3.0 Hz, 1 H, 1α-H), 5.87 (m, 1 H, OCH2CH2CH2), 5.38 (dd, J2,3 = 3.4, J2,1 = 2.0 Hz, 1 H, 21-H), 5.28 (dd, J2,1 = 18.0, J2,ω = 1.5 Hz, 1 H, trans OCH2CH2CHH), 5.18 (q, J5,6 = J5,4 = 6.0 Hz, 1 H, 5α-H), 5.12 (dd, J5,6 = 10.0, J5,ω = 1.5 Hz, 1 H, cis OCH2CH2CHH), 4.94 (d, J5,ω = 11.0 Hz, 1 H, OCHHHPh), 4.91 (d, J1,2 = 2.0 Hz, 1 H, 1β-H), 4.64 (m, 3 H, 3 OCHHHPh), 4.43 (t, J3,4 = J3,2 = 5.4 Hz, 1 H, 3α-H), 4.20 (m, 2 H, 4α-H, OCHHCHCH2), 4.09 (m, 2 H, 5α-H, OCHHHCHCH2), 3.96 (m, 2 H, 2α-H, 3β-H), 3.49 (t, J4,5 = J4,3 = 9.0 Hz, 1 H, 4β-H), 2.08 (s, 3 H, CH3CO), 2.06 (s, 3 H, CH3CO), 1.31 (d, J6,5 = 6.0 Hz, 3 H, 6α-H), 1.21 (d, J6,5 = 6.0 Hz, 3 H, 6β-H) ppm. 13C NMR (CDCl3, 50 MHz): δ = 169.9, 169.8, 165.5 (3 CO), 138.6, 137.1 (2 C=O), 134.6 (OCH2CH=CH2), 133.1 (C=O), 129.9–127.6 (C-Ar), 117.3 (OCH2CH=CH2), 99.5, 98.3 (C-1α, C-1β), 81.4, 80.8, 79.7, 77.4, 76.6, 75.0, 72.5, 70.6, 69.7, 68.6, 68.5 (C-2α, C-2β, C-3α, C-3β, C-4α, C-4β, C-5α, C-5β, 2 OCH3Ph,
Acetyl 3-O-Allyl-2-O-benzoyl-4-O-benzyl-α-D-rhamnopyranosyl-(1→3)-5-O-acetyl-2-O-benzyl-α-D-rhamnofuranoside (388): [α]D = -6 (c = 0.3, CH2Cl2). 1H NMR (500 MHz, CDCl3): δ = 8.07–7.28 (m, 15 H, Ar-H), 6.25 (d, J1,2 = 4.5 Hz, 1 H, 1α-H), 5.88 (m, 1 H, OCH2CH=CH2), 5.36 (dd, Jvic = 18.0, Jgem = 1.6 Hz, 1 H, trans OCH2CH=CHH), 5.30 (dd, J2,3 = 3.4, J2,1 = 2.0 Hz, 1 H, 2α-H), 5.13 (m, 2 H, 5α-H, cis OCH2CH=CH2), 5.30 (dd, J2,3 = 3.4, J2,1 = 2.0 Hz, 1 H, 2β-H), 5.13 (m, 2 H, 5α-H, cis OCH2CH=CH2), 4.95 (d, J2,3 = 3.4, J2,1 = 2.0 Hz, 1 H, 2α-H), 4.85 (d, J1,2 = 1.8 Hz, 1 H, OCH2CH=CH2), 4.69 (d, J2,3 = 3.4, J2,1 = 2.0 Hz, 1 H, 2β-H), 4.63 (d, J2,3 = 3.4, J2,1 = 2.0 Hz, 1 H, 2β-H), 4.32 (dq, J5,4 = 10.0, J5,6 = 6.0 Hz, 1 H, 5β-H), 4.23 (m, 2 H, 3α-H, OCH2HCH=CH2), 4.10 (dd, J5,6 = 10.0, J5,6 = 6.0 Hz, 1 H, 5β-H), 4.00 (dd, J3,4 = 10.0, J3,2 = 3.4Hz, 1 H, 3α-H), 3.94 (dd, J4,5 = 8.5, J4,3 = 4.0 Hz, 1 H, 4α-H), 3.85 (tr, J4,5 = 4.5 Hz, 1 H, 1β-H), 3.50 (dd, J4,5 = 8.5, J4,3 = 4.0 Hz, 1 H, 4α-H), 2.11 (s, 3 H, CH3CO), 2.09 (s, 3 H, CH3CO), 1.36 (d, J6,5 = 6.0 Hz, 3 H, 6α-H), 1.15 (d, J6,5 = 6.0Hz, 3 H, 6β-H) ppm. 13C NMR (CDCl3, 125 MHz): δ = 170.5, 170.3, 165.6 (3 CO), 138.9, 137.1 (2 Cgem-Bn), 135.2, 134.8 (OCH2CH=CH2, Cgem-Bz), 129.9–127.4 (C-Ar), 117.3 (OCH2CH=CH2), 99.8, 93.2 (C-1α, C-1β), 82.7, 80.1, 77.7, 77.2, 76.6, 75.0, 72.6, 70.1, 69.8, 68.6, 68.3 (C-2α, C-2β, C-3α, C-3β, C-4α, C-4β, C-5α, C-5β, 2 OCH2Ph, OCH2CH=CH2), 21.4, 21.3 (2 CH3CO), 18.1, 16.9 (C-6α, C-6β) ppm. MS (MALDI-TOF): calcd. for C48H66O12 [M]+ 718.30; found 740.56 [M + Na]+. C48H66O12 (718.79): calcd. C 66.84, H 6.45; found C 66.55, H 6.25.
Acetyl 3-O-Allyl-2-O-benzoyl-4-O-benzyl-α-D-rhamnopyranosyl-(1→3)-2,4-di-O-benzyl-α-D-rhamnopyranoside (39): $[\alpha]_D = -2.5$ (c = 1.2, CH$_2$Cl$_2$). $^1$H NMR (200 MHz, CDCl$_3$): δ = 8.07–7.16 (m, 20 H, Ar-H), 6.14 (d, $J_{1,2} = 2.0$ Hz, 1 H, 1α-H), 5.84 (m, 1 H, OCH$_2$CH=CH$_2$), 5.68 (dd, $J_{2,1} = 1.6, J_{2,3} = 3.4$ Hz, 1 H, 2α-H), 5.25–5.21 (m, 2 H, 1β-H, trans OCH$_2$CH=CHH), 5.08 (dd, $J_{4,5} = 10.2, J_{4,6} = 1.8$ Hz, 1 H, cis OCH$_2$CH=CHH), 4.95 (d, $J_{4,6} = 11.0$ Hz, 1 H, OCHHPh), 4.79 (d, $J_{4,5} = 12.0$ Hz, 1 H, OCHHPh), 4.72–4.62 (m, 3 H, 3 OCHHPh), 4.19 (dd, $J_{4,5} = 12.0, J_{4,6} = 5.0$ Hz, 1 H, OCHHCH=CH$_2$), 4.12–4.05 (m, 2 H, 3α-H, OCHHCH=CH$_2$), 3.98 (dd, $J_{3,2} = 3.2, J_{3,4} = 9.2$ Hz, 1 H, 3β-H), 3.91–3.65 (m, 4 H, 2α-H, 4α-H, 5α-H, 5β-H), 3.51 (t, $J_{4,5} = J_{4,6} = 9.6$ Hz, 1 H, 4β-H), 2.06 (s, 3 H, CH$_3$CO), 1.32 (d, $J_{6,5} = 6.2$ Hz, 3 H, 6β-H), 1.29 (d, $J_{6,5} = 6.2$ Hz, 3 H, 6α-H) ppm. $^{13}$C NMR (CDCl$_3$, 50 MHz): δ = 169.2, 165.5 (2 CO), 138.6, 137.8, 137.5 (3 C$_{\text{p,n}}$-Bn), 134.6 (OCH2CH=CH2), 133.1 (C$_{\text{p,n}}$-Bz), 129.8–125.3 (C-Ar), 117.1 (OCH$_2$CH=CH$_2$), 99.5 (C-1b), 91.2 (C-1α), 80.1, 79.9, 77.6, 77.4, 76.7, 75.5, 75.2, 72.6, 72.5, 70.6, 69.6, 68.4 (C-2α, C-2β, C-3α, C-3β, C-4α, C-4β, C-5α, C-5β, 3 OCH$_3$Ph, OCH$_2$CH=CH$_2$), 21.0 (CH$_3$CO), 18.2, 18.1 (C-6α, C-6β) ppm. MS (MALDI-TOF): calcd. for C$_{49}$H$_{30}$O$_{11}$ [M]$^+$ 766.34; found 789.42 [M + Na]$^+$. C$_{49}$H$_{30}$O$_{11}$ (766.87): calcd. C 70.48, H 6.57; found C 70.28, H 6.34.

Acetyl 2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-O-benzyl- L-fucopyranoside (41): $^1$H NMR (300 MHz, CDCl$_3$) (α-anomer): δ = 7.42–7.32 (m, 10 H, Ar-H), 6.44 (d, $J_{1,2} = 2.7$ Hz, 1 H, 1α-H), 5.41 (dd, $J_{5,4} = 9.9, J_{5,6} = 3.6$ Hz, 1 H, 3α-H), 5.20 (dd, $J_{2,3} = 3.6, J_{2,1} = 1.8$ Hz, 1 H, 2α-H), 5.06 (t, $J_{4,5} = J_{4,6} = 9.9$ Hz, 1 H, 4β-H), 5.00 (s, 1 H, 1β-H), 4.93 (d, $J_{4,5} = 11.1$ Hz, 1 H, OCHHPh), 4.71 (d, $J_{4,5} = 11.4$ Hz, 1 H, OCHHPh), 4.62 (d, $J_{4,5} = 11.4$ Hz, 1 H, OCHHPh), 4.60 (d, $J_{4,5} = 11.1$ Hz, 1 H, OCHHPh), 4.18
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(\text{dq}, J_{5,4} = 9.9, J_{5,6} = 6.3 \text{ Hz}, 1 \text{ H}, 5_\beta-\text{H}), 4.12 (\text{d}, J_{4,3} = 2.4 \text{ Hz}, 1 \text{ H}, 4_\alpha-\text{H}), 3.99 (\text{q}, J_{5,6} = 6.6 \text{ Hz}, 1 \text{ H}, 5_\alpha-\text{H}), 3.69-3.61 (\text{m}, 2 \text{ H}, 2_\alpha-\text{H}, 3_\alpha-\text{H}), 2.16, 2.11, 2.03, 2.00 (4 \text{ s}, 12 \text{ H}, 4 \text{ CH}_2\text{CO}), 1.18 (\text{d}, J_{6,5} = 6.6 \text{ Hz}, 3 \text{ H}, 6_\alpha-\text{H}), 0.98 (\text{d}, J_{6,5} = 6.3 \text{ Hz}, 3 \text{ H}, 6_\beta-\text{H}) \text{ ppm}.

\text{\textsuperscript{13}C NMR} (\text{CDCl}_3, 75 \text{ MHz}) (\alpha \text{ anomer}): \delta = 170.3, 169.9, 169.7, 169.3 (4 \text{ CO}), 137.9, 137.7 (2 \text{ C}_{\text{ipso}}-\text{Bn}), 128.9-127.7 (\text{C}-\text{Ar}), 94.1 (\text{C}-1_\beta), 90.6 (\text{C}-1_\alpha), 76.1, 75.6, 74.3, 73.4, 73.0, 70.9, 70.3, 69.0, 68.9, 66.5 (\text{C}-2_\alpha, \text{C}-2_\beta, \text{C}-3_\alpha, \text{C}-3_\beta, \text{C}-4_\alpha, \text{C}-4_\beta, \text{C}-5_\alpha, \text{C}-5_\beta, 2 \text{ OCH}_2\text{Ph}), 21.0-20.7 (4 \text{ CH}_2\text{CO}), 17.0, 16.8 (\text{C}-6_\alpha, \text{C}-6_\beta) \text{ ppm}.

\text{MS (MALDI-TOF)}: \text{calcd. for C}_{34}\text{H}_{42}\text{O}_{13}\ [\text{M}]^+ 658.26; \text{found 680.88 [M + Na]}^+.

\text{C}_{34}\text{H}_{42}\text{O}_{13} (658.69): \text{calcd. C 62.00, H 6.43; found C 62.18, H 6.61.}

\text{4,5-di-O-acetyl-2,3-di-O-benzyl-D-quinovose (42): \{[\alpha]_D +10 (c 0.4; CH}_2\text{Cl}_2); \text{\textsuperscript{1}H NMR (400 MHz, CDCl}_3): \delta 9.73 (s, 1\text{H}, \text{CHO}), 7.36-7.23 (\text{m}, 10\text{H}, \text{H}-\text{Ar}), 5.35 (\text{dd}, 1\text{H}, J_{4,5} 6.4 \text{ Hz}, J_{4,3} 3.0 \text{ Hz}, \text{H}-\text{4}), 5.10 (\text{quintet, 1\text{H}}, J_{5,4}=J_{5,6} 6.4 \text{ Hz}, \text{H}-\text{5}), 4.77 (\text{d}, 1\text{H}, J_{\text{gem}} 12.0 \text{ Hz}, \text{OCCH}_2\text{Ph}), 4.57 (\text{s, 2\text{H}}, \text{OCH}_2\text{Ph}), 4.52 (\text{d}, 1\text{H}, J_{\text{gem}} 12.0 \text{ Hz}, \text{OCCH}_2\text{Ph}), 3.92 (\text{m}, 2\text{H}, \text{H}-\text{2, H}-\text{3}), 2.02 (\text{s, 3\text{H}}, \text{CH}_3\text{CO}), 1.97 (\text{s, 3\text{H}}, \text{CH}_3\text{CO}), 1.19 (\text{d}, 3\text{H}, J_{6,5} 6.4 \text{ Hz}, \text{H}-\text{6}); \text{\textsuperscript{13}C NMR (100 MHz, CDCl}_3): \delta 201.1 (\text{CHO}), 169.9, 169.7 (2 \text{ CO}), 136.9, 136.8 (2 \text{ C}_{\text{ipso}}-\text{Bn}), 128.6-128.0 (\text{C}-\text{Ar}), 80.6, 77.5, 74.1, 73.2, 72.1, 68.6 (\text{C}-2, \text{C}-3, \text{C}-4, \text{C}-5, 2 \text{ OCH}_2\text{Ph}), 21.2, 20.8 (2 \text{ CH}_2\text{CO}), 15.8 (\text{C}-6). \text{MALDI TOF-MS: caleld for C}_{24}\text{H}_{28}\text{O}_7 (m/z), 428.18; \text{found, 451.01 [M+Na]}^+. \text{Anal Calcd for C}_{24}\text{H}_{28}\text{O}_7: \text{C}, 67.28; \text{H, 6.59. Found: C, 67.09; H, 6.49.}

\text{1,1,4,5-tetra-O-acetyl-2,3-di-O-benzyl-D-quinovose acetal (43): \{[\alpha]_D +19 (c 0.5; CH}_2\text{Cl}_2); \text{\textsuperscript{1}H NMR (400 MHz, CDCl}_3): \delta 7.34-7.28 (\text{m}, 10\text{H}, \text{H}-\text{Ar}), 6.97 (\text{d}, 1\text{H}, J_{1,2} 3.2 \text{ Hz}, \text{H}-\text{1}), 5.24 (\text{d}, 1\text{H}, J_{4,5} 5.9 \text{ Hz}, J_{4,3} 3.2 \text{ Hz}, \text{H}-\text{4}), 5.16 (\text{quintet, 1\text{H}}, J_{5,4}=J_{5,6} 6.2 \text{ Hz}, \text{H}-}\)
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1.11 1,5-di-\(\alpha\)-acetyl-2,3-di-\(\alpha\)-benzyl-D-quinovofuranose (44): \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.36-7.24 (m, 10H\(_a\) + 10H\(_b\), H-Ar), 6.35 (d, 1H\(_a\), \(J_{1,2}\) 4.2 Hz, H-1\(_a\)), 6.16 (bs, 1H\(_b\), H-1\(_b\)), 5.24 (dq, 1H\(_b\), \(J_{5,6}\) 7.4 Hz, \(J_{5,6}\) 6.3 Hz, H-5\(_b\)), 5.18 (dq, 1H\(_a\), \(J_{5,6}\) 6.2 Hz, \(J_{5,4}\) 4.5 Hz, H-5\(_a\)), 4.64 (m, 2H\(_a\), 2 OCH\(_2\)Ph\(_a\) + OCH\(_2\)Ph\(_b\)), 4.58 (d, 1H\(_a\), \(J_{gem}\) 12.0 Hz, OCH\(_2\)Ph\(_a\)), 4.55 (d, 1H\(_b\), \(J_{gem}\) 12.0 Hz, OCH\(_2\)Ph\(_b\)), 4.41 (d, 1H\(_a\), \(J_{gem}\) 12.0 Hz, OCH\(_2\)Ph\(_b\)), 4.36 (dd, 1H\(_a\), \(J_{4,5}\) 5.8 Hz, \(J_{4,5}\) 4.5 Hz, H-4\(_a\)), 4.29 (dd, 1H\(_b\), \(J_{4,5}\) 7.4 Hz, \(J_{4,3}\) 5.0 Hz, H-4\(_b\)), 4.12 (m, 2H\(_a\), H-2\(_a\), H-3\(_a\)), 4.04 (bs, 1H\(_b\), H-2\(_b\)), 4.03 (dd, 1H\(_b\), \(J_{3,2}\) 5.0 Hz, \(J_{3,2}\) 1.3 Hz, H-3\(_b\)), 2.09 (s, 3H\(_a\), CH\(_2\)CO\(_a\)), 2.04 (s, 3H\(_b\), CH\(_2\)CO\(_b\)), 1.97 (s, 3H\(_a\), CH\(_2\)CO\(_a\)), 1.91 (s, 3H\(_b\), CH\(_2\)CO\(_b\)), 1.34 (d, 3H\(_a\), \(J_{6,5}\) 6.2 Hz, H-6\(_a\)), 1.30 (d, 3H\(_b\), \(J_{6,5}\) 6.2 Hz, H-6\(_b\)), \(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 170.1, 169.9 (2 CO\(_a\)), 170.1, 169.8 (2 CO\(_b\)), 137.6, 137.2 (2 C\(_{\text{quar-Bn}}\)_a), 137.4, 137.1 (2 C\(_{\text{quar-Bn}}\)_b), 128.5-127.5 (C-Ar), 100.2 (C-1\(_b\)), 94.4 (C-1\(_a\)), 84.5, 84.2, 80.5, 72.1, 72.0, 68.6 (C-2\(_b\), C-3\(_b\), C-4\(_b\), C-5\(_b\), 2 OCH\(_2\)Ph\(_b\)), 83.2, 80.0, 77.0, 73.3, 72.6, 69.0 (C-2\(_a\), C-3\(_a\), C-4\(_a\), C-5\(_a\), 2 OCH\(_2\)Ph\(_a\)), 21.2-21.1 (2 CH\(_3\)CO + 2 CH\(_3\)CO), 17.2 (C-6\(_b\)), 15.8 [M+Na]\(^{+}\). Anal Calcd for C\(_{26}\)H\(_{34}\)O\(_{10}\): C, 63.39; H, 6.46. Found: C, 63.11; H, 6.24.
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(C-6\textsubscript{a}). MALDI TOF-MS: calcd for C\textsubscript{24}H\textsubscript{28}O\textsubscript{7} (m/z), 428.18; found, 451.39 [M+Na\textsuperscript{+}]. Anal Calcd for C\textsubscript{24}H\textsubscript{28}O\textsubscript{7}: C, 67.28; H, 6.59. Found: C, 66.99; H, 6.38.

2,3,4-Tri-\textit{O}-acetyl-\textit{\alpha}-L-rhamnopyranosyl-(1→3)-1,1,4,5-tetra-\textit{O}-acetyl-2-\textit{O}-benzyl-D-quinovose Acetal (46): \([\alpha]_D = -15 \text{ (c = 0.1, CH}_2\text{Cl}_2\text{).}\) \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): \(\delta = 7.36\) (m, 5 H, Ar-H), 6.95 (d, \(J_{1,2} = 3.2\) Hz, 1 H, 1\textsubscript{\alpha}-H), 5.33 (s, \(J_{3,4} = 2.3\) Hz, 1 H, 4\textsubscript{\alpha}-H), 5.28 (dd, \(J_{3,4} = 10.0, J_{3,2} = 4.8\) Hz, 1 H, 3\textsubscript{\beta}-H), 5.19 (dd, \(J_{3,4} = 4.8, J_{2,1} = 3.2\) Hz, 1 H, 2\textsubscript{\beta}-H), 5.11 (q, \(J_{\beta,\alpha} = 6.3\) Hz, 1 H, 5\textsubscript{\alpha}-H), 5.06 (t, \(J_{4,5} = 10.0\) Hz, 1 H, 4\textsubscript{\beta}-H), 5.03 (br. s, 1 H, 1\textsubscript{\beta}-H), 4.75 (d, \(J_{\text{gem}} = 11.6\) Hz, 1 H, OCH\textsubscript{2}Ph), 4.70 (d, \(J_{\text{gem}} = 11.5\) Hz, 1 H, OCH\textsubscript{2}Ph), 4.06 (dd, \(J_{3,2} = 6.8, J_{3,4} = 2.3\) Hz, 1 H, 3\textsubscript{\alpha}-H), 4.00 (dq, \(J_{5,4} = 10.0, J_{5,6} = 6.4\) Hz, 1 H, 5\textsubscript{\beta}-H), 3.80 (dd, \(J_{2,3} = 6.8, J_{2,1} = 3.2\) Hz, 1 H, 2\textsubscript{\alpha}-H), 2.10, 2.07, 2.06, 2.05, 2.04, 2.03, 1.97 (7 s, 21 H, 7 CH\textsubscript{3}CO), 1.24 (d, \(J_{6,5} = 6.3\) Hz, 3 H, 6\textsubscript{\alpha}-H), 1.21 (d, \(J_{6,5} = 6.4\) Hz, 3 H, 6\textsubscript{\beta}-H) ppm. \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 100 MHz): \(\delta = 170.1, 170.0, 169.9, 169.7, 168.6, 168.5, 168.4\) (7 CO), 134.7 (C\textsubscript{\textalpha\textomega}-Bn), 128.4–127.9 (C-Ar), 100.8, 98.9 (C-1\textsubscript{\alpha}, C-1\textsubscript{\beta}), 87.7, 74.5, 74.1, 72.9, 70.9, 69.6, 69.0, 68.8, 67.5 (C-2\textsubscript{\alpha}, C-2\textsubscript{\beta}, C-3\textsubscript{\alpha}, C-3\textsubscript{\beta}, C-4\textsubscript{\alpha}, C-4\textsubscript{\beta}, C-5\textsubscript{\alpha}, C-5\textsubscript{\beta}, OCH\textsubscript{2}Ph), 21.1–20.7 (7 CH\textsubscript{3}CO), 17.3, 15.8 (C-6\textsubscript{\alpha}, C-6\textsubscript{\beta}) ppm. MS (MALDI-TOF): calcd. for C\textsubscript{33}H\textsubscript{44}O\textsubscript{17} [M\textsuperscript{+}] 712.29; found 734.61 [M + Na\textsuperscript{+}]. C\textsubscript{33}H\textsubscript{44}O\textsubscript{17} (712.69): calcd. C 55.61, H 6.22; found C 55.42, H 6.10.

Acetyl 2,3,4-Tri-\textit{O}-acetyl-\textit{\alpha}-L-rhamnopyranosyl-(1→3)-5-\textit{O}-acetyl-2-\textit{O}-benzyl-D-quinovofuranoside (47): \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) (\(\beta\)-anomer): \(\delta = 7.31\) (m, 5 H, Ar-H), 6.21 (s, 1 H, 1\textsubscript{\alpha}-H), 5.25–5.00 (m, 4 H, 2\textsubscript{\alpha}-H, 3\textsubscript{\beta}-H, 4\textsubscript{\beta}-H, 5\textsubscript{\alpha}-H), 4.76 (s, 1 H, 1\textsubscript{\beta}-H), 4.69 (d, \(J_{\text{gem}} = 11.7\) Hz, 1 H, OCH\textsubscript{2}Ph), 4.60 (d, \(J_{\text{gem}} = 11.7\) Hz, 1 H, OCH\textsubscript{2}Ph), 4.36 (dd, \(J_{4,5} = 7.2, J_{4,3} = 4.5\) Hz, 1 H, 4\textsubscript{\alpha}-H), 4.29 (d, \(J_{3,4} = 4.5\) Hz, 1 H, 3\textsubscript{\alpha}-H), 4.08 (s, 1
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H, 2\(_\alpha\)-H), 3.79 (dq, \(J_{\delta,\gamma} = 9.3, J_{\delta,\delta} = 6.3\) Hz, 1 H, 5\(_\beta\)-H), 2.15, 2.09, 2.06, 2.04, 1.98 (5 s, 15 H, 5 CH\(_2\)CO), 1.38 (d, \(J_{\delta,\delta} = 6.3\) Hz, 3 H, 6\(_\alpha\)-H), 1.24 (d, \(J_{\delta,\delta} = 6.3\) Hz, 3 H, 6\(_\beta\)-H) ppm.

\(^{13}\)C NMR (CDCl\(_3\), 75 MHz) (\(\beta\)-anomer): \(\delta = 170.1–169.6\) (5 CO), 136.8 (C\(_{\text{p-}}\)Bn), 128.5–127.7 (C-Ar), 99.2 (C-1\(_b\)), 95.8 (C-1\(_a\)), 84.2, 83.6, 76.9, 72.4, 70.6, 69.8, 68.9, 68.5, 67.5 (C-2\(_a\), C-2\(_b\), C-3\(_a\), C-3\(_b\), C-4\(_a\), C-4\(_b\), C-5\(_a\), C-5\(_b\), OCH\(_2\)Ph), 21.3–20.7 (5 CH\(_2\)CO), 17.6, 17.2 (C-6\(_a\), C-6\(_b\)) ppm. MS (MALDI-TOF): calcd. for C\(_{29}\)H\(_{38}\)O\(_{14}\) [M]\(^+\) 610.23; found 632.97 [M + Na]\(^+\).

Acetyl 2,3,4-Tri-\(\alpha\)-acetyl-\(\alpha\)-L-rhamnopyranosyl-(1→3)-2,4-di-\(\beta\)-benzyl-D-quinovopyranoside (48): \(^1\)H NMR (400 MHz, CDCl\(_3\)) (\(\alpha\)-anomer): \(\delta = 7.34–7.24\) (m, 10 H, Ar-H), 6.25 (d, \(J_{\gamma,\delta} = 3.6\) Hz, 1 H, 1\(_\gamma\)-H), 5.39–5.28 (m, 3 H, 1\(_b\)-H, 2\(_b\)-H, 3\(_b\)-H), 4.99 (t, \(J_{\gamma,\nu} = 9.9\) Hz, 1 H, 1\(_\alpha\)-H), 4.86 (d, \(J_{\gamma,\nu} = 11.1\) Hz, 1 H, OCH\(_2\)Ph), 4.72 (d, \(J_{\gamma,\nu} = 11.1\) Hz, 1 H, OCH\(_2\)Ph), 4.63 (d, \(J_{\gamma,\nu} = 11.1\) Hz, 1 H, OCH\(_2\)Ph), 4.50 (d, \(J_{\gamma,\nu} = 11.1\) Hz, 1 H, OCH\(_2\)Ph), 4.10 (dq, \(J_{\gamma,\mu} = 9.6\) Hz, 1 H, 3\(_\alpha\)-H), 4.05 (dq, \(J_{\gamma,\nu} = 9.9, J_{\gamma,\delta} = 6.3\) Hz, 1 H, 3\(_\beta\)-H), 3.66 (dd, \(J_{\gamma,\nu} = 6.3\) Hz, 1 H, 5\(_\beta\)-H), 3.91 (dq, \(J_{\gamma,\mu} = 9.6, J_{\gamma,\delta} = 6.3\) Hz, 1 H, 5\(_\alpha\)-H), 2.13, 2.07, 2.01, 1.94 (4 s, 12 H, 4 CH\(_3\)CO), 1.32 (d, \(J_{\delta,\mu} = 6.3\) Hz, 3 H, 6\(_\gamma\)-H), 0.91 (d, \(J_{\delta,\delta} = 6.3\) Hz, 3 H, 6\(_\beta\)-H) ppm.

\(^{13}\)C NMR (CDCl\(_3\), 75 MHz) (\(\alpha\)-anomer): \(\delta = 170.0, 169.8, 169.7, 169.4\) (4 CO), 137.6, 136.9 (2 C\(_{\text{p-}}\)Bn), 128.9–127.3 (C-Ar), 97.6 (C-1\(_b\)), 89.0 (C-1\(_a\)), 81.9, 79.8, 75.4, 75.0, 72.8, 71.0, 69.6, 69.5, 69.2, 66.4 (C-2\(_a\), C-2\(_b\), C-3\(_a\), C-3\(_b\), C-4\(_a\), C-4\(_b\), C-5\(_a\), C-5\(_b\), 2 OCH\(_2\)Ph), 21.0–20.8 (4 CH\(_3\)CO), 18.0, 17.1 (C-6\(_a\), C-6\(_b\)) ppm. MS (MALDI-TOF): calcd. for C\(_{34}\)H\(_{42}\)O\(_{13}\) [M]\(^+\) 658.26; found 681.15 [M + Na]\(^+\). C\(_{34}\)H\(_{42}\)O\(_{13}\) (658.69): calcd. C 62.00, H 6.43; found C 62.20, H 6.56.
Chapter 3: Synthesis of biologically relevant peptidoglycan fragments

3.1 The prokaryotic cell-wall

The cell wall is the layer, usually fairly rigid, that lies just outside the plasma membrane. It is one of the most important part of a prokaryotic cell for several reasons. Most bacteria have strong walls that give them shape and protect them from the osmotic lysis and its components can contribute to the pathogenicity of the bacteria. Moreover, the wall can protect the cell from toxic substances and is the site of action of several antibodies.

Bacteria can be divided into two major groups based on their response to the Gram-

![Fig. 3.1: Gram-Positive And Gram-Negative Cell Walls.](image) The Gram-positive envelope is from *Bacillus licheniformis* (left), and the Gram-negative micrograph is of *Aquaspirillum serpens* (right).

stain procedure: Gram-positive and Gram-negative bacteria. The advent of the transmission electron spectroscopy made clear the structural differences between these two types of bacteria. The Gram-positive bacterial cell wall consists of a single 20 to 80 nm thick homogeneous peptidoglycan or murein layer lying outside the plasma membrane. In contrast, Gram-negative cell wall is quite complex: it has in fact a 2 to 7 nm
peptidoglycan layer surrounded by a 7 to 8 nm outer membrane.

3.1.1 **Gram-positive Cell Walls**

The thick homogeneous cell wall of Gram-positive bacteria is composed primarily of peptidoglycan. Usually Gram-positive cell walls contain large amounts of teichoic acids, polymers of glycerol or ribitol joined by phosphate groups. Amino acids such as D-alanine or sugars like glucose are attached to the glycitol or ribitol groups (FIG. 3.2). The teichoic acids are connected to either the peptidoglycan itself by a covalent bond with hydroxyl of N-acetylmuramic acid or to plasma membrane lipids: in the latter case they are called lipoteichoic acids. Teichoic acids appear to extend to the surface of peptidoglycan, and, because they are negatively charged, give the Gram-positive cell wall its negative charge. The functions of these molecules are still unclear, but they may be important in maintaining the structure of the wall. Teichoic acids are not present in Gram-negative bacteria.

FIG. 3.2: The Gram-positive envelope.

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3.1.2 **Gram-negative Cell Walls**

The Gram-negative cell wall (Fig. 3.3) is much more complex than Gram-positive cell wall. The thin peptidoglycan layer next to the plasma membrane may constitute not more than 5 to 10% of the cell wall. The outer membrane lies outside the thin peptidoglycan layer. The most abundant protein present in Gram-negative cell wall is the Brown’s protein, a small lipoprotein covalently joined to the underlying peptidoglycan and embedded in the outer membrane by its hydrophobic end. The outer membrane and peptidoglycan are so firmly linked by this lipoprotein that they can be isolated as one unit. The most particular component of the Gram-negative cell wall are the lipopolysaccharides (LPS). These large macromolecules contain both lipid and carbohydrate moieties and consist of three parts: (1) lipid A; (2) the core oligosaccharide and (3) the O-side chain. The **lipid A** is formed by two units of glucosamine linked together by a $\beta$-(1→6) glycosidic bond. Each glucosamine residue has three fatty acids and phosphate or pyrophosphate groups attached. It is bound to the outer membrane and the remainder of LPS molecule projects from the surface. The **core**
oligosaccharide is joined both to lipid A and to the O-antigen. It is constructed of a limited number of sugars some of which are peculiar for this region such as KDO (3-deoxy-D-manno-2-octululonic acid). The core oligosaccharides possess a more conservative structure when compared with those of the O-specific polysaccharides. The O-side chain or O-antigen is a polysaccharide chain extending outward from the core. It is constituted of several sugars and varies even in the same bacterial strain. Although O-side chains are readily recognized by host antibodies, Gram-negative bacteria may thwart host defenses by changing the nature of their O-side chains to avoid detection. LPS is important for several reasons other than the avoidance of host defenses. Since the core polysaccharides contain charged sugars and phosphates, LPSs help stabilize membrane structure. Furthermore, lipid A is often toxic; as a result LPS can act as endotoxin and causes some of the symptoms that arise in Gram-negative bacterial infections.

3.1.3 Peptidoglycan: a closer look

As anticipated before peptidoglycan (PGN) is one of the most important component of the bacterial cell-wall. Its main function is to preserve cell integrity by withstanding the turgor; it also contributes to the maintenance of a well defined shape and serves as a scaffold for anchoring other cell wall components such as proteins and teichoic acids and is involved in the division processes.

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Chapter 3: Synthesis of biologically relevant peptidoglycan fragments

The main structural features of peptidoglycan are linear glycan strands cross-linked by short peptides. The glycan strands are made up of alternating $\beta$-1→4 linked N-acetyl-D-glucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) residues cross-linked by peptide chains. Four to five amino acids are linked to the lactyl group of each MurNAc residue. The amino acids occur in alternating L. and D. isomers (whose presence protects against the attack of most peptidase) and include $\gamma$-bonded-D-glutamic acid, non-protein amino acids like diaminopimelic acid (DAP), ornithine or lanthionine, in combinations typical for the bacterial species. These amino acids cross-link the glycan backbones, usually via the free amino group of a basic amino acid, such as L-lysine (Lys) or meso-DAP, for Lys-type or DAP-type PGN, and a terminal free carboxyl group, frequently D-alanine.\textsuperscript{178} The peptide structure of a Gram-negative bacterium is commonly L-Ala-$\gamma$-D-Glu-meso-DAP-D-Ala-D-Ala, where the dibasic meso-

DAP is the cross-linking peptide. The typical structure of PGN of Gram-positive bacteria is L-Ala-γ-D-Glu-L-Lys-D-Ala-D-Ala, with an interpeptide bridge often made up of a chain of five Gly between the Lys side chain and the Ala of neighboring chain (Fig. 3.6).  

Peptidoglycan is not only an essential structural component of the bacterial cell wall, but is also involved in diverse biological processes, such as microbial pathogenesis, symbiotic associations and a range of interactions between bacteria and other organisms. In particular the importance of peptidoglycan detection in the host immune response has acquired great relevance nowadays. It has been demonstrated in fact that peptidoglycan fragments generated during bacterial division and growth can be detected in mammals, insects and plants, by PGRP proteins (“**PG Recognition Protein**”) and can stimulate innate immunity that is the first line of defense against infections by microbes: PGN fragments are in fact considered PAMPs (“**Pathogen Associated Molecular Patterns**”) and are therefore recognized by PRRs (“**Pattern Recognition Receptors**”). These recognition process lead, through activation of transcription factors such as NF-κB and IRF3, to the production of pro- and anti-inflammatory cytokines, chemokines.

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and to the expression of co-stimulatory molecules on antigen presenting cells. The PRRs involved in the recognition mechanism are different for different species. In FIG. 3.7, it is schematically reported the way of interaction of PGN fragments in flies (such as Drosophila melanogaster) and in mammals and it is clearly showed that the way of interaction and the receptors involved are completely different. In the case of plants it has been also demonstrated that peptidoglycan can be recognized but the mechanism of interaction and the species involved are still unclear. In order to impair the detection by host innate immune receptors and to enhance the resistance to antibiotics and host degradative enzymes that target the bacterial cell wall, PGN structures can be modified in both the peptide stem (FIG. 3.8) and bridge regions and in the glycan strands.

**FIG. 3.8: Most common variation in peptide stem (a) and in the cross-linking (b and c).** Amino acids with a star (*) are formed by a secondary modification during peptidoglycan biosynthesis.

The most common modifications of the glycan strands are reported in FIG. 3.9: these include N-deacetylation (at GlcN, MurN or both), N-glycolylation (at MurN), O-acetylation (at MurNAc), the formation of a muramic-δ-lactam (in all Gram-negative

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Chapter 3: Synthesis of biologically relevant peptidoglycan fragments

**Figure 3.9:** Modification in the glycan strands. The name of some species containing the modifications in their peptidoglycan are indicated.
bacteria), the attachment of surface polymers (e.g. teichoic acids or capsular polysaccharides via phosphodiester bonds to their GlcNAc or MurNAc residues). Among these modifications N-deacetylation has acquired great relevance because of its influence on recognition processes. The presence of deacetylated sugars in peptidoglycan strands strongly reduces the activity of the muramidase lysozyme, the enzyme that is involved in the hydrolysis of the glycan strands. The absence of acetyl groups indeed destabilizes the interaction between a hexasaccharide glycan strand and amino acids in the long groove of the lysozyme reducing its activity. It is worth noting that the activity of the lysozyme can be restored by chemical acetylation of the substrate. Moreover, it is likely that the effect of peptidoglycan deacetylation on the interaction with host might be multifactorial and not restricted to the effect on lysozyme activity. Deacetylation introduces additional positive charges into the cell wall, potentially affecting the binding of specific cell-wall proteins and other compounds such as capsular polysaccharides. In addition, increasing the positive charge of the cell wall is likely to increase the resistance of the bacterium to cationic antimicrobial peptides, which are important elements of the innate immune system. The role of peptidoglycan in virulence has been largely investigated for the human-pathogen Gram-positive Lysteria monocytogenes where it has been demonstrated that the N-deacetylation enhances the resistance of the bacterium to the lysozyme. Recently, partially N-deacetylated PGN fragments have been also isolated from the phytopathogenic Gram-

negative bacterium *Xanthomonas campestris* pv *campestris* but in this case it seems that the N-deacetylation at GlcN site seems to enhance the immune response of the plant model *Arabidopsis thaliana*.\(^{190}\) A fully comprehension of the influence of N-deacetylation is therefore not well defined and further studies are going on to clarify exactly how this modification affects the response of the immune system.

### 3.1.3.1 Peptidoglycan hydrolysis and bacterial resuscitation

The interest toward peptidoglycan structures has increased recently because it has been found that peptidoglycan can exert a controlling influence on non-growth states and is involved in reactivation process of several bacteria from dormancy.\(^{191}\) In particular several human pathogen bacteria such as *Escherichia coli*, *Helicobacter pylori*, *Legionella pneumophila*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Vibrio cholera* and the most important medically *Mycobacterium tuberculosis*\(^{192}\) can enter in a dormant state in which, crucially, they are insensitive to antibiotics.\(^{193}\) Peptidoglycan seems to be involved in such processes because in the dormant states cells have an altered peptidoglycan structure (similar modifications are found also in bacterial spores).\(^{194}\) For example, *Escherichia coli* in the dormant state has a substantial increase in the degree of peptidoglycan cross-linking, an increase in lipoprotein-bearing muropeptides and a


\(^{192}\) Oliver, J.D. *J. Microbiol.* 2005, 43, 93.


\(^{194}\) Atrih, A.; Foster, S.J. *Antonie Van Leeuwenhoek* 1999, 75, 299.
shortening of the chain length of glycan strands. Also *Enterococcus faecalis* exhibits qualitatively similar modifications in peptidoglycan and, recently, it has been shown that also O-acetylation is increased.

The most important discovery in the analysis of the resuscitation process has been the understanding of non-growth states for the high G+C Gram-positive *Actinobacter Micrococcus luteus (lysokeiktoikus)*, whose cells could be resuscitated by exposure to non-dormant *Micrococcus luteus*, this suggesting that cell-cell interaction are required to exit from dormancy. It was established that resuscitation was caused by a protein which was therefore called Rpf (resuscitation promoting factor). The Rpf protein was found to be extremely potent and exhibited activity at low picomolar concentrations. Inactivation of the *Micrococcus luteus rpf* gene was not possible, which indicated the essential nature of Rpf for cell survival. In the case of *Mycobacterium tuberculosis*, five resuscitation promoting factors have been found (RpfA-E), which are responsible for the revival of the bacterium from dormancy. A key enzyme for *M. tuberculosis* cell division is resuscitation promoting factor interacting protein (RipA), which is believed to cleave peptidoglycan cross-links but its mechanism of action is still unknown and

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198 The *H+G bacteria* are a family of bacteria that are phylogenetically related for a high content of guanine and cytosine in the sequence of 16S rRNAs isolated from 30S subunits of prokaryotic ribosomes. The advantages of this phylogenetic classification are due to the low variations of their structure with time, presumably because of their constant and critical role: rRNAs are almost ideal for studies of microbial evolution and relatedness since they are essential to a critical organelle found in all microorganisms.
is object of several studies. It is in fact still unclear how peptidoglycan hydrolysis is linked to the reactivation growth. One of the most accredited possibility is that cleavage of the cell walls of the dormant bacteria is required, perhaps in a specific region such as the septum, and that the resuscitation factor overcomes a physical requirement for cell expansion or division. This activity could be provided by Rpf during normal growth but these enzymes are downregulated during stationary phase and become limiting for further growth. In this case, cell-cell signaling is carried out by diffusion of RpfS themselves. In confirmation of this, two of the five M. tuberculosis RPFs (RpfC and RpfB) are secreted to the medium, whereas the others are likely to be attached to the peptidoglycan of the producing cell. Moreover, it seems in some cases that the exit from dormancy can be favored also by a change of the external conditions or simply by the presence of muropeptides released by other growing microorganism.

**Fig. 3.10: Exit from dormancy triggered by growing cells.** a) Dormant cells (beige) may sense some aspect of the environment before exiting dormancy and initiating growth (green cells). b) The presence of secreted signaling molecules such as cell wall muropeptides from growing cells (pale green) could serve as an indication that growth-permissive conditions are present and could thereby stimulate exit from dormancy.

3.2 Results and Discussion

In order to investigate the function of N-deacetylation in peptidoglycans and to obtain suitably substrates for the analysis of Rpf action in the resuscitation process, it has been developed a new strategy for the synthesis of a disaccharide moiety that could lead to the obtainment of both partially N-deacetylated peptidoglycan fragments at GlcN site and of a muramyl fragment in which two disaccharide units were linked by a peptide chain repeating the peptide bridge of the Gram-positive bacterium *Micrococcus lysodeikticus*. Although in literature are present several chemical syntheses of peptidoglycan fragments, there was no report on the synthesis of N-dacetylated-GlcN-containing structures. The known syntheses of peptidoglycan fragments could not be applied to the synthesis of peptidoglycan fragments with a N-deacetylated GlcN unit because all necessitated to cleave the GlcN nitrogen atom protecting group and to acetylate it before coupling with the peptide chain. Therefore, GlcN and MurN nitrogen atoms had to be protected with orthogonal protecting groups, the GlcN one being able to liberate the amine at the final stage of the synthesis. Moreover, in order to open an access not only to peptidodisaccharide fragments but also to higher oligomers, the anomeric position of MurNAc unit and the position 4 of GlcN had to be protected with orthogonal protecting groups too. Finally, the eventuality of side-reactions involving the

(R)-lactyl moiety during the manipulation of MurNAc building blocks (racemization, intramolecular lactonization at 4-hydroxy position), \(^{207}\) suggested to introduce the lactyl ether at a late stage in the synthesis. All these constrains designed A as a proper disaccharide building block (FIG. 3.11). It could be obtained by a stereoselective coupling between suitably protected GlcN donor B and acceptor C.

The glycosylation of a 4-hydroxy group in glucosamine acceptors presents some well-known difficulties related to its low nucleophilicity. \(^{208}\) Some methods were reported to address this problem; \(^{209}\) among these protocols, we firstly focused our attention on the use of a N-acetyl-2,3-oxazolidinone protection \(^{210}\) on the glycosyl acceptor. Several building blocks were developed as glycosyl donors for the stereoselective synthesis of \(\beta\)-2-amino-2-deoxyglucosides. \(^{211}\) They generally present a N-2 protecting group capable of efficient participation via acyloxonium ion that guarantees 1,2-\(\text{trans}\) stereoselectivity. These are very usually amido-, carbamato- or imido protecting groups. Unfortunately they could present some problems here in surviving as stable protecting groups — that is, during the cleavage of oxazolidinone — and/or in their final deprotection to free amine in the


Fig. 3.12: Reagents and conditions: a) ref. 13; b) triphosgene, 2:1 v/v CH₂CN/sat. aq. NaHCO₃, rt, 60%; c) Ac₂O, DIPEA, CH₂Cl₂, rt, 92%; d) Et₃SiH, TFA, AW-300 4Å MS, CH₂Cl₂, 5°C, 57% for 4, 74% for 8, 77% for 13; e) i. TfN₃, CuSO₄, Et₃N, py, 0°C; ii. BnBr, NaH, DMF, rt, 71% over two steps; f) PdCl₂, 1:1 v/v CH₂Cl₂/MeOH, rt, 58% for 6, 80% for 10; g) Cl₃CCN, DBU, CH₂Cl₂, rt, 78% for 7, 70% for 11; h) PMBCl, NaH, DMF, 0°C, 92%; i) i. TrocCl, NaHCO₃, 2:1 H₂O/CH₂CN, rt; ii. CbzCl, DMAP, CH₂Cl₂, rt, 70% over two steps.
presence of the peptide chain. To avoid any protecting group interconversion at a
disaccharide level, an azide was selected as amino-masking group. Even if azide is
incapable of neighboring group participation, it can be easily transformed into a free
amine during final hydrogenolysis deprotection step. The known compound 1 \(^{212}\) was
prepared in three steps from N-acetyl-glucosamine and served as key building block for
the synthesis of both acceptor 4 and donor 7 (FIG. 3.12). An aliquot of 1 was treated
with triphosgene \(^{213}\) to give oxazolidinone 2 in 60% yield. Subsequent N-acetylation
(92%) and regioselective benzylidene ring opening under reductive conditions
(Et\(_3\)SiH/TFA in CH\(_2\)Cl\(_2\); 57%) \(^{214}\) afforded acceptor 4. Conversely, the treatment of 1
with triflyl azide in pyridine \(^{215}\) and subsequent benzyla of the 3-hydroxy group gave
5 (71% over two steps), which was de-O-allylated with PdCl\(_2\) (58%) and then converted
into the \(\alpha\)-trichloroacetimidate donor 7. Unfortunately the coupling between 4 and 7 in
a \(\beta\)-directing nitrile solvent gave no disaccharide (Table 3.1, entries 1 and 2). Given the
torsional and electronic disarming effect of a 4,6-benzylidene protection on glycosyl
donors, \(^{216}\) a more reactive 2-azido-2-deoxyglucosyl donor was synthesized, having a
benzyl at position 6 and a selectively cleavable \(p\)-methoxybenzyl group at position 4. It
was obtained in four steps from 5 (FIG. 3.12). Regioselective reductive opening of the
benzylidene ring gave 8 (74%), that was then treated with PMBCl and NaH to afford 9
in 92% yield. De-O-allylation (80%) and subsequent treatment with Cl\(_3\)CCN and DBU
afforded the desired \(\alpha\)-trichloroacetimidate 11 in 70% yield.

2003, 3603.
Again, the reaction of 4 and 11 in nitrile solvent gave no coupling (entry 3). Some scattered examples in the literature report the β-glycosylation of 2-azido-2-

![Chemical structures]

Table 3.1: Glycosylation Reactions (Donor/acceptor molar ratio = 1.6 unless otherwise stated; Promoter equivalents calculated with respect to the donor; Isolated yield unless otherwise stated; detected by TLC and MALDI analysis; Anomeric ratio measured by the isolation of two anomers; Donor/acceptor molar ratio = 2.3).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acceptor</th>
<th>Donor</th>
<th>Promoter</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Disaccharide product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>7</td>
<td>TMSOTf</td>
<td>CH₃CN</td>
<td>20°C</td>
<td>No reaction</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>7</td>
<td>TMSOTf</td>
<td>CH₃CN</td>
<td>rt</td>
<td>14 (traces)</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>11</td>
<td>TMSOTf</td>
<td>Pivalonitrile</td>
<td>rt</td>
<td>15 (traces)</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>11</td>
<td>BF₃·OEt₂</td>
<td>3:2 v/v CH₂Cl₂/hexane</td>
<td>-30°C</td>
<td>15 (68%; β/α 1:1)</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
<td>11</td>
<td>BF₃·OEt₂</td>
<td>3:2 v/v CH₂Cl₂/hexane</td>
<td>-60°C</td>
<td>15 (68%; β/α 1.3:1)</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>7</td>
<td>BF₃·OEt₂</td>
<td>3:2 v/v CH₂Cl₂/hexane</td>
<td>-80°C</td>
<td>16 (traces)</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>11</td>
<td>BF₃·OEt₂</td>
<td>3:2 v/v CH₂Cl₂/hexane</td>
<td>-80°C</td>
<td>17 (81% only β)</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>11</td>
<td>BF₃·OEt₂</td>
<td>3:2 v/v CH₂Cl₂/hexane</td>
<td>-30°C</td>
<td>17 (40% only β)</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>11</td>
<td>BF₃·OEt₂</td>
<td>3:2 v/v CH₂Cl₂/CH₃CN</td>
<td>-30°C</td>
<td>17 (43% only β)</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>11</td>
<td>TMSOTf</td>
<td>3:2 v/v CH₂Cl₂/hexane</td>
<td>-80°C</td>
<td>17 (71% β/α 1:4)</td>
</tr>
</tbody>
</table>
deoxyglycosyl-\(\alpha\)-trichloroacetimidates under S\(_\text{N}\)2 conditions. \(^{217}\) Therefore, the coupling between 4 and 11 was attempted at low temperature in a CH\(_2\)Cl\(_2\)-hexane solvent mixture with BF\(_3\)OEt\(_2\) as catalyst (entry 4). Disaccharide 15 was obtained in 68% yield, but without any stereoselectivity. Higher donor/acceptor molar ratio and even milder activation conditions gave the same yield with an only slight excess of \(\beta\)-anomer (entry 5). The low \(\beta/\alpha\) stereoselectivity is not really surprising for glycosylations involving 2,3-oxazolidinone GlcN acceptors. \(^{37}\) Therefore, a new glycosyl acceptor (13) was designed, with the amino group protected as a trichloroethoxycarbamate (Troc), which affords greater GlcN-4-hydroxy reactivity than other carbamato- or imidooxycarbonyl protecting groups. \(^{218}\) Compound 13 was synthesized from key building block 1 by N-trichloroethoxycarbonylation and protection of the 3-hydroxy as benzoxycarbonyl group (12, 70% over two steps), which were then subjected to Et\(_3\)SiH/TFA benzylidene ring opening (77% for 13) (Fig. 3.12). The coupling between 13 and 7 was unsatisfying (entry 6), whereas glycosylation between 13 and 11 under BF\(_3\)OEt\(_2\) catalysis in a CH\(_2\)Cl\(_2\)-hexane solvent system at -78°C (typical S\(_\text{N}\)2 reaction conditions) afforded disaccharide product 17 with a good yield and (entry 7). A higher temperature, a more polar solvent system or a stronger activator considerably reduced the yield of 17 (entries 8–10). Disaccharide 17 is a highly versatile building block. Exploiting the orthogonality of the allyl and PMB protecting group, it has been possible to obtain from 17 the disaccharide glycosyl acceptor 18 and the hemiacetals 19 from which a disaccharide glycosyl donor can be easily obtained installing a leaving group on the hemiacetal anomeric oxygen. These two molecules will be used for the synthesis of tetra- and octasaccharide muramyl fragments (Fig. 3.13).

Disaccharide 17 was readily transformed also into alcohol 20 by cleavage of Troc- and Cbz-protection groups with 2 M KOH and subsequent N-acetylation (67% over two steps) (Fig. 3.13). For the insertion of the lactoyl moiety several conditions were tested using both a (S)-2-chloropropionic acid (entries 1 and 2, Table 3.2) and the triflate of ethyl (S)-lactate (entries 3 and 4, Table 3.2). As it is possible to see from Table 3.2, the best results were obtained using 3 equivalents of NaH and only 2 equivalents of ethyl (S)-lactate. Using the conditions reported in entry 3, it was obtained instead the unexpected product 22, whose mechanism of formation is still unclear and needs further investigations.

Table 3.2: Screening of the conditions for the lactoyl moiety insertion.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reaction conditions</th>
<th>Products (isolated yield %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(S)-2-chloropropionic acid (5eq.), NaH (11.2 eq), THF, 40°C, on.</td>
<td>No product</td>
</tr>
<tr>
<td>2</td>
<td>(S)-2-chloropropionic acid (5eq.), NaH (15.6 eq), 1:1 v/v DMF/dioxane, 95°C, on.</td>
<td>21 R=OH, R'=Ac (14%)</td>
</tr>
<tr>
<td>3</td>
<td>Triflyl-(S)-2-propionic acid ethyl ester (3 eq.), NaH (9 eq), CH₂Cl₂, rt, on.</td>
<td>22 R=Et, R'=H (50%)</td>
</tr>
<tr>
<td>4</td>
<td>Triflyl-(S)-2-propionic acid ethyl ester (2 eq.), NaH (3 eq), CH₂Cl₂, rt, 90 min.</td>
<td>23 R=Et, R'=H (63%)</td>
</tr>
</tbody>
</table>
GlcN–MurNAc deprotected disaccharide was then obtained as ethyl ester by hydrogenolysis of 23 (65%). It is worth noting that this deprotected disaccharide unit 24 and its N-diacetylated derivative 25 seem to be recognized from the innate immune system of the plant model Arabidopsis thaliana by preliminary phytopathological tests conducted in the laboratory of prof. M.A. Newmann of the Department of Plant Biology & Biotechnology of the University of Copenhagen. Further studies are in course to analyze which mechanisms are involved in these recognition processes.

The disaccharide 23 has also been used for the synthesis of a muramyl fragments from the bacterium Micrococcus luteus (lysodeiktikus). The ester group has been hydrolyzed to give the free carboxylic acid 21 and then coupled with a pentadecapetide which represents the peptide stem of Micrococcus luteus (lysodeiktikus). The synthesis of the pentadecapetide unit 26 and the coupling reaction have been performed in the laboratory of the Prof. R. Berisio of the Institute of Biostructures and Bioimaging of the CNR of Naples. The unoptimized yield (≈20%) for the coupling reaction was detected using HPLC. The formation of the muramyl fragment (27) has been detected using MALDI and further experiments are now in progress in order to have the complete spectroscopic characterization. Because the muramyl fragments of the bacterium Micrococcus luteus (lysodeiktikus) are good substrate also for RipA, the Rpf involved in
the resuscitation of *Mycobacterium tuberculosis* from dormancy, glycopeptide 27 will be completely deprotected and its interaction with RipA will be evaluated in the laboratory of Dr. Daniela Marasco of Institute of Biostructures and Bioimaging of the CNR of Naples.

**Fig. 3.15**: Reagents and conditions: (a) LiOH, 2:1:0.5 v/v/v THF/dioxane/H2O, rt, 82%; (b) PyBOP, DIPEA, CH2Cl2, rt, on.
3.3 Conclusions
The synthesis of a highly versatile GlcN-MurNAc building block was reported. Since glycosylation presented several difficulties and constrains (low reactivity at position 4 of GlcN acceptors; necessity of gain 1,2-trans stereoselectivity without the use of amido-, imido- or carboxamido neighboring protecting group; a protecting group pattern suitable for disaccharide oligomerization) a careful study of optimal glycosyl donor and acceptor and coupling conditions was carried out. The disaccharide was finally obtained in high yield and complete β-stereoselectivity. It is suitable for further manipulations towards the first synthesis of N-deacetylated-GlcN–containing peptidoglycan fragments, that are interesting molecules for the study of host innate immune system evasion mechanism in bacteria. From the fully protected disaccharide moiety a muramyl fragment from the bacterium *Micrococcus luteus* (*lysodeikticus*) has been also synthesized for the investigation of the mechanisms involved in the resuscitation of bacteria from dotmancy.

3.4 Experimental Section

**General methods:** $^1$H and $^{13}$C NMR spectra were recorded with Varian XL-200 ($^1$H: 200 MHz; $^{12}$C: 50 MHz), Varian Gemini-300 ($^1$H: 300 MHz; $^{13}$C:75 MHz), Bruker DRX-400 ($^1$H: 400 MHz; $^{13}$C:100 MHz), Varian INOVA 500 ($^1$H: 500 MHz; $^{13}$C:125 MHz), CryoProbe Bruker 600 ($^1$H: 600 MHz; $^{13}$C:150 MHz) instruments in CDCl$_3$ (CHCl$_3$ as internal standard: $^1$H: CHCl$_3$ at $\delta=7.26$ ppm; $^{13}$C: CDCl$_3$ at $\delta=77.0$ ppm). Assignment of the proton chemical shifts was based on 1D HOHAHA experiments. Positive MALDI-TOF MS spectra were recorded with an Applied Biosystem Voyager DE-PRO MALDI-TOF mass spectrometer in the positive mode: compounds were dissolved in CH$_3$CN at
concentration of 1 mg/mL and one microliter of these solutions were mixed with one microliter of a 20 mg/mL solution of 2,5-dihydroxybenzoic acid in 7:3 CH$_2$CN/water. Optical rotations were measured with a JASCO P-1010 polarimeter. Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with Merck Silica Gel 60 F$_{254}$ as the adsorbent. The plates were developed with a 10% H$_2$SO$_4$ ethanolic solution and by heating to 130°C. Column chromatography was performed on Merck Kiesegel 60 (63-200 mesh).

**Allyl 4,6-O-benzylidene-2,3-N$_3$O-carbonyl-2-deoxy-α-D-glucopyranoside (2):**
Amino alcohol 1 (325.2 mg, 1.05 mmol) was dissolved in 2:1 v/v CH$_2$CN/sat. NaHCO$_3$ (14.6 mL) and treated at T=0°C with triphosgene (430.5 mg, 1.45 mmol). The solution was stirred at room temperature for 30 min and then a solution of ethylenediamine in ethyl acetate (195 μL of ethylenediamine in 15 mL of ethyl acetate) was added. The mixture was then diluted with AcOEt (15 mL) and washed with 1 M NaHCO$_3$. The organic layer was collected, dried with Na$_2$SO$_4$, filtered and purified by chromatography (1% methanol in dichloromethane) to give 2 as a white powder (209.6 mg, 60%). $[\alpha]_D$ +33 (c 0.3; CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$): δ 7.51-7.36 (m, 5H, H-Ar), 5.91 (m, 1H, OCH$_2$CH=CH$_2$), 5.62 (s, 1H, CHPh), 5.35 (ddd, 1H, $J_{\text{vic}}$ 17.2 Hz, $J_{\text{H,H}}$ 3.0 Hz, $J_{\text{gem}}$ 1.5 Hz, trans OCH$_2$CH=CHH), 5.28 (dd, 1H, $J_{\text{vic}}$ 10.4 Hz, $J_{\text{gem}}$ 1.5 Hz, cis OCH$_2$CH=CHH), 5.15 (d, 1H, $J_{\text{H,2}}$ 2.9 Hz, H-1), 5.07 (bs, 1H, NH), 4.84 (dd, 1H, $J_{\text{J,2}}$=11.3 Hz, $J_{\text{H,2}}$=10.2 Hz, H-3), 4.29 (m, 2H, H-6a, OCHHCH=CH$_2$), 4.09 (m, 2H, H-4, OCHHCH=CH$_2$), 3.90 (m, 2H, H-5, H-6b), 3.74 (dd, 1H, $J_{\text{H,2}}$ 11.3 Hz, $J_{\text{H,2}}$ 2.9 Hz, H-2); $^{13}$C NMR (125 MHz, CDCl$_3$): δ 159.1 (C=O), 136.5 (C=O), 132.8 (OCH$_2$CH=CH$_2$), 129.2, 128.3, 126.2 (C-Ar), 118.5 (OCH$_2$CH=CH$_2$), 101.4 (CHPh),
Chapter 3: Synthesis of biologically relevant peptidoglycan fragments

95.5 (C-1), 80.1, 75.6, 69.1, 68.5, 65.5, 59.5 (C-2, C-3, C-4, C-5, C-6, OCH₂CH=CH₂).

MALDI TOF-MS: calcd for C₁₇H₁₉NO₆ (m/z), 333.12; found, 334.16 [M+H]^+.

Anal Calcd for C₁₇H₁₉NO₆: C, 61.26; H, 5.75; N, 4.20. Found: C, 61.06; H, 5.58; N 4.11.

Allyl 2-acetamido-4,6-O-benzylidene-2-N,3-O-carbonyl-2-deoxy-α-D-glucopyranoside (3): Compound 2 (207.4 mg, 0.623 mmol) was dissolved in CH₂Cl₂ (5.1 mL) and treated with DIPEA (542 μL, 3.11 mmol) and then with AcCl (221 μL, 3.11 mmol). The solution was stirred for 60 min at room temperature and then diluted with CH₂Cl₂ and washed with 1 M NaHCO₃. The organic layer was collected, dried with Na₂SO₄, filtered and purified by chromatography (16% ethyl acetate in petroleum ether) to give 3 as a amorphous solid (206.5 mg, 83.1%).

¹H NMR (600 MHz, CDCl₃): δ 7.50-7.34 (m, 5H, H-Ar), 5.85 (m, 1H, OCH₂CH=CH₂), 5.80 (d, 1H, Jₓ₋ₓ=2.7 Hz, H-1), 5.58 (s, 1H, CHΦ), 5.31 (d, 1H, Jₓ₋ₓ=17.6 Hz, trans OCH₂CH=CHH), 5.27 (d, 1H, Jₓ₋ₓ 10.3 Hz, cis OCH₂CH=CHH), 4.75 (t, 1H, J₃₋₄= 11.7 Hz, H-3), 4.32 (dd, 1H, Jₓ₋ₓ=16.2 Hz, J₆₋₆=10.2 Hz, H-6a), 4.27 (dd, 1H, Jₓ₋ₓ= 12.6 Hz, Jₓ₋ₓ=5.2 Hz, OCH/HCH=CH₂), 4.23 (dd, 1H, Jₓ₋ₓ= 12.6 Hz, Jₓ₋ₓ=6.24 Hz, OCH/HCH=CH₂), 4.7 (t, 1H, J₄₋₅ = J₃₋₄ = 11.7 Hz, H-4); 3.95-3.91 (m, 2H, H-5, H6b), 3.88 (dd, 1H, Jₓ₋ₓ=2.7 Hz, Jₓ₋ₓ=11.7 Hz, H-2).

¹³C NMR (50 MHz, CDCl₃): δ 171.11 (C OCH₃), 152.8 (NCOO), 136.3 (Cᵧᵧᵧ), 132.9 (OCH₂CH=CH₂), 129.1, 128.1, 125.2 (C-Ar), 118.2 (OCH₂CH=CH₂), 101.0 (CHPh), 95.8 (C-1), 79.4, 73.4, 69.4, 68.3, 65.0, 60.6 (C-2, C-3, C-4, C-5, C-6, OCH₂CH=CH₂), 23.6 (COCH₃). MALDI TOF-MS: calcd for C₁₇H₁₉NO₆ (m/z), 357.13; found, 358.16 [M+H]^+.

Anal Calcd for C₁₇H₁₉NO₆: C, 60.79; H, 5.64; N, 3.73. Found: C, 60.82; H, 5.67; N 3.53.
Allyl 2-acetamido-6-O-benzyl-2-N,3-O-carbonyl-2-deoxy-α-D-glucopyranoside (4): compound 3 (184.6 mg, 0.492 mmol) was co-evaporated three times with toluene (4 mL). The residue was dried, mixed with freshly activated AW-300 molecular sieves (4Å) under Ar, cooled to 0°C and dissolved in CH₂Cl₂ (6.0 mL). Et₃SiH (393 μL, 2.46 mmol) and a 2.4 M solution of TFA in CH₂Cl₂ were then added and the mixture was stirred at 5°C over night. The mixture was then filtered, diluted in CH₂Cl₂ (10 mL) and washed with a 1 M NaHCO₃. The organic layer was collected, dried with Na₂SO₄, filtered and purified by chromatography (20% ethyl acetate in petroleum ether) to give 4 as a yellow oil (98.6 mg, 53.1%).

1H NMR (400 MHz, CDCl₃): δ 7.35-7.38 (m, 5H, H-Ar), 5.85 (m, 1H, OCH₂CH=CH₂), 5.68 (d, 1H, J₈₂=2.7 Hz, H-1), 5.24 (dd, 1H, J₈,Vic=17.2 Hz, trans OCH₂CH=CHH), 5.19 (dd, 1H, J₈,Vic=10.3 Hz, cis OCH₂CH=CHH), 4.64-4.59 (m, 3H, H-3, OCHHPh), 4.54 (d, 1H, J₉₁=12.1 Hz, OCHHPh), 4.18 (dd, 1H, J₈₁,J₉₁=12.7 Hz, H₈₆), 4.10-4.03 (m, 2H, H-4, OCHHCH=CH₂), 3.80 (dd, 1H, J₈₆,J₉₆=9.5 Hz, H₈₆), 3.75-3.66 (m, 3H, H-2, H-5, H-6b), 2.48 (s, 3H, NCOCH₃).

13C NMR (50 MHz, CDCl₃): δ 171.3 (COCH₃), 152.3 (NCOO), 136.3 (Cipso), 133.2 (OCH₂CH=CH₂), 128.5, 127.9, 127.7 (C-Ar), 118.1 (OCH₂CH=CH₂), 95.1 (C-1), 76.6, 73.7, 72.1, 70.1, 69.2, 59.6 (C-2, C-3, C-4, C-5, C-6, CH₂Ph OCH₂CH=CH₂), 23.7 (COCH₃). MALDI TOF-MS: calcd for C₁₉H₂₃NO₇ (m/z), 377.15; found, 378.23 [M+H]⁺. Anal Calcd for C₁₉H₂₁NO₇: C, 60.47; H, 6.14; N, 3.71. Found: C, 60.7; H, 6.27; N 3.75.

Allyl 2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (5): Compound 2 (1.0071 gr, 3.28 mmol) were dissolved in pyridine (5.0 mL) and treated with Et₃N (930 μL, 6.67 mmol) and CuSO₄ (54.8 mg, 0.343 mmol). A 0°C a 0.71 M solution
of triflyl azide (5.6 mL, 3.97 mmol) was added and the mixture was stirred for 2h at room temperature. The pyridine was co-evaporated with toluen and a yellow oil was obtained. The residue was then dissolved in DMF (8 mL) and to the solution BnBr (1.640 mL, 13.78 mmol) and NaH (60% in oil, 555.1 mg, 13.80 mmol) were added. The mixture was stirred over night, then cooled to 0 °C and treated dropwise with a little water until production of gas ceased. The mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and washed with water. The organic layer was collected, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and purified by chromatography (4 to 6% ethyl acetate in petroleum ether) to give 5 as a yellow oil (984.6 mg, 71.1%):<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 7.56-7.36 (m, 10H, H-Ar), 6.0 (m, 1H, OCH<sub>2</sub>CH=CH<sub>2</sub>), 5.63 (s, 1H, CHΦ), 5.41 (d, 1H, J<sub>vic</sub>=17.0 Hz, trans OCH<sub>2</sub>CH=CHH), 5.01 (d, 1H, J<sub>gem</sub>= 11 Hz, OC<sub>H</sub>HPh), 4.98 (d, 1H, J<sub>1,2</sub>=3.0 Hz, H-1), 4.86 (d, 1H, J<sub>gem</sub>= 11 Hz, OCH<sub>H</sub>HPh), 4.33 (dd, J<sub>gem</sub>= 10 Hz, J<sub>5,6</sub>=5.5 Hz, H-6a), 4.26 (dd, 1H, J<sub>vic</sub>= 12.7 Hz, J<sub>gem</sub>=4.5 Hz, OCHHCH=CH<sub>2</sub>), 4.17 (t, 1H, H-3), 4.10 (dd, 1H, J<sub>vk</sub>= 12.7 Hz, J<sub>gem</sub>=6 Hz, OCHH(CH=CH<sub>2</sub>), 3.97 (m, 1H, H-5), 3.82-3.74 (m, 2H, H-4, H-6b), 3.46 (dd, 1H, J<sub>1,2</sub>= 3 Hz, J<sub>2,3</sub>= 10 Hz, H-2). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 137.8, 137.1 (C<sub>ipso</sub>), 133.0 (OCH<sub>2</sub>CH=CH<sub>2</sub>), 128.9, 128.4, 128.3, 128.2, 128.1, 128.0, 128.7, 126.0, 125.9 (C-Ar), 118.1 (OCH<sub>2</sub>CH=CH<sub>2</sub>), 101.3 (CHPh), 97.4 (C-1), 82.7, 76.1, 74.9, 68.8, 68.6, 62.9, 62.7 (C-2, C-3, C-4, C-5, C-6, CH<sub>3</sub>Ph OCH<sub>2</sub>CH=CH<sub>2</sub>). MALDI TOF-MS: calcd for C<sub>23</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub> (m/z), 423.18; found, 423.8 [M+H]<sup>+</sup>. Anal Calcd for C<sub>23</sub>H<sub>21</sub>N<sub>3</sub>O<sub>5</sub>: C, 65.24; H, 5.95; N, 9.92. Found: C, 65.5; H, 5.89; N 9.95.

2-Azido-3-<i>O</i>-benzyl-4,6-<i>O</i>-benzylidene-2-deoxy-<i>z</i>-D-glucopyranose (6): A solution of 5 (99.1 mg, 0.234 mmol) in 9:1 v/v MeOH/CH<sub>2</sub>Cl<sub>2</sub> (1.46 mL) was treated
with PdCl$_2$ (17.52 mg, 95.6 μmol). The mixture was vigorously stirred at room temperature for 1 h and then filtered over a Celite pad, diluted with CH$_2$Cl$_2$ (8 mL) and washed with water (8 mL). The organic layer was collected, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated. The residue was subjected to column chromatography (17 to 25% ethyl acetate in petroleum ether) to give 6 (51.5 mg, 58% 6α/6β≈1:1) as a colourless oil: $^1$H NMR (400 MHz, CDCl$_3$): δ 7.50-7.32 (m, 20H, H-Ar), 5.60 (s, 1H, CH(Φ)), 5.58 (s, 1H, CH(Φ)), 5.25 (d, 1H, $J_{1,2}$=3.5 Hz, H-1α), 4.98 (d, 1H, $J_{gem}$=11.2 Hz, OCH(Ph)), 4.94 (d, 1H, $J_{gem}$=11.2 Hz, OCH(Ph)), 4.82 (d, 1H, $J_{gem}$=11.2 Hz, OCH(Ph)), 4.81 (d, 1H, $J_{gem}$=11.2 Hz, OCH(Ph)), 4.59 (d, 1H, $J_{1,2}$=4.6 Hz, H-1β), 4.35-4.27 (m, 2H, H-3α, H-3β), 4.14-4.09 (m, 2H, H-6α', H-6β'), 3.81-3.70 (m, 4H, H-6α', H-4α, H-4β, H-5β), 3.60 (t, 1H, H-6β''), 3.49 (dd, 1H, $J_{gem}$=3.6 Hz, $J_{gem}$=9.8 Hz, H-2). MALDI TOF-MS: calcd for C$_{20}$H$_{21}$N$_3$O$_5$ (m/z), 384.15; found, 384.8 [M+H]$^+$. Anal Calcd for C$_{20}$H$_{21}$N$_3$O$_5$: C, 62.65; H, 5.52; N, 10.96. Found: C, 62.5; H, 5.89; N 9.95.

2-Azido-3-$\alpha$-benzyl-4,6-$\alpha$-benzyldiene-2-deoxy-$\alpha$-D-glucopyranosyl trifluoroacetimidate (7): to a solution of compound 6 (51.5 mg, 0.134 mmol) in CH$_2$Cl$_2$ (900 μL) Cl$_3$CCN (200 μL, 2.01 mmol) and a 0.40 M solution of DBU in CH$_2$Cl$_2$ (100 μL, 40.2 μmol) wev added. After 2 h the mixture is concentrated and subjected to column chromatography (12.5 to 20% ethyl acetate in n-hexane) to give 7 (63.5 mg, 94%) as a yellow oil: $^1$H NMR (400 MHz, CDCl$_3$): δ 8.74 (s, 1H, NH), 7.51-7.32 (m, 5H, H-Ar), 6.39 (d, 1H, $J_{1,2}$=3.7 Hz, H-1), 5.63 (s, 1H, CH(Φ)), 5.03 (d, 1H, $J_{gem}$=10.8Hz, OCH(Ph)), 4.85 (d, 1H, $J_{gem}$=10.8Hz, OCH(Ph)), 4.34 (dd, 1H, $J_{6,5}$=4.9 Hz, $J_{gem}$=7.4 Hz, H-6α), 4.18 (t, 1H, $J_{3,2}$ = $J_{3,4}$= 9.4 Hz, H-3), 4.08 (m, 1H, H-5), 3.84 (t, 1H, $J_{gem}$= $J_{6,}}
\begin{align*}
\delta=10.8 \text{Hz, H-6b)}, 3.80 \text{ (t, 1H, H-4), 3.73 \text{ (dd, 1H, H-}1_{i-2}=3.7 \text{ Hz, } J_{2,3}=9.9 \text{ Hz, H-2).} \\
\text{MALDI TOF-MS: unstable. Anal Calcd for C}_{22}\text{H}_{21}\text{Cl}_{3}\text{N}_{4}\text{O}_{5}: C, 50.06; H, 4.01; Cl 20.15; N, 10.62. Found: C, 50.13; H, 4.21; Cl 20.00; N, 10.81.}
\end{align*}

**Allyl 2-azido-3,6-di-O-benzyl-2-deoxy-\alpha-D-glucopyranoside (8):** Compound 5 (225.4 mg, 0.532 mmol) was co-evaporated three times with toluene (5 mL). The residue was dried, mixed with freshly activated AW-300 molecular sieves (4Å) under Ar, cooled to 0°C and dissolved in CH\textsubscript{2}Cl\textsubscript{2} (6.5 mL). Et\textsubscript{3}SiH (425 μL, 2.66 mmol) and a 2.4 M solution of TFA in CH\textsubscript{2}Cl\textsubscript{2} (1 mL, 2.66 mmol) were then added and the mixture was stirred at 5°C over night. The mixture was then filtered, diluted in CH\textsubscript{2}Cl\textsubscript{2} (5 mL) and washed with a 1 M NaHCO\textsubscript{3}. The organic layer was collected, dried with Na\textsubscript{2}SO\textsubscript{4}, filtered and purified by chromatography (11% ethyl acetate in petroleum ether) to give 8 as a yellow oil (155.3 mg, 69%): \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): \delta 7.43-7.32 \text{ (m, 10H, H-Ar)}, 5.93 \text{ (m, 1H, OCH\textsubscript{2}CH=CH\textsubscript{2})}, 5.36 \text{ (dd, 1H, } J_{\text{vic}}=17.5 \text{ Hz, } J_{\text{gem}}=1.5 \text{ Hz, trans OCH\textsubscript{2}CH=CHH}), 5.27 \text{ (dd, 1H, } J_{\text{vic}}=12.6 \text{ Hz, } J_{\text{gem}}=1 \text{ Hz, cis OCH\textsubscript{2}CH=CHH}), 4.97 \text{ (d, 1H, } J_{1,2}=3.5 \text{ Hz, H-1}), 4.94 \text{ (d, 1H, } J_{\text{vic}}=11.5 \text{ Hz, OCHHPh}), 4.82 \text{ (d, 1H, } J_{\text{vic}}=11 \text{ Hz, OCHHPh}), 4.62 \text{ (d, 1H, } J_{\text{vic}}=12 \text{ Hz, OCHHPh}), 4.55 \text{ (d, 1H, } J_{\text{vic}}=12 \text{ Hz, OCHHPh}), 4.22 \text{ (dd, 1H, } J_{\text{vic}}=12.6 \text{ Hz, } J_{\text{gem}}=5.0 \text{ Hz, OCHHCH=CH\textsubscript{2}}), 4.06 \text{ (dd, 1H, } J_{\text{vic}}=12.6 \text{ Hz, } J_{\text{gem}}=6.5 \text{ Hz, OCHHCH=CH\textsubscript{2}}), 3.87 \text{ (t, 1H, H-6a)}, 3.80 \text{ (m, 1H, H-5)}, 3.73 \text{ (m, 3H, H-3, H-4, H-6b)}, 3.35 \text{ (dd, 1H, } J_{1,2}=3.5 \text{ Hz, } J_{2,3}=10 \text{ Hz, H-2). MALDI TOF-MS: caled for C}_{23}\text{H}_{27}\text{N}_{3}\text{O}_{5} (m/z), 425.20; found, 448.2 \text{ [M+Na]}^+. \text{ Anal Calcd for C}_{23}\text{H}_{27}\text{N}_{3}\text{O}_{5}: C, 64.93; H, 6.40; N, 9.88. Found: C, 64.72; H, 6.22; N, 9.74.}
Allyl 2-azido-3,6-di-O-benzyl-4-p-methoxybenzyl-2-deoxy-α-D-glucopyranoside (9): Compound 8 (155.3 mg, 0.366 mmol) was dissolved in DMF (1 mL) and to the solution p-methoxybenzyl bromide (100 μL, 0.732 mmol) and NaH (60% in oil, 28.1 mg, 0.702 mmol) were added. The mixture was stirred for 3 h, then cooled to 0 °C and treated dropwise with a little water until production of gas ceased. The mixture was diluted with CH₂Cl₂ (6 mL) and washed with water. The organic layer was collected, dried with Na₂SO₄, filtered and purified by chromatography (16 to 7% ethyl acetate in petroleum ether) to give 5 as a yellow oil (181.1 mg, 92%): ¹H NMR (300 MHz, CDCl₃): δ 7.60-7.50 (m, 10H, H-Ar), 7.25 (d, 2H, Jᵥic = 14 Hz, H-Ar), 7.02 (d, 2H, Jᵥic = 13.5 Hz, H-Ar), 6.12 (m, 1H, OCH₂C=CH₂), 5.54 (dd, 1H, Jᵥic = 17.1 Hz, J_gem = 0.9 Hz, trans OCH₂CH=CH₂), (dd, 1H, Jᵥic = 10.5 Hz, cis OCH₂CH=CH₂), 5.19 (d, 1H, J₁₂ = 3.3 Hz, H-1), 5.10 (s, 2H, CH₃PhOMe), 4.95 (d, 1H, Jᵥic = 10.5 Hz, OCH₃HPh), 4.86 (d, 1H, J_gem = 12.3 Hz, OCH₃HPh), 4.71 (d, 1H, J_gem = 12.3 Hz, OCH₃HPh), 4.66 (d, 1H, J_gem = 10.2 Hz, OCH₃HPh), 4.41 (dd, 1H, Jᵥic = 12.9 Hz, J_gem = 4.8 Hz, OCH₃HCH=CH₂), 4.28-4.19 (m, 2H, H-3, OCH₃HCH=CH₂), 4.06-3.84 (m, 7H, H-6a, H-6b, H-4, H-5, OCH₃₂), 3.62 (dd, J₁₂ = 3.6 Hz, J₂₃ = 10.2 Hz, H-2). MALDI TOF-MS: calcd for C₃₁H₅₅N₃O₆ (m/z), 524.25; found, 547.77 [M+Na]+. Anal Calcd for C₃₁H₅₅N₃O₆: C, 68.24; H, 6.47; N, 7.70. Found: C, 68.36; H, 6.44; N, 7.22.

2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranose trichloroacetimidate (10α) and 2-Azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranose (10β): A solution of 9 (101.1 mg, 0.185 mmol) in 1:1 v/v MeOH/CH₂Cl₂ (1.8 mL) was treated with PdCl₂ (16.6 mg, 93 μmol). The mixture was vigorously stirred at room temperature for 2 h, diluted with CH₂Cl₂ (20 mL) and then
filtered over a Celite pad and concentrated. The residue was subjected to column chromatography (20% ethyl acetate in petroleum ether) to give 10α and 10β in a 1:1 ratio (74.9 mg, 80%) as a colourless oil: 1H NMR (400 MHz, CDCl₃): δ 7.40-7.34 (28H, H-Ar), 7.06 (t, 4H, H-Ar PMB), 6.82 (d, 4H, H-Ar PMB), 5.30 (bs, 1H, H-1α), 4.90-4.88 (m, 3H, OCH₂HPh, OCH₂OMePh), 4.82 (d, 1H, J_gem=10.8 Hz, OCH₂HPh), 4.73 (t, 2H, OCH₂OMePh), 4.61-4.41 (m, 7H, 6 OCH₂HPh, H-1β), 4.05-3.97 (m, 2H), 3.81 (s, 6H, 2 OCH₂OMePh), 3.65-3.37 (m, 10H). 13C NMR (100 MHz, CDCl₃): δ 159.3 (C-OMe), 159.2 (C-OMe), 137.8, 137.5, 137.4 (C_ipso), 129.8-127.7 (C-Ar), 113.8 (C-Ar PMB), 95.8 (C-1β), 91.84 (C-1α), 83.0, 80.0, 78.2, 77.3, 75.3, 74.6, 73.3, 70.3, 68.5, 67.2, 63.8, 55.2, 4 CH₂Bn, 2 CH₂PhOCH₃, 2 CH₂PhOCH₃. MALDI TOF-MS: calcd for C₂₈H₃₁N₃O₆ (m/z), 505.56; found, 548.77 [M+Na]+. Anal Calcd for C₂₈H₃₁N₃O₆: C, 66.52; H, 6.18; N, 8.31. Found: C, 66.36; H, 6.44; N, 8.22.

2-Azido-3,6-di-O-benzyl-4-O-pmethoxy benzyl-2-deoxy-α-D-glucopyranose trichloroacetimide (11): to a solution of compound 10α and 10β (74.9 mg, 0.15 mmol) in CH₂Cl₂ (1 mL) Cl₃CCN (220 µL, 2.19 mmol) and a 0.40 M solution of DBU in CH₂Cl₂ (110.4 µL, 44 µmol) were added. After 2 h the mixture is concentrated and subjected to column chromatography (12.5 to 20% ethyl acetate in n-hexane) to give 11 (67.7 mg, 70.2%) as a yellow oil: 1H NMR (400 MHz, CDCl₃): δ 8.70 (N-H), 7.40-7.36 (10H, H-Ar), 7.07 (d, 2H, J_o-o=8.6 Hz, H₆PMB), 6.82 (d, 2H, J_o-o=8.6 Hz, H₆PMB), 6.44 (1H, J₁,₂=3.5 Hz, H-1), 4.96 (d, 1H, J_gem=10.8 Hz, OCH₂HPh), 4.90 (d, 1H, J_gem=10.7 Hz, OCH₂HPh), 4.75 (d, 1H, J_gem=10.3 Hz, OCH₂HPhOMe), 4.64 (d, 1H, J_gem=12 Hz, OCH₂HPh), 4.50 (d, 1H, J_gem=10.3 Hz, OCH₂HPhOMe), 4.49 (1H, J_gem=12
Hz, OCHHPh), 4.05-3.97 (m, 2H, H-6a, H-3), 3.89-3.79 (m, 5H, H-6b, H-5, OCH₃), 3.71-3.65 (m, 2H, H-2, H-4). ¹³C NMR (100 MHz, CDCl₃): δ 160.6 (C=NH), 159.4 (C-OMe), 137.7 (Cqυ), 129.7-127.7 (C-Ar), 113.8 (CmPMB), 94.8 (C-1), 79.9, 77.3, 75.3, 74.8, 73.5, 73.4, 67.7, 62.9, 55.1 (C-2, C-3, C-4, C-5, C-6, 2 CH₂Bn, CH₃PhOCH₃, CH₂PhOCH₃). MALDI TOF-MS: unstable. Anal Calcd for C₃₀H₃₁Cl₃N₄O₆ (649.95): C, 55.44; H, 4.81; Cl 16.36; N, 8.62. Found: C, 55.13; H, 4.21; Cl 16.00; N, 8.81.

**Allyl 4,6-O-benzylidene-3-O-benzyloxy carbonyl-2-N-trichlorothioxycarbonyl-α-D-glucopyranoside (12):** Compound 2 (265.1 mg, 0.865 mmol) was dissolved in 2:1 v/v H₂O/CH₃CN (5.7 mL) and treated with NaHCO₃ (214.9 mg, 2.55 mmol) and TrocCl (300 μL, 2.2 mmol). The mixture was vigorously stirred at room temperature for 1 h and then with AcOEt (8 mL). The organic layer was collected, dried over anhydrous Na₂SO₄, filtered and concentrated. The residue was dissolved in CH₂Cl₂ and treated with DMAP (212.1 mg, 1.74 mmol) and CbzCl (245 μL, 1.72 mmol). The mixture was stirred for 90 min and concentrated. The residue was subjected to column chromatography (14% ethyl acetate in n-hexane) to give 12 (361.3 mg, 70%) as a colourless oil: [α]D +30.2 (c 1.05 in CH₂Cl₂); ¹H NMR (200 MHz, CDCl₃): δ 7.46-7.31 (10 H, m, H-Ar), 5.90 (s, 1 H, OCH₂CH=CH₂), 5.52 (s, 1H, CHΦ), 5.44-5.06 (m, 6H, H-3, NH, trans OCH₂CH=CHH, cis OCH₂CH=CHH, COCH₂Cl), 4.94 (d, 1-H, H-1, J₁₂=3.6 Hz), 4.66 (s, 2H, COCH₂Φ), 4.34-3.91 (m, 6-H, H-2, H-6a, H-5, OCHHCH=CH₂), 3.83-3.71 (m, 2H, H-4, H-6b). ¹³C NMR (100 MHz, CDCl₃): 155.13 (C=O), 154.23 (C=O), 136.84 (Cqυ), 132.94 (Cqυ), OCH₂CH=CH₂, 129.07-126.16 (C-Ar), 118.65 (OCH₂CH=CH₂), 101.54 (C-Φ), 97.05 (C-1), 79.02, 74.51, 74.16, 69.99, 68.82, 68.73, 62.98, 54.55 (C-2, C-3, C-4, C-5, C-6,
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CH₂Φ, CH₂CCl₃, OCH₂CH=CH₂). MALDI TOF-MS: calcd for C₁₇H₂₉Cl₂NO₉ (m/z), 615.08; found, 538.69 [M+Na]⁺. Anal Calcd for C₁₇H₂₉Cl₂NO₉: C, 52.57; H, 4.58; Cl, 17.24; N, 2.27. Found: C, 52.36; H, 4.44; Cl, 17.39, N, 2.22.

Allyl 4-O-benzyl-3-O-benzylxycarbonyl-2-N-trichloroethoxycarbonylamino-2-deoxy-α-D-glucopyranoside 13: Compound 12 (345.6 mg, 0.560 mmol) was co-evaporated three times with toluene (5 mL). The residue was dried, mixed with freshly activated AW-300 molecular sieves (4Å) under Ar, cooled to 0°C and dissolved in CH₂Cl₂ (7.5 mL). Et₃SiH (465 μL, 3.01 mmol) and a 5.1 M solution of TFA in CH₂Cl₂ (545 μL, 2.8 mmol) were then added and the mixture was stirred at 5°C over night. The mixture was then filtered, diluted in CH₂Cl₂ (10 mL) and washed with a 1 M NaHCO₃. The organic layer was collected, dried with Na₂SO₄, filtered and purified by column chromatography (17% ethyl acetate in petroleum ether) to give 12 as a yellow oil (266.0 mg, 77%): [α]D +56.3 (c 0.88 in CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): δ 7.37-7.26 (m, 10H, H-Ar), 5.87 (m, 1H, OCH₂CH=CH₂), 5.35 (d, 1H, JNH-H₂=9.8 Hz), 5.29 (dd, 1H, Jvv=17.2 Hz, Jgem 1.4 Hz, trans OCH₂CH=CHH), 5.22 (dd, 1H, Jvv=10.3 Hz, Jgem 1.2 Hz, cis OCH₂CH=CHH), 5.15 (s, 2H, COC₃H₂Φ), 4.99 (m, 1H, H-3), 4.91 (d, 1H, J₁₂=3.6 Hz, H-1), 4.69-4.61 (m, 3H, CHHΦ, CH₂Cl₂), 4.55 (d, 1H, CHHΦ, Jgem =12.0 Hz), 4.19 (dd, 1H, Jvv= 5.27 Hz, Jgem= 12.8 Hz, OCH₂CH=CH₂), 4.04-3.97 (m, 2H, H-2, OCH₂CH=CH₂), 3.84-3.83 (bs, 2H, H-4, H-5), 3.78 (dd, 1H, Jgem= 10.7 Hz, J₆a,₅= 2.8 Hz, H-6a), 3.71 (dd, 1H, Jgem= 10.4 Hz, J₆b,₅= 2.6 Hz, H-6b). ¹³C NMR (100 MHz, CDCl₃): 155.74 (C=O), 154.15 (C=O), 137.60 (C₆p), 134.77 (C₆p), 133.12 (OCH₂CH=CH₂), 128.55-127.61 (C-Ar), 118.24 (OCH₂CH=CH₂), 96.34 (C-1), 77.86, 74.40, 73.61, 70.26, 70.09, 69.86, 69.39, 68.48, 53.77 (C-2, C-3, C-4, C-5, C-6, CH₂Φ,
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\[ CH_2Cl_2, OCH_2CH=CH_2 \]. MALDI TOF-MS: calcd for C_{27}H_{35}ClNO_6 (m/z), 617.10; found, 540.53 [M+Na]^+.

Anal Calcd for C_{27}H_{35}ClNO_6: C, 52.40; H, 4.89; Cl, 17.19; N, 2.26. Found: C, 52.38; H, 4.92; Cl, 17.33; N, 2.18.

**Allyl 2-azido-3,6-di-O-benzyl-4-O-p-methoxybenzyl-2-deoxy-α-D-glucopyranosyl-(1→4)-2-acetamido-6-O-benzyl-2-N,3-O-carbonyl-2-deoxy-α-D-glucopyranoside (15α)** and **allyl 2-azido-3,6-di-O-benzyl-4-O-p-methoxybenzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-6-O-benzyl-2-N,3-O-carbonyl-2-deoxy-α-D-glucopyranoside (15β)**: a mixture of acceptor 4 (8.96 mg, 24 μmol) and of donor 11 (27.8 mg, 42.8 μmol) was co-evaporated three times with toluene. The residue was dried, mixed with freshly activated AW-300 molecular sieves (4Å) under Ar, cooled to -30°C and dissolved in 2:1.3 v/v CH_2Cl_2/n-Hex (441 μL). A 1.4 M solution of BF_3·OEt_2 in CH_2Cl_2 (18.5 μL, 26.7 μmol) was the added and the mixture was stirred at -30°C for 1 h. Et_3H (10 μL) was added and the mixture was filtered on a Celite pad. The mixture was purified by column chromatography (10 to 15% ethyl acetate in n-hexane) to give 15α (7.3 mg, 35%) and 15β (6.9 mg, 33%) both as a white crystalline compounds:

15β [α]D +29.1 (c 0.54 in CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): δ 7.37-7.21 (m, 15H, H-Ar), 7.10 (d, 2H, J_{ortho} 8.6 Hz, H-Ar PMB), 6.82 (d, 2H, J_{ortho} 8.6 Hz, H-Ar PMB), 5.85 (m, 1H, OCH_2CH=CH_2), 5.72 (d, 1H, J_{1,2}=2.7 Hz, H-1_β), 5.26 (dd, 1H, J_{vic}=18.7 Hz, J_{gem} 1.4 Hz, trans OCH_2CH=CHH), 5.21 (dd, 1H, J_{vic}=10.4 Hz, J_{gem} 1.3 Hz, cis OCH_2CH=CHH), 4.83 (s, 2H, OCH_2PhOMe), 4.26-4.65 (m, 5H, H-3_α, CH_2Ph), 4.57 (d, 1H, J_{gem}=13.4 Hz, CHHΦ), 4.51 (d, 1H, CHHΦ, J_{vic}=10.8 Hz), 4.20-4.14 (m, 3H, H-1_βs, H-3_βs, OCH_2CH=CH_2); 4.07 (dd, 1H, J_{vic}= 5.27 Hz, J_{gem} = 12.8 Hz, OCH_2CH=CH_2), 3.90 (dd, 1H, J_{gem} = 10.4 Hz, J_{6Ba,5b}=2.6 Hz, H-6_βa), 3.81-3.59 (m, 10H, H-2_α, H-4_α, H-
5₅, H-6₅a, H-6₅b, H-4₅b, H-6₅b, OCH₃), 2.45 (s, 3H, COCH₃). ¹²C NMR (75 MHz, CDCl₃): δ 171.2 (C=O), 159.4 (Cₚsp², PMB), 152.9 (C=O), 138.6, 137.8, 135.1 (3 Cₚsp³, Bn), 133.2 (OCH₂CH=CH₂), 130.8 (Cₚsp², PMB), 130.1-125.1 (C-Ar), 118.4 (OCH₂CH=CH₂), 113.8 (C-Ar), 101.2 (C-1₅b), 95.0 (C-1₅a), 83.2 (C-3₅), 75.6, 75.5, 75.2, 74.5, 73.7, 73.7, 72.4, 69.5, 68.5, 67.6, 66.4, 59.9 (C-4₅b, C-3₅, C-5₅b, OCH₃, OMePh, C-4₅a, 3 OCH₂Ph, C-5₅, C-6₅b, OCH₂CH=CH₂, C-6₅, C-2₅b, C-2₅a) 55.3 (OMe), 29.7 (CH₃CO).


**Compound 15x**: [α]D +29.6 (c 0.47 in CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): δ 7.38-7.21 (m, 15H, H-Ar), 7.03 (d, 2H, J ortho 8.4 Hz, H-Ar PMB), 6.81 (2 H, d, J ortho 8.6 Hz, H-Ar PMB), 5.84 (m, 1H, OCH₂CH=CH₂), 5.73 (d, 1H, J 2.8 Hz, H-1₅a), 5.41 (d, 1H, J 2.8 Hz, H-1₅b), 5.25 (dd, 1H, J σ 17.2 Hz, J gem 1.5 Hz, trans OCH₂CH=CHH), 5.20 (dd, 1H, J σ 10.4 Hz, J gem 1.2 Hz, σ OCH₂CH=CHH), 4.85 (s, 2H, OCH₃PhOMe), 4.76 (t, 1H, J 3,4 = J 3,2 = 9.9 Hz, H-3₅, H-3₅a), 4.68 (d, 1H, J gem 12 Hz, CHHΦ), 4.56 (d, 1H, J gem 12.0 Hz, CHHΦ), 4.52 (d, 1H, J gem 12.0 Hz, CHHΦ), 4.46 (d, 1H, J gem 12.0 Hz, CHHΦ), 4.40 (d, 1H, J gem 12.0 Hz, CHHΦ), 4.35 (t, 1H, J 4,3 = J 4,5 = 10 Hz, H-4₅a), 4.30 (d, 1H, J gem 12.0 Hz, CHHΦ), 4.20 (dd, 1H, J vic 5.27 Hz, J gem 12.8 Hz, OCH₂CH=CH₂), 4.07 (dd, 1H, J vic 5.33 Hz, J gem 12.6 Hz, OCH₂CH=CH₂), 3.82-3.76 (m, 6H, H-3₅b, H-5₅a, H-2₅a, OCH₃), 3.71-3.55 (m, 4H, H-4₅b, H-5₅b, H-6₅a, H-6₅b), 3.45 (dd, 1H, J gem 10.6 Hz, J 6₅b-2₅b = 2.8 Hz, H-6₅b), 3.45 (dd, 1H, J 1,2 = 2.8 Hz, J 3,2 = 9.5 Hz, H-2₅b), 3.35 (d, 1H, J gem 10.6 Hz, H-6₅b), 2.5 (s, 3H, COCH₃). ¹³C NMR (75 MHz, CDCl₃): δ 171.2 (C=O), 160.0 (Cₚsp², PMB), 152.9 (C=O), 137.9, 135.2, 133.2 (3 Cₚsp³, Bn), 130.9 (OCH₂CH=CH₂), 130.2, 129.6, 128.8, 128.5, 128.3, 127.9, 127.9, 127.7, 127.4 (C-Ar), 118.1 (OCH₂CH=CH₂), 113.8 (C-Ar), 96.3 (C-1₅b), 95.0 (C-1₅a), 80.2 (C-3₅b), 77.6,

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75.4, 74.7, 73.6, 73.5, 71.9, 71.8, 70.7, 69.5, 68.2, 67.8, 63.3, 59.9 (C-4_B, C-3_A, C-5_B, OCH_2OMePh, C-4_A, 3 OCH_2Ph, C-5_A, C-6_B, OCH_2CH=CH_2, C-6_A, C-2_B, C-2_A) 55.3 (OMe), 29.7 (CH_3CO).

MALDI TOF-MS: calcd for C_{47}H_{52}N_4O_{12} (m/z), 864.36; found, 898.88 [M+Na]^+. Anal Calcd for C_{47}H_{52}N_4O_{12}: C, 65.27; H, 6.06; N, 6.48. Found: C, 65.82; H, 6.00; N, 6.23.

Allyl 2-azido-3,6-di-\( \text{\textgreek{O}} \)-benzyl-4-\( \text{\textgreek{O}} \)-methoxybenzyl-2-deoxy-\( \beta \)-D-glucopyranosyl-(1→4)-6-\( \text{\textgreek{O}} \)-benzyl-3-\( \text{\textgreek{O}} \)-benzyloxycarbonyl-2-N-trichloroethoxy-carbonylamino-2-deoxy-\( \alpha \)-D-glucopyranoside (17): a mixture of acceptor 13 (13.5 mg, 21.8 \( \mu \)mol) and of donor 11 (22.8 mg, 35.0 \( \mu \)mol) was co-evaporated three times with toluene. The residue was dried, mixed with freshly activated AW-300 molecular sieves (4Å) under Ar, cooled to -30°C and dissolved in 2:1.3 v/v CH_2Cl_2/n-Hex (370 \( \mu \)L). A 31.1 mM solution of BF_3·OEt_2 in CH_2Cl_2 (230 \( \mu \)L, 7.45 \( \mu \)mol) was the added and the mixture was stirred at -80°C for 30 min. Et_3H (5 \( \mu \)L) was then added and the mixture was filtered on a Celite pad. The mixture was purified by column chromatography (10 to 15% ethyl acetate in n-hexane) to give 17 (7.3 mg, 35%) as a white crystalline compound: \([\alpha]_D^2 +7 (c 0.6 in CH_2Cl_2); ^1H NMR (400 MHz, CDCl_3): \delta 7.37-7.25 (20 H, m, H-Ar), 7.08 (2 H, d, \_J_{ortho}=8.4 Hz, H-Ar PMB), 6.80 (2 H, d, \_J_{ortho}=8.4 Hz, H-Ar PMB), 5.86 (1 H, m, OCH_2CH=CH_2), 5.28 (2 H, m, NH, \text{\textit{trans}} OCH_2CH=CHH), 5.22 (1 H, br d, \_J_{\text{vic}}=10.4 Hz, \_J_{\text{cis}} OCH_2CH=CHH), 5.08 (1 H, t, \_J_{1,2}=10.1 Hz, H-3_\text{A}), 5.06 (1 H, d, \_J_{\text{gem}}=12.5 Hz, OCHHClCl), 4.97 (1 H, d, \_J_{\text{gem}}=12.5 Hz, OCHHClCl), 4.93 (1 H, dd, \_J_{1,2}=3.5 Hz, H-1_\text{A}), 4.81 (2 H, s, OCH_2OMePh), 4.69-4.60 (4 H, m, 4 OCHHPh), 4.51 (1 H, d, \_J_{\text{gem}}=12.1 Hz, OCHHPh), 4.47 (2 H, s, OCH_2Ph), 4.45 (1 H, d, \_J_{\text{gem}}=10.5 Hz, OCHHPh), 4.21 (1 H, dd, \_J_{\text{gem}}=12.5 Hz, \_J_{\text{vic}}=5.2 Hz, OCHHCH=CH_2), 4.13 (1 H, d, \_J_{1,2}
7.2 Hz, H-1δ), 4.07-3.91 (5 H, m, H-2α, H-4α, H-5α, H-6α, OCH\textsubscript{2}HCH=CH\textsubscript{2}), 3.79 (3 H, s, OMe), 3.72 (1 H, dd, J\textsubscript{gem} = 9.8 Hz, J\textsubscript{6b,5} 1.7 Hz, H-6bδ), 3.61 (1 H, d, J\textsubscript{gem} 10.8 Hz, J\textsubscript{6a,5} 1.7 Hz, H-6aδ), 3.47 (2 H, m, H-4β, H-6bβ), 3.23 (3 H, m, H-2β, H-3β, H-5β, H-6bβ). \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): δ 159.3 (C\textsubscript{ipso} PMB), 155.3, 154.1 (O\textsubscript{C}OOCH\textsubscript{2}Ph, N\textsubscript{C}OOCH\textsubscript{2}CCl\textsubscript{3}), 138.4, 138.0, 137.7, 135.2 (4 C\textsubscript{ipso} Bn), 133.1 (OCH\textsubscript{2}CH=CH\textsubscript{2}), 130.1 (C\textsubscript{ipso} PMB), 129.4-125.0 (C-Ar), 129.6 (OCH\textsubscript{2}CH=CH\textsubscript{2}), 123.6, 123.3 (C-1α), 96.3 (C-1δ), 83.3 (C-3α), 77.6 (C-4α), 75.7 (C-3β), 75.4 (C-5δ), OCH\textsubscript{2}OMePh, 74.7 (OCH\textsubscript{2}Ph), 74.5 (C-4α), OCH\textsubscript{2}Ph), 73.5 (2 OCH\textsubscript{2}Ph), 70.3 (C-5β), 69.5 (OCH\textsubscript{2}CCl\textsubscript{3}), 68.7 (C-6δ), 68.6 (OCH\textsubscript{2}CH=CH\textsubscript{2}), 67.7 (C-6α), 66.4 (C-2β), 55.3 (OMe), 54.1 (C-2δ). MALDI TOF-MS: calcd for C\textsubscript{55}H\textsubscript{59}Cl\textsubscript{3}N\textsubscript{4}O\textsubscript{14} (m/z), 1104.31; found, 1127.07 [M+Na]\textsuperscript{+}. Anal Calcd for C\textsubscript{55}H\textsubscript{59}Cl\textsubscript{3}N\textsubscript{4}O\textsubscript{14}: C, 59.70; H, 5.37; N, 5.06. Found: C, 59.55; H, 5.34; N, 4.99.

**Allyl 2-azido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-6-O-benzyl-3-O-benzoxycarbonyl-2-N-trichloroethoxy-carbonylamino-2-deoxy-α-D-glucopyranoside (18):** Compound 17 (46 mg, 0.041 mmol) was dissolved in 18:1 v/v CH\textsubscript{2}Cl\textsubscript{2}/MeOH (5.2 mL) and the solution was treated with DDQ (12.8 mg, 0.056 mmol). After stirring for 3 h at room temperature, the mixture was diluted with CH\textsubscript{2}Cl\textsubscript{2} and washed with water. The mixture was diluted with CH\textsubscript{2}Cl\textsubscript{2} and then washed with water. The organic layer was collected and treated with Na\textsubscript{2}SO\textsubscript{4}, filtered and the solvent was evaporated. The residue was purified by column chromatography (30% ethyl acetate in n-hexane) to give 18 as a white oil (23.5 mg, 58%). \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}): δ 7.40-7.23 (m, 20H, H-Ar), 5.86 (m, 1H, OCH\textsubscript{2}CH=CH\textsubscript{2}), 5.31-5.21 (m, 3H, H-3A, OCH\textsubscript{2}CH=CH\textsubscript{2}), 5.05 (d, 1H, J\textsubscript{gem} = 8.5 Hz, COOCH\textsubscript{2}Ph), 5.00 (d, 1H, J\textsubscript{gem} = 8.6 Hz,
COOCH\textsubscript{3}C\textsubscript{2}H\textsubscript{5}O; H, 43%, \(\alpha/\beta\) 9:1). Chromatography (10 to 12.5/ toluene in ethyl acetate) to give was evaporated and the product was isolated by column chromatography (10 to 12.5/ toluene in ethyl acetate) to give

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\[ \text{Compound 17 (46.9 mg, 42.3 \(\mu\)mol) was dissolved in 1:1 v/v CH}_{2}\text{Cl}_2/\text{MeOH(500 \(\mu\)L) and the solution was treated with PdCl}_2 (3.5 mg, 19 \(\mu\)mol) and stirred for 2 h. The mixture was then diluted with CH}_{2}\text{Cl}_2 (4.5 mL) and filtered on a Celite pad. The solvent was evaporated and the produc was isolated by column chromatography (10 to 12.5/ toluene in ethyl acetate) to give 19 as a yellow oil (19.4 mg, 43%, \(\alpha/\beta\) 9:1).} \]

\[ \text{\textsuperscript{1}H NMR (400 MHz, CDCl}_3; \delta 7.42-7.18 (m, 15H, H-Ar), 7.06 (2H, d,} \]
After 4 h the mixture was concentrated and the product was purified by column chromatography (20% of n-hexane in ethyl acetate) to give 21 as a white powder (17 mg,
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67%. [α]D = +34.0 (c 1.2 in CH2Cl2); 1H NMR (400 MHz, CDCl3): δ 7.37-7.31 (m, 15H, H-Ar), 7.06 (2 H, d, Jortho = 8.6 Hz, H-Ar PMB), 6.81 (2 H, d, Jortho = 8.6 Hz, H-Ar PMB), 5.83 (m, 1H, OCH2CH=CH2), 5.62 (d, 1H, JNHR=8.6 Hz, NH), 5.28 (dd, 1H, Jvic = 17.2 Hz, Jgem 1.5 Hz, trans OCH2CH=CHH), 5.20 (dd, 1H, Jvic = 10.4 Hz, Jgem = 1.4 Hz, cis OCH2CH=CHH), 4.96 (1 H, d, J1,2 = 3.6 Hz, H-1a), 4.84 (s, 2H, OCH2PhOMe), 4.69 (d, 1 H, Jgem = 10.1 Hz, OCH2PhH), 4.67 (d, 1 H, Jgem = 12.0 Hz, OCH2PhH), 4.55 (d, 1 H, Jgem = 11.9 Hz, OCH2PhH), 4.53 (d, 1 H, Jgem = 11.9 Hz, OCH2PhH), 4.45 (d, 1 H, Jgem = 11.9 Hz, OCH2PhH), 4.44 (d, 1 H, Jgem = 11.9 Hz, OCH2PhH), 4.12 (m, 4H, OCH2HCH=CH2, H-3a, H-1b, H-2b), 4.95 (dd, 1H, Jvic = 5.33 Hz, Jgem = 12.6 Hz, OCH2HCH=CH2), 3.86-3.75 (m, 6H, OCH3, H-6'a, H-6'b, H-5'α), 3.70-3.59 (m, 3H, H-4'b, H-6'a, H-6'b), 3.35-3.33 (m, 3H, H-3b, H-5'b, H-2'b), 2.00 (COCH3). 13C NMR (75 MHz, CDCl3): δ 170.9 (C=O), 159.5 (Cips PMB), 138.2, 137.7, 133.7 (3 Cips Bn), 129.8 (OCH2CH=CH2), 129.6, 128.5, 128.4, 127.9, 127.8 (C-Ar), 117.5 (OCH2CH=CH2), 113.9 (C-Ar), 101.7 (C-1b), 96.5 (C-1a), 83.1, 81.1, 75.6, 74.7, 73.3, 70.7, 69.7, 68.5, 68.4, 68.2, 66.5, 55.3 (C-3b, C-4b, C-3a, C-5b, OCH2OMePh, C-4a, 3 OCH2Ph, C-5a, C-6b, OCH2CH=CH2, C-6a, C-2b, C-2a), 53.3 (OMe), 23.5 (CH3CO). MALDI TOF-MS: calcd for C46H41N4O11 (m/z), 838.38; found, 862.38 [M+Na]+. Anal Calcd for C46H41N4O11: C, 65.86; H, 6.49; N, 6.68. Found: C, 65.82; H, 6.00; N, 6.21.

Allyl 2-azido-3,6-di-O-benzyl-4-O-p-methoxybenzyl-2-deoxy-β-D-glucopyranosyl-(1→4)- 2-acetamido-6-O-benzyl-3-O-((R)-1′-carboxyethyl)-2-deoxy-x-D-glucopyranoside (21): Compound 20 (231.1 mg, 0.27 mmol) was co-evaporated three times with toluene and then under Ar dissolved in 1:1 v/v DMF/dioxane (2 mL). To the solution NaH (60% in oil, 167.1 mg, 4.17 mmol) was added and stirred for 30
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min at 95°C. The mixture was treated with (3)-2-chloropropionic acid (1.35 mmol) and stirred for 3 h, then cooled to 0 °C and treated dropwise with a little water until production of gas ceased. The mixture was diluted with CH₂Cl₂ and then washed with water. The organic layer was collected and treated with Na₂SO₄, filtered and the solvent was evaporated. The residue was purified by column chromatography (2% methanol in dichloroethane) to give 21 as a white oil (42.4 mg, 14%). [α]D +13 (c 0.3 in CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): δ 7.36-7.26 (15H, m, H-Ar), 7.06 (2 H, d, Jortho 8.5 Hz, H-Ar PMB), 6.81 (2 H, d, Jortho 8.5 Hz, H-Ar PMB), 5.86 (1 H, m, OCH₂C=CH₂), 5.26 (1 H, br d, Jvic 17.0 Hz, trans OCH₂CH=CHH), 5.21 (1 H, d, J1,2 3.0 Hz, H-1A), 5.18 (1 H, br d, Jvic 11.0 Hz, cis OCH₂CH=CHH), 4.81 (2 H, s, OCH₂OMePh), 4.71 (1 H, d, Jgem 12.1 Hz, OCH₂HPh), 4.68 (1 H, d, Jgem 10.5 Hz, OCH₂HPh), 4.63 (1 H, q, Jvic 6.5 Hz, CH₃CO), 4.54-4.45 (4 H, m, 4 OCH₂HPh), 4.25 (1 H, d, J1,2 7.5 Hz, H-1B), 4.12 (1 H, dd, Jgem 3.64 (6 H, m, H-3A, H-4bH, H-5A, H-6bA, H-6aA, H-6bA), 3.28 (1 H, br d, J5,4 9.6 Hz, H-5b), 3.61 (1H, br d, J3,4 9.5 Hz, H-3b), 3.23 (1 H, t, J3,2 9.5 Hz, H-3b), 3.16 (1H, br d, J5,4 9.6 Hz, H-5b), 2.02 (3H, s, CH₃CO), 1.41 (3H, d, Jvic 6.5 Hz, CH₃CHO); ¹³C NMR (100 MHz, CDCl₃): δ 177.0 (COOH), 171.1 (NHCOCH₂), 159.5 (Cipso PMB), 137.9, 137.8, 137.6 (3 Cipso Bn), 133.8 (OCH₂CH=CH₂), 130.2 (Cipso PMB), 129.4-127.7 (C-Ar), 117.5 (OCH₂CH=CH₂), 113.8 (C-Ar), 100.7 (C-1b), 96.0 (C-1a), 83.3 (C-3b), 77.5 (C-3b), 76.9 (C-4A), 76.1 (C-4b), 75.4 (OCH₂OMePh), 75.2 (CH₂CH), 74.7 (C-5b), 74.5 (OCH₂Ph), 73.5 (OCH₂Ph), 73.3 (OCH₂Ph), 70.6 (C-5b), 70.5 (C-6b), 68.6 (OCH₂CH=CH₂), 67.8 (C-6a), 66.6 (C-2b), 55.3 (OMe), 54.0 (C-2A), 23.1 (CH₂CO), 18.3 (CH₂CH). MALDI TOF-MS: calcd for
C\textsubscript{49}H\textsubscript{58}Cl\textsubscript{3}N\textsubscript{4}O\textsubscript{13} (m/z), 910.40; found, 933.29 [M+Na]. Anal Calcd for C\textsubscript{49}H\textsubscript{58}N\textsubscript{4}O\textsubscript{13}: C, 64.60; H, 6.42; N, 6.15. Found: C, 64.44; H, 6.34; N, 6.06.

Compound 21 could be obtained also through a basic hydrolysis of the ethyl ester 23: Compound 23 (41.2 mg, 44.0 μmol) was dissolved in 2:1:0.5 v/v/v THF/dioxane/water. LiOH (1.39 mg, 58 μmol) was then added and the mixture was stirred 2 h. Dowex-H\textsuperscript{+} resin was added until a pH=7. The mixture was filtered, the solvent was evaporated and the product was purified by column chromatography (98% to 96% dichloromethane in methanol) to give 21 as a white oil (32.8 mg, 82%).

**Allyl 2-azido-3,6-di-\textit{O}-benzyl-4-\textit{O}-p-methoxybenzyl-2-deoxy-\textbeta\textsuperscript{-}D-glucopyranosyl-(1→4)-2-amino-6-\textit{O}-benzyl-3-\textit{O}-(\textit{R})-1’-(ethoxycarbonyl)ethyl)-2-deoxy -\textalpha\textsuperscript{-}D-glucopyranoside (22):** Compound 20 (342 mg, 0.407 mmol) was co-evaporated three times with toluene and then under Ar dissolved in CH\textsubscript{2}Cl\textsubscript{2} (6.8 mL). To the solution NaH (60% in oil, 154 mg, 3.9 mmol) was added and stirred for 15 min at room temperature. The mixture was treated with triflyl (S)-2-propionic acid methyl ester (989.1 mg, 3.95 mmol) and stirred on at room temperature, then cooled to 0 °C and treated dropwise with a little water until production of gas ceased. The mixture was diluted with CH\textsubscript{2}Cl\textsubscript{2} and then washed with water. The organic layer was collected and treated with Na\textsubscript{2}SO\textsubscript{4}, filtered and the solvent was evaporated. The residue was purified by column chromatography (70% ethyl acetate in \textit{n}-hexane) to give 22 as a white oil (209.4 mg, 50%). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): \delta 7.37-7.32 (15H, m, H-Ar), 7.09 (2H, d, \textit{J}_{\text{ortho}} = 8.5 Hz, H-Ar PMB), 6.84 (2H, d, \textit{J}_{\text{ortho}} = 8.5 Hz, H-Ar PMB), 5.88 (1H, m, OCH\textsubscript{2}CH=CH\textsubscript{2}), 5.29 (1H, d, \textit{J}_{\text{vic}}=17.0 Hz, trans OCH\textsubscript{2}CH=CHH), 5.22 (d, 1H, \textit{J}_{\text{vic}} = 11.0 Hz, cis OCH\textsubscript{2}CH=CHH), 5.18 (d, 1H, \textit{J}_{1,2}=3.5 Hz, H-1\textalpha), 4.84-4.82 (s, 2H, ...
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OCH₃(OMePh), 4.74-4.70 (m, 3H), 4.63-4.44 (m, 7H), 4.23-3.95 (m, 4H), 3.80-3.74 (m, 8H), 3.30-3.18 (m, 4H), 1.18 (3 H, t, J vic = 7.5 Hz, OCH₂CH₂). ¹³C NMR (125 MHz, CDCl₃): δ 175.3 (COOEt), 159.3 (C ipso PMB), 137.9-137.5 (3 C ipso Bn), 133.4 (OCH₂CH=CH₂), 130.0 (C ipso PMB), 129.3-127.6 (C-Ar), 118.2 (OCH₂CH=CH₂), 113.8 (C-Ar), 100.4 (C-1b), 95.1 (C-1a), 83.3, 78.3, 77.0, 76.4, 75.5, 75.3, 75.1, 74.5, 74.4, 73.4, 73.3, 70.4, 69.0, 68.2, 67.5, 66.5 (C-2a, C-3a, C-3b, C-4a, C-4b, C-5a, C-5b, C-6a, C-6b, OCHCH₃, 3 OCH₂Ph, OCH₂PhOMe, OCH₂CH=CH₂), 61.2 (OCH₂CH₃), 60.9, 55.2, 54.6 (C-2a, OCH₃), 18.4 (CH₃CH), 14.0 (OCH₂CH₃). MALDI TOF-MS: calcd for C₄₉H₆₀N₄O₁₃ (m/z), 868.39; found, 891.21 [M+Na]. Anal Calcd for C₄₉H₆₀N₄O₁₃: C, 65.61; H, 6.74; N, 6.25. Found: C, 65.48; H, 6.30; N, 6.27.

Allyl 2-azido-3,6-di-O-benzyl-4-O-pmethoxy benzyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-6-O-benzyl-3-O-((R)-1'-(ethoxycarbonyl)ethyl)-2-deoxy-α-D-glucopyranoside (23): Compound 20 (83.5 mg, 0.099 mmol) was co-evaporated three times with toluene and then under Ar dissolved in CH₂Cl₂ (1 mL). To the solution NaH (60% in oil, 11.8 mg, 2.9 mmol) was added and stirred for 15 min at room temperature. The mixture was treated with triflyl (S)-2-propionic acid methyl ester (48.5 mg, 0.194 mmol) and stirred for 1 h, then cooled to 0 °C and treated dropwise with a little water until production of gas ceased. The mixture was diluted with CH₂Cl₂ and then washed with water. The organic layer was collected and treated with Na₂SO₄, filtered and the solvent was evaporated. The residue was purified by column chromatography (50% ethyl acetate in n-hexane) to give 23 as a white oil (53.9 mg, 60%). [α]D +25.0 (c 2.0 in CH₂Cl₂); ¹H NMR (500 MHz, CDCl₃): δ 7.37-7.30 (15H, m, H-Ar), 7.07 (2 H, d, J ortho = 8.5 Hz, H-Ar PMB), 6.81 (2 H, d, J ortho = 8.5 Hz, H-Ar PMB), 5.86 (1
H, m, OCH₂CH=CH₂), 5.37 (d, 1H, J₁₂ 3.5 Hz, H-1β), 5.25 (1 H, br d, Jvic 17.0 Hz, trans OCH₂CH=CHH), 5.15 (1 H, br d, Jvic 11.0 Hz, cis OCH₂CH=CHH), 4.83 (2 H, s, OCH₂OMePh), 4.73 (1 H, d, Jgem 12.0 Hz, OCH₂PhH), 4.69 (1 H, d, Jgem 10.5 Hz, OCH₂PhH), 4.66 (1 H, q, Jvic 7.5 Hz, CH₂CHO), 4.54 (1 H, d, Jgem 11.5 Hz, OCH₂PhH), 4.50 (1 H, d, Jgem 10.5 Hz, OCH₂PhH), 4.46 (1 H, d, Jgem 12.0 Hz, OCH₂PhH), 4.43 (1 H, d, Jgem 11.5 Hz, OCH₂PhH), 4.25 (1 H, d, J₁₂ 8.0 Hz, H-1α), 4.22 (2 H, m, OCH₂CH₃), 4.11 (1 H, dd, Jvic 13.5 Hz, Jvic 5.5 Hz, OCH₂CH=CH₂), 4.06 (1 H, t, J₂,₃ 10.0 Hz, H-4B), 3.99 (1 H, dd, Jvic 13.5 Hz, Jvic 5.5 Hz, OCH₂CH=CH₂), 3.95 (dd, 1H, J₂,₁ 9.0 Hz, J₂,₁ 3.5 Hz, H-2α), 3.80-3.64 (m, 10H, H-3A, H-4B, H-5A, H-6aA, H-6bA, H-6bB, OCH₃), 3.30 (1 H, t, J₂,₃ 8.0 Hz, H-2B), 3.25 (1 H, t, J₁₄,₁₃ 9.5 Hz, H-3B), 3.16 (1 H, br d, J₅,₄ 9.6 Hz, H-5B), 2.04 (3H, s, CH₃CO), 1.36 (3 H, d, Jvic 7.5 Hz, CH₂CHO), 1.30 (3 H, t, Jvic 7.5 Hz, OCH₂CH₂); ¹³C NMR (125 MHz, CDCl₃): δ 176.3 (C=OEt), 170.1 (NHCOCH₃), 159.3 (C₃-OMe Ph), 137.9-137.7 (3 C₃-OMe Ph), 134.1 (OCH₂CH=CH₂), 130.2 (C₃-OMe Ph), 129.3-127.6 (C-Ar), 116.9 (OCH₂CH=CH₂), 113.8 (C-Ar), 100.6 (C-1B), 95.9 (C-1A), 83.3, 77.3, 76.8, 75.4, 75.2, 75.1, 74.5, 74.4, 73.4, 73.1, 70.6, 68.7, 68.3, 67.8, 66.7 (C-2A, C-3A, C-3B, C-4A, C-4B, C-5A, C-5B, C-6A, C-6B, OCH₃), 3 OCH₃Ph, OCH₃PhOMe, OCH₂CH=CH₂), 61.2 (OCH₂CH₃), 55.2, 54.4 (C-2A, OCH₃), 23.1 (CH₃CO), 18.5 (CH₃CH₂), 14.1 (OCH₂CH₃). MALDI TOF-MS: calcd for C₅₁H₆₂N₄O₁₃ (m/z), 938.43 [M+H]⁺; found, 961.21 [M+Na]. Anal Calcd for C₅₁H₆₂N₄O₁₃: C, 65.23; H, 6.65; N, 5.97. Found: C, 65.35; H, 6.55; N, 6.01.

Propyl 2-amino-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-((R)-1′-(ethoxycarbonyl)ethyl)-2-deoxy-α-D-glucopyranoside (24): Compound 23 (24.8 mg, 0.026 mmol) was dissolved in 2.2:1 v/v MeOH/CH₂Cl₂ (2.4 mL).Pd(OH)₂ (26.1 mg)
and AcOH (165 μL, 2.88 mmol) were then added. The solution was stirred in H\(_2\) atmosphere for 10 h. The mixture was then filtered on a Celite pad and the solvent was evaporated to give 24 as a white solid (8.6 mg, 65%). \(^1\)H NMR (500 MHz, D\(_2\)O): \(\delta\) 4.86 (d, 1H, \(J_{1-2}=3\) Hz, H-1\(_A\)), 4.44 (q, 1H, \(J_{gem}=7\) Hz, CH(CH\(_3\))COOEt), 4.16-4.05 (m, 2H), 3.96-3.13 (m, 11H), 3.00 (t, \(J_{2-3}=J_{1-2}=8.5\) Hz, H-2\(_B\)), 1.92 (s, 3H, COCH\(_3\)), 1.46 (m, 2H, OCH\(_2\)CH\(_2\)CH\(_3\)), 1.31 (d, 3H, \(J_{gem}=7\) Hz, CH(CH\(_3\))COOEt), 1.24 (s, 3H, COCH\(_3\)), 0.77 (t, 3H, OCH\(_2\)CH\(_2\)CH\(_3\)). MALDI TOF-MS: calced for C\(_{22}\)H\(_{40}\)N\(_2\)O\(_{12}\) (m/z), 524.56; found, 546.28 [M+Na\(^+\)]. Anal Calced for C\(_{22}\)H\(_{40}\)N\(_2\)O\(_{12}\): C, 50.37; H, 7.59; N, 5.34. Found: C, 50.46; H, 7.73; N, 5.22.

Propyl 2-acetamido-2-deoxy-\(\beta\)-D-glucopyranosyl-(1\(\rightarrow\)4)- 2-acetamido-3-\(\alpha\)-(\((R)\)-1\(\prime\)-(ethoxycarbonyl)ethyl)-2-deoxy-\(\alpha\)-D-glucopyranoside (25): Compound 24 (3.9 mg, 7.43 μmol) was dissolved in 9:1 v/v MeOH/Ac\(_2\)O and the mixture was stirred at room temperature for 2 h. The solvent was evaporated and 25 could be obtained as a white solid (2.8 mg, 95%). \(^1\)H NMR (500 MHz, D\(_2\)O): \(\delta\) 4.85 (d, 1H, \(J_{1-2}=3\) Hz, H-1\(_A\)), 4.55 (q, 1H, \(J_{gem}=7\) Hz, CH(CH\(_3\))COOEt), 4.39 (d, 1H, \(J_{1-2}=8.5\) Hz, H-1\(_B\)), 4.11 (q, 2H, \(J_{gem}=7\) Hz, COOCH\(_2\)CH\(_3\)), 3.82 (dd, 1H, \(J_{6a-5a}=2\) Hz, \(J_{gem}=12.5\) Hz), 3.75-3.41 (m, 10H), 3.32-3.29 (m, 3H), 1.92 (s, 3H, COCH\(_3\)), 1.91 (s, 3H, COCH\(_3\)), 1.44 (m, 2H, OCH\(_2\)CH\(_2\)CH\(_3\)), 1.33 (d, 3H, \(J_{gem}=7\) Hz, CH(CH\(_3\))COOEt), 1.16 (t, 3H, COOCH\(_3\)CH\(_3\)), 0.76 (t, 3H, \(J_{gem}=7\) Hz, OCH\(_2\)CH\(_2\)CH\(_3\)). MALDI TOF-MS: calced for C\(_{24}\)H\(_{42}\)N\(_2\)O\(_{13}\) (m/z), 567.27 [M+H\(^+\)]; found, 590.32 [M+Na\(^+\)]. Anal Calced for C\(_{24}\)H\(_{42}\)N\(_2\)O\(_{13}\): C, 50.88; H, 7.47; N, 4.94. Found: C, 50.92 H, 7.53; N, 4.88.
Chapter 4: Synthesis of Urea Neosaccharides

4.1 Introduction

Although the preparation of urea and its derivatives dates back to the early history of organic chemistry, the enormous chemical and biological potential of the ureido linkage has been largely exploited only in the last decades: urea itself is capable of forming inclusion complexes with long-chain alkanes and some functionalized molecules. In such complexes, the urea molecules self-assemble through intramolecular hydrogen bonds giving rise to a helical lattice into which the guest compounds can be oriented and accommodated. Urea moieties also act as strong hydrogen-bond donors and acceptors, and have been frequently used as functional groups for the formation of supramolecular architectures. Substituted ureas and bis-ureas have also been shown to give gels with organic liquids and water, and in some cases gels are also thermally reversible and require very low concentrations.

In the last decade, growing and growing efforts were also dedicated to the obtainment of ureido sugar mimics (a new class of “pseudosaccharides”) which have the acid-labile glycosidic bonds substituted with ureido-linkages (in this case the incorporation of a pseudo-amide intersaccharide linkage has an additional feature because this functional groups is isosteric with phosphodiester group occurring in

Some carbohydrate-based ureas exhibit in fact relevant biological properties such as the antibiotics SF-1993, CV-1, and the family of glycocinnamoylspermidines (Fig. 4.1, 1-4). In 2005, Ichikawa and co-workers described the total synthesis of the glycocinnasperemicin D, a broad spectrum antibiotic against Gram-negative organisms, which contains two highly functionalised aminosugars connected by an urea linkage. While exploring the synthesis of this target molecule, this group established a new method for the stereoselective synthesis of novel β-urea-linked pseudooligosaccharides, which involves the reaction of amine-glycoside with Steyemark-type gluco- and galactopyranosyl oxazolidinones. Another example of

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natural disaccharide analogue containing a pseudoamide linkage is trehazolin 7,\textsuperscript{229} which possesses a cyclic isourea functionality between the α-D-glucose and aminocyclopentitol rings. Trehazolin is a potent trehalase inhibitor in which an aminocyclopentitol ring replaces the glucopyranosyl cation postulated as an intermediate in the enzymatic hydrolysis of α,α\textsuperscript{-}trehalose. Although several synthetic methodologies had been successfully applied to prepare this inhibitor, Chiara and co-workers have described a novel complementary approach in which the oxazoline ring is generated by S\textsubscript{N}2 nucleophilic displacement reaction from the β-hydroxyurea 5 via the triflate intermediate 6 (Fig. 4.2).\textsuperscript{230}

\[ \begin{align*}
\text{5} & \xrightarrow{\text{a}} \text{6} \\
\text{7} & \xrightarrow{\text{b}} \text{5}
\end{align*} \]

**Fig. 4.242: Synthesis of trehazoline, via triflate, from β-hydroxyurea:** *Reagents and conditions:* (a) Tf\textsubscript{2}O, py, CH\textsubscript{2}Cl\textsubscript{2}, from -20°C to rt; (b) i. H\textsubscript{2}, Pd(OH)\textsubscript{2}/C, EtOH, TFA; ii. 2N HCl, rt.

Most part of urea-linked pseudosaccharides presents a tether involving at least one anomeric position; there are two general strategies to access these oligosaccharide...


mimics: i) nucleophilic addition of sugar derivatives to carbohydrate isocyanate and ii) conversion of sugar azides into glycosyl carbodiimides via a tandem Staudinger-aza-Wittig type reaction with triphenylphosphine and an isothiocyanate, followed by the addition of a nucleophile (generally $H_2O$). The experimental difficulties in handling isocyanates have led to the preferential use of sugar carbodiimides as key intermediates for the preparation of glycosylureido sugars (FIG. 4.3).

![FIG. 4.3: Approaches to access pseudoamyde-type oligosaccaride mimics.](image)

A particular area of interest in glycomimetics is that of neosaccharides, that are oligosaccharides linked together without using the anomeric centre. To the best of our knowledge, urea-linked neosaccharides were reported only twice. Herein, it is reported an access to a novel class of neosaccharides starting from an unprecedented urea-linked glucosamine dimer obtained from amino-alcohol through a modification of the known oxazolidinone closure reaction.

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4.2 Results and Discussion

As mentioned in Chapter 3, for the synthesis of peptidoglycan fragments it was firstly proposed to use as glycosyl acceptor a derivative of glucosamine in which the hydroxyl group at position 3 and the amino group at position 2 were protected with a 2,3 oxazolidinone protecting group. A procedure alternative to that reported in Chapter 3 for the installation of the oxazolidinone protecting group (triphosgene, base) is based on the reaction between a 2,3-aminoalcohol sugar derivative with 4-nitrophenyl chloroformate (NPCC) at 0°C for 3 hours, producing a mixture of the desired oxazolidinone and uncyclised carbamate, that is converted to the fully protected material by an additional step with Amberlyst IR-120 Na ion exchange resin. Performing the reaction between the amino alcohol 8 and NPCC with shorter times (30 min instead of 180 min) an uncyclised carbamate 9 was exclusively obtained. In order to have the desired oxazolidinone derivative, it was thought to treat 9 with a strong base (such as sodium hydride) in N,N-dimethylformamide but the product obtained was unexpectedly constituted by two oxazolidinone protected glucosamine derivatives held together by a ureido-linkage (10). This unprecedented system has been then largely studied and the

![Diagram](image_url)

**Fig. 4.4: Reagents and Conditions:** (a) $p$NO$_2$PhOCOCl, NaHCO$_3$, 3:2 v/v CH$_3$CN/H$_2$O, 0°C; (b) NaH, DMF.

methodology has been applied also for the synthesis of galactose ureido-sugars. The results obtained are described in the next pages of this chapter.

### 4.2.1 Glucosamine dimerization

It was firstly studied the behavior of the reaction of 8 – easily available from N-acetyl glucosamine - with 4-nitrophenyl chloroformate in dependence of the temperature. By performing the reaction at 40°C, no uncyclised carbamate 9 was detected by $^1$H NMR analysis of the crude mixture and 2,3-oxazolidinone 11 was recovered in 80% isolated yield. On the contrary, at 0°C only uncyclised carbamate 9 was obtained when reaction was conducted for short times (Fig. 4.5 and Table 4.1).

Compound 9 was recovered by simple extraction and then treated with NaH in DMF at 30°C. No oxazolidinone 2 was recovered, whereas a new compound was isolated as major product (62% yield over two steps) together with N-aryloxycarbonyl-oxazolidinone 12 (10% from 8). $^1$H NMR analysis of the unknown compound presented a single set of carbinolic signals, with H-2 and H-3 ones downfield shifted at 3.99 and 4.94 ppm, respectively. $^{13}$C NMR spectrum was characterized by two signals at 150.4 and 150.0 ppm, with the first one approximately double than the second. MALDI-MS spectrum gave a peak at 715 m/z ([M+Na$^+$]). According to these data, two $C_2$-symmetric dimeric structures could be hypothesized, with two glucosamines linked by an ureido (structure 10) or an ureido and two carbamate bridges (13). To assign an unequivocal structure to the new compound, the benzylidene rings were cleaved with CSA in dioxane/water to afford the water-soluble tetraol 14/15 (73% yield), which was subjected to detailed NMR analysis and molecular mechanics and dynamics calculations.

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FIG. 4.5: Synthesis of urea-linked neodisaccharide from amino alcohol 8. Reagents and conditions: (a) \( p-\text{NO}_2\text{PhOCOCl} \), 3:2 CH\(_3\)CN/H\(_2\)O, 40°C, 80%; (b) \( p-\text{NO}_2\text{PhOCOCl} \), 3:2 CH\(_3\)CN/H\(_2\)O, 0°C; (c) NaH, DMF, 30°C, 72% from 8, (10/13:12=6.2:1); (d) CSA, 4:1 dioxane/H\(_2\)O, 60°C, 73%.

Table 4.1: Oxazolidinone 11 versus uncyclized carbamate 9 formation from 8 with NPCC (5 equiv.) and NaHCO\(_3\) (5 equiv.) in 2:3 (v/v) H\(_2\)O/CH\(_3\)CN.

<table>
<thead>
<tr>
<th>entry</th>
<th>T</th>
<th>time</th>
<th>11/9*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40°C</td>
<td>30 min</td>
<td>100:0</td>
</tr>
<tr>
<td>2</td>
<td>20°C</td>
<td>3 hours</td>
<td>19:81</td>
</tr>
<tr>
<td>3</td>
<td>0°C</td>
<td>3 hours</td>
<td>17:83</td>
</tr>
<tr>
<td>4</td>
<td>0°C</td>
<td>2 hours</td>
<td>6:94</td>
</tr>
<tr>
<td>5</td>
<td>0°C</td>
<td>50 min</td>
<td>0:100</td>
</tr>
<tr>
<td>6</td>
<td>0°C</td>
<td>30 min</td>
<td>0:100</td>
</tr>
</tbody>
</table>

*Percent molar ratio determined by \(^1\)H NMR analysis of the crude mixture
in order to discriminate between the two possible structures and, in addition, to have an evaluation of the conformational behavior of the novel neodi saccharide in aqueous solution. The molecular mechanics and dynamics calculations have been carried out by Dr Alba Silipo of the Department of Organic Chemistry and Biochemistry of the University of Naples “Federico II”. No specific details in the choice of parameters and force field used will be given here, but it is worth noting that the experimental $\tilde{J}_{\text{C-H}}$ measured in NMR experiments were in good agreement with the calculated $\tilde{J}_{\text{C-H}}$ for 14. The formation of 15 was therefore excluded because the calculated data were in discordance with those measured in the NMR experiments (Table 4.2).

Table 4.2: Experimental and theoretical values for selected $\tilde{J}_{\text{C,H}}$ coupling constants [Hz] of structures 14 and 15.

<table>
<thead>
<tr>
<th>atom pairs</th>
<th>compound 14/15 (NMR data)</th>
<th>compound 14 (MD data)$^a$</th>
<th>compound 15 (MD data)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\tilde{J}_{\text{C,amido, H-2}}$</td>
<td>1.6/1.7$^b$</td>
<td>1.6</td>
<td>5.1</td>
</tr>
<tr>
<td>$\tilde{J}_{\text{C, carbamate, H-2}}$</td>
<td>absent</td>
<td>0.9</td>
<td>6.5</td>
</tr>
<tr>
<td>$\tilde{J}_{\text{C, carbamate, H-3}}$</td>
<td>absent</td>
<td>0.9</td>
<td>7.7</td>
</tr>
</tbody>
</table>

$^a$The values correspond to the weighted average $\tilde{J}_{\text{C,H}}$ coupling constants calculated from the molecular dynamics simulation.

$^b$The two values correspond to the $\tilde{J}_{\text{C,H}}$ coupling constants obtained from HSQC and 1D-MBC experiments, respectively.

4.2.2 Mechanism Investigation

The dimerization process of aminoalcohol 8 into 10 is strictly dependent on the presence of excess NPCC in the reaction mixture. Indeed, when uncyclised carbamate 9 was recovered pure by column chromatography (87% yield from 8) and then subjected to NaH/DMF treatment, no dimer 10 was detected in this case. The reaction produced

$^{238}$ For the complete treating of the molecular mechanics and dynamic calculations, see Ref. 236.
exclusively oxazolidinone 11 in 91% yield, accordingly with known similar transformations. On the basis of this result a mechanism was firstly hypothesized (Fig. 4.6), in which, after the initial formation of 11 through reactive isocyanate 17, N-

![Diagram](image_url)

**FIG. 4.6**: Proposed mechanism for the formation of 10.

aryloxy carbonyl-oxazolidinone 12 is produced and then coupled to 9 (FIG. 4.6). Nonetheless, by mixing pure 9 and 12 in the presence of NaH in DMF, no dimer species were detected. Moreover, by treating pure 11 under dimerization conditions in the presence of excess NPCC, only compound 12 was recovered together with starting oxazolidinone. These results suggested that this pathway had to be discarded. Instead, the formation of derivative 16 was proposed as a pathway in competition with oxazolidinone formation. Compound 16 could then be converted into isocyanate 18, which cannot give any intramolecular reaction as for 17. On the contrary, it can be subjected to nucleophilic addition by oxazolidinone 11 to a dimer specie, which could easily react further to give final product 10. Indeed when pure compound 16 was mixed with 11 in the presence of excess NaH, dimer 10 was obtained exclusively. However, a deeper study is in progress to confirm in details the proposed mechanism.

4.2.3 Galactosamine dimerization

The methodology developed for the synthesis of the glucosamine neodisaccharide 10 has been also applied for the synthesis of an analogous galactosamine neodisaccharide. The aminoalcohol 21 has been synthesized using a known procedure from N-acetyl glucosamine and then the reaction with excess of NPCC was performed at 0°C for 50 min and after a simple extractive work-up the desired uncyclised carbamate 22 (FIG. 4.7) was obtained together with not reacted NPCC as it could be demonstrated by 1H-NMR (the amount of the NPCC were dependent on the number of equivalents used in the reaction). On the crude reaction mixture several reactions (Table 4.3) were performed in order to have the urea-linked galactose neodisaccharide 25. It is worth

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240 Compound 16 was obtained by reacting 9 with DMAP and excess NPCC in DMF at 5°C.
Fig. 4.7: Synthesis of galctosamine derived neocaccharide. Reagents and conditions: (a) \( p\)-NO\(_2\)PhOCOCl, 3:2 CH\(_3\)CN/H\(_2\)O; (b) NaH, DMF.

Table 4.3: Screening of the reaction conditions for the synthesis of 25.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Concentration of 22</th>
<th>( p)-NO(_2)PhOCOCl eq.</th>
<th>Base (eq.)</th>
<th>T (°C)</th>
<th>23</th>
<th>24</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.16 M</td>
<td>4</td>
<td>NaH (5)</td>
<td>0</td>
<td>33%</td>
<td>46%</td>
<td>8%</td>
</tr>
<tr>
<td>2</td>
<td>0.16 M</td>
<td>4</td>
<td>NaH (5)</td>
<td>30</td>
<td>43%</td>
<td>30%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.16 M</td>
<td>4</td>
<td>NaH (5)</td>
<td>50</td>
<td>27%</td>
<td>43%</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.16 M</td>
<td>4</td>
<td>NaH (5)</td>
<td>70</td>
<td>27%</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.05 M</td>
<td>4</td>
<td>NaH (5)</td>
<td>30</td>
<td>64%</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.46 M</td>
<td>4</td>
<td>NaH (5)</td>
<td>30</td>
<td>13%</td>
<td>78%</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.46 M</td>
<td>4</td>
<td>NaH (5)</td>
<td>50</td>
<td>10%</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.46 M</td>
<td>0</td>
<td>NaH (5)</td>
<td>30</td>
<td>90%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.46 M</td>
<td>1.01</td>
<td>NaH (5)</td>
<td>0</td>
<td>85%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.46 M</td>
<td>4</td>
<td>DBU (5)</td>
<td>30</td>
<td>20%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( ^{\dagger} \) the equivalents of \( p\)-NO\(_2\)PhOCOCl have been verified by \(^{1}H\)-NMR integration.
noting that the reaction conditions that lead to the formation of the glucosamine ureido-
neodisaccharide gave 25 in only 30% yield together with 43% of 24 (entry 2). Thus, a
large screening of the reaction conditions was mandatory by varying the temperature,
the base, the concentration of both NPCC and of the uncyclised carbamate 31 in the
dimerization reaction. As it is possible to see from the Table 4.3, the best results for the
synthesis of 25 were obtained at 30°C and with a more concentrated solution of the
substrate (entry 6, Table 4.3) with respect to glucosamine dimerization reaction (in this
case the preferential formation of the dimeric structure is probably due to the more
favorite intermolecular process respect to the intramolecular cyclization for the closure
of the oxazolidinone ring); as it could be easily expected performing the reaction in a
more diluted solution (entry 5) the N-aryloxy-oxazolidinone-derivative 24 is
preferentially formed. In accordance with the proposed mechanism, also in the case of
galactosamine derived neosaccharides the role of NPCC seems to be crucial for the
formation of the galactosamine dimer: in entries 8 and 9, in which no NPCC or a small
excess of NPCC was used, dimer product 25 could be detected in 0% or 17% yield,
respectively. The role of temperature is also determinant for the obtainment of 25: in
general from entries 1, 2, 3 and 4 it is clear that increasing the temperature the
intermolecular process is slightly favoured (the yield of 25 also increases). It is worth
noting that a simultaneous increase of both concentration of 22 and temperature (entry
7) has an adverse effect on the yield of 25: the reasons of the mismatching behaviour of
this two factors is not yet fully understood and will be object of further investigations.
4.2.4 Chemoselective Reactions on the Oxazolidinone-Urea-Oxazolidinone System

A case study of selective reactions on the oxazolidinone-urea-oxazolidinone system of dimer 10 was then pursued. To the best of our knowledge, this system is unprecedented. Mild conditions were searched for the chemoselective cleavage of one or both oxazolidinone cycles with respect to the ureido bridge or vice versa. Solvolysis of 10 was conducted under several conditions, as indicated Fig. 4.8. Reaction conditions employed for the chemoselective deprotection of a N-acetyl-2,3-N,O-oxazolidinone derivative of glucosamine \(^{242}\) gave unsatisfying results on 10 (Entries 1-3). Interestingly, chemoselective cleavage of one or both carbamates with respect to the ureido bridge was accomplished by employing mild Et\(_3\)N-mediated hydrolysis or methanolysis (entries 4-6). A very interesting compound obtained by mild solvolysis was alcohol 26a (entry 5), which could be used as glycosyl acceptor to access higher neooligosaccharides. For example, a glycosylation reaction between 26a and fucosyl donor 28 \(^{150}\) afforded neotrisaccharide 29 (76\%), that was deprotected in two steps (Fig. 4.9). A first

chemoselective cleavage of the residual oxazolidinone ring was possible under the conditions developed above. Indeed, hydrolysis in 6:2:1 v/v dioxane/water/Et₃N gave alcohol 30 (58%). Then, a transfer hydrogenation under Perlin conditions furnished water-soluble urea-linked neotrisaccharide 31 in 72% yield.

Alcohol 26a was an useful building block to access also a first representative example of a novel class of carbamate-linked macrocycles analogues to cyclodextrins. Indeed, when treated with DBU in DMF, 26a gave carbamate-linked macrocycle 32 (43%; 51% based on recovered starting material) (FIG. 4.10). The detection of oxazolidinone 2 as the sole byproduct in the crude reaction mixture suggested that the mechanism could

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proceed through the attack of the alcoholate ion on the ureido carbonyl group, giving the macrocycle 27 after a subsequent rearrangement of the amine intermediate (path a,a) or directly the oxazolidinone 11 (path b). The latter compound could be obtained also through the amine intermediate (path a,b). Benzylidene cleavage on 32 with CSA in 4:1 v/v dioxane/water at 60°C afforded the water-soluble macrocycle 33 (98%).

Compound 33 has been fully characterized and through molecular mechanics and dynamic calculation it has been shown that this cyclodextrin mimic is characterized by an elliptic shape in which the major axis has an average length of 4.43 Å, the minor one of 3.03 Å (FIG. 4.10).

![Synthesis of carbamate-linked cyclic neosaccharide 33. Reaction and conditions: (a), DBU, DMF, 50°C; (b) CSA, T=60°C, 98%.](image)

**Fig. 4.10:** View of the cyclic neodisaccharide 33.
4.2.5 Self-assembling properties of urea derivatives

Urea-urea hydrogen bonding motif has already proven to be a reliable and predictable self-assembling motif. This versatile urea-urea motif has been employed to direct the formation of sheets, fibers, polymers, gels and two-dimensional molecular solids. The properties of self-assembling have been also valuated for urea-linked disaccharides 27a and 34 (the latter was obtained using on neodisaccharide 25 the same reaction conditions used for 27a, Fig. 4.12).

![Image of compounds 25 and 34](image)

**Fig. 4.12: Synthesis of galactosamine urea-linked disaccharide**

$^1$H-NMR experiments at different conditions have been conducted on compounds 27a and 34 to investigate if these compounds could self-assemble by measuring the variation

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of the chemical shift on the NH of the urea moieties in CDCl$_3$. In the graphs below is reported the variation of the chemical shift on the NH in function of the logarithm of the concentration (the experiments have been made by successive dilutions of a saturated solution of 27a and 34). As it is possible to see in Fig. 4.13 with both urea-derivatives, at low concentration there is plateau, which suggests that at these concentrations the molecules are un-associated monomers. Increasing the concentration of the urea-derivatives the variation of the chemical shift is more pronounced for the GalN-urea derivative respect to the GlcN-urea derivative as it can be detected both through the trend of the blue rhombs and directly from the analysis of the $^1$H-NMR spectra (with the arrow is represented the signal of NH-urea, Fig. 4.14). The absence of a plateau is clearly evident for 34 also at higher concentrations, indicating that the association did not reach the equilibrium even in saturated solution. Although the different behaviour of the two urea derivatives 27a and 34 is not fully understood, it is probable that the difference between the value of chemical shift for 27a and 34 and the different increase of chemical shift of NHs in function of the concentration is due to the possibility of 27a to form intramolecular hydrogen bonding in the unassociated monomers.
monomer at low concentrations (the NH in 27a are more downfield than those of 34). Further experiments are in progress to clarify the different behaviour of these urea-derivatives through NMR, IR, molecular mechanics and dynamic calculations.

![Chemical shift of the NH of the urea derivative 34. Both spectra were recorded on a 500MHz instrument in CDCl₃ at 297K. A) [34]=76.7 mM; B) [34]=2.29 mM.]

Fig. 4.14: Chemical shift of the NH of the urea derivative 34. Both spectra were recorded on a 500MHz instrument in CDCl₃ at 297K. A) [34]=76.7 mM; B) [34]=2.29 mM.

4.3 Conclusions

In conclusion, it has been developed a new synthetic methodology for the synthesis of a novel urea-tethered glucosamine and galactosamine neodisaccharide building block by dimerization of a simple 2,3-amino-alcohol monomer of gluco and galactosamine through a modification of standard oxazolidinone closure reaction. The glucosamine dimer was fully characterized by NMR spectroscopy as well as molecular mechanics and dynamics calculations. The behavior of the oxazolidinone-urea-oxazolidinone moiety of the dimer under mild solvolysis conditions was studied. The chemoselective cleavage of only one oxazolidinone of the glucosamine
neodisaccharide afforded an alcohol building block, that was used for the synthesis of a higher neoligosaccharide as well as a carbamate-bridged cyclic neosaccharide. The possibility of the urea-linked neodisaccharides to form hydrogen-bonding interactions has been also evaluated by measuring the NMR chemical shift of the NH proton of the sugar moieties.

4.4 Experimental Section

$^1$H and $^{13}$C NMR spectra were recorded on 200, 400 or 500 MHz instruments in CDCl$_3$ (CHCl$_3$ as internal standard, $^1$H: CHCl$_3$ at $\delta = 7.26$; $^{13}$C: CDCl$_3$ at $\delta = 77.0$) Compounds 7, 19 and 21 were analyzed in D$_2$O (acetone as internal standard, $^1$H: (CH$_3$)$_2$CO at $\delta$ 2.22; $^{13}$C: (CH$_3$)$_2$CO at $\delta$ 31.0). Extensive NMR analysis on compounds 7 and 21 were made on a 600 MHz instrument equipped with a cryo probe. Positive MALDI-MS spectra were recorded on a MALDI-TOF mass spectrometer in the positive mode: compounds were dissolved in CH$_3$CN at a concentration of 1 mg/mL and one microliter of these solutions was mixed with one microliter of a 20 mg/mL solution of 2,5-dihydroxybenzoic acid in 7:3 CH$_3$CN/0.1 M trifluoroacetic acid. Analytical thin layer chromatographies (TLC) were performed on aluminium plates precoated with silica gel as the adsorbent. The plates were developed with 5% H$_2$SO$_4$ ethanolic solution and then heating to 130°C. Flash chromatographies were performed on silica gel (63-200 mesh).

**Allyl 4,6-O-benzylidene-2,3-N,$\alpha$-carbonyl-2-deoxy-$\alpha$-D-glucopyranoside (11).**

Compound 8 (95.4 mg, 0.310 mmol) was suspended in 2:1 v/v water/CH$_3$CN (1.12 mL) and then heated to 40°C. NaHCO$_3$ (128 mg, 1.52 mmol) and then a solution of 4-nitrophenyl chloroformate (314 mg, 1.56 mmol) in CH$_3$CN (750 $\mu$L) were added. After
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30 min stirring at 40°C, the mixture was diluted with ethyl acetate (50 mL) and washed with 1M NaHCO₃. The organic layer was collected, dried over anhydrous Na₂SO₄ and concentrated. A flash-chromatography (silica gel; 5:1 to 1:1 petroleum ether-ethyl acetate) afforded 11 (82.3 mg, 80%) as a white powder. [α]D +33 (c 0.3; CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): δ 7.51-7.36 (m, 5H, H-Ar), 5.91 (m, 1H, OCH₂CH=CH₂), 5.62 (s, 1H, CHPh), 5.35 (ddd, 1H, Jvic 17.2 Hz, JH,H 3.0 Hz, Jgem 1.5 Hz, trans OCH₂CH=CH₂), 5.28 (dd, 1H, Jvic 10.4 Hz, Jgem 1.5 Hz, cis OCH₂CH=CH₂), 5.15 (d, 1H, J1,2 2.9 Hz, H-1), 5.07 (bs, 1H, NH), 4.84 (dd, 1H, J1,2=11.3 Hz, J3,4=10.2 Hz, H-3), 4.29 (m, 2H, H-6a, OCH₂HCH=CH₂), 4.09 (m, 2H, H-4, OCH₂HCH=CH₂), 3.90 (m, 2H, H-5, H-6b), 3.74 (dd, 1H, J2,3 11.3 Hz, J2,1 2.9 Hz, H-2); ¹³C NMR (125 MHz, CDCl₃): δ 159.1 (NCOO), 136.5 (Cipso), 132.8 (OCH₂CH=CH₂), 129.2, 128.3, 126.2 (C-Ar), 118.5 (OCH₂CH=CH₂), 101.4 (CHPh), 95.5 (C-1), 80.1, 75.6, 69.1, 68.5, 65.5, 59.5 (C-2, C-3, C-4, C-5, C-6, OCH₂CH=CH₂). MALDI TOF-MS: calcd for C₁₇H₁₉N₆O₆ (m/z), 333.12; found, 334.16 [M+H]+. Anal Calcd for C₁₇H₁₉N₆O₆: C, 61.26; H, 5.75; N, 4.20. Found: C, 61.06; H, 5.58; N 4.11.

Allyl 4,6-O-benzylidene-2,3-N,O-carbonyl-2-deoxy-2-(p-nitrophenoxycarbonylamino)-x-D-glucopyranoside (12) and N,N'-Bis(1-O-allyl-4,6-O-benzylidene-2,3-N,O-carbonyl-2-deoxy-x-D-glucopyranos-2-yl)urea (10).

Compound 8 (430 mg, 1.40 mmol) was suspended in 2:1 v/v water/CH₃CN (6.0 mL) and then cooled to 0°C. NaHCO₃ (585 mg, 6.96 mmol) and then a solution of 4-nitrophenyl chloroformate (1.83 g, 6.80 mmol) in CH₃CN (4.0 mL) were added. After 45 min stirring at 0°C, the mixture was diluted with ethyl acetate (300 mL) and washed
with 1M NaHCO$_3$. The organic layer was collected, dried over anhydrous Na$_2$SO$_4$ and concentrated to give a white gummy solid.

To obtain pure allyl 4,6-O-benzylidene-2-deoxy-2-(p-nitro-phenoxy carbonylamino)-α-D-glucopyranoside 9 for analytical purposes, a flash-chromatography (silica gel; 15:1 to 2:1 petroleum ether-ethyl acetate) was performed: [α]$_D$ +22.4 (c 1.4; CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$): δ 8.25 (d, 2H, $J_{3',2'}$ 8.8 Hz, 2 H-3’ pNO$_2$-Ar), 7.51-7.35 (m, 7H, H-Ar benzylidene, 2 H-2’ pNO$_2$-Ar), 5.94 (m, 1H, OCH$_2$CH=CH$_2$), 5.58 (s, 1H, CHPh), 5.47 (d, 1H, $J_{H,NH}$ 7.8 Hz, NH), 5.35 (bd, 1H, $J_{vic}$ 17.2 Hz, trans OCH$_2$CH=CH$_2$), 5.29 (bd, 1H, $J_{vic}$ 10.4 Hz, cis OCH$_2$CH=CH$_2$), 4.25 (dd, 1H, $J_{gem}$ 12.3 Hz, $J_{vic}$ 4.7 Hz, OCHHCH=CH$_2$), 4.06 (dd, 1H, $J_{gem}$ 12.3 Hz, $J_{vic}$ 4.7 Hz, OCHHCH=CH$_2$), 4.02 (m, 2H, H-2, H-3), 3.89 (dr, 1H, $J_{3a}=J_{5b}$ 10.2 Hz, $J_{5a}$ 4.7 Hz, H-5), 3.78 (t, 1H, $J_{gem}=J_{6b,5}$ 10.2 Hz, H-6b), 2.58 (bs, 1H, OH); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 155.7 (NCOO), 153.3, 145.1, 136.9 (3 C$_{ipso}$), 133.1 (OCH$_2$CH=CH$_2$), 129.4, 128.4, 126.2, 125.1, 121.9 (C-Ar), 118.4 (OCH$_2$CH=CH$_2$), 102.0 (CHPh), 97.0 (C-1), 81.8, 70.2, 68.8, 68.7, 62.7, 55.7 (C-2, C-3, C-4, C-5, C-6, OCH$_2$CH=CH$_2$). MALDI TOF-MS: calcd for C$_{23}$H$_{24}$N$_2$O$_9$ (m/z), 472.15; found, 495.28 [M+Na]$^+$. Anal Calcd for C$_{23}$H$_{24}$N$_2$O$_9$: C, 58.47; H, 5.12; N, 5.93. Found: C, 58.30; H, 5.01; N 5.85.

The crude gummy solid was dissolved in DMF (6.4 mL) and heated to 30°C. NaH (60% dispersion in oil) (261 mg, 6.53 mmol) was then added portionwise to avoid a sudden overheating. The yellow mixture was stirred for 45 min at 30°C, then cooled to 0°C and treated dropwise with some mL of water until production of gas ceased. The mixture was diluted with CH$_2$Cl$_2$ (150 mL) and washed with water. The organic layer was collected, dried and concentrated. The residue was subjected to flash-chromatography.
(silica gel; 6:1 to 2:3 hexane-ethyl acetate) to give, as first eluted compound, 12 (69.0 mg, 10%) as white amorphous crystals. \( [\alpha]_D +64.7 \) (c 1.0; CH\(_2\)Cl\(_2\)); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 8.29 (d, 2H, \( J_{3',2'} = 9.1 \) Hz, 2 H-3', \( p\)NO\(_2\)-Ar), 7.51-7.38 (m, 7H, benzylidene, 2 H-2', \( p\)NO\(_2\)-Ar), 5.88 (m, 1H, OCH\(_2\)CH=CH\(_2\)), 5.64 (s, 1H, CHPh), 5.63 (d, 1H, \( J_{1,2} = 2.7 \) Hz, H-1), 5.32 (bd, 1H, \( J_{\text{vic}} = 17.2 \) Hz, \( \text{trans} \) OCH\(_2\)CH=CH\(_2\)), 5.22 (bd, 1H, \( J_{\text{vic}} = 10.3 \) Hz, \( \text{cis} \) OCH\(_2\)CH=CH\(_2\)), 4.90 (t, 1H, \( J_{3,2} = J_{3,4} = 11.0 \) Hz, H-3), 4.30 (m, 2H, H-6a, OCH\(_2\)CH=CH\(_2\)), 4.11 (m, 2H, H-4, OCH\(_2\)CH=CH\(_2\)), 4.05 (dd, 1H, \( J_{2,3} = J_{2,1} = 2.7 \) Hz, H-2), 3.92 (m, 2H, H-5, H-6b); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 154.3, 145.8 (2 C\(_{\text{ipso}}\)), 150.3, 148.9 (2 N\(_{\text{NCOO}}\)), 136.2 (C\(_{\text{ipso}}\) benzylidene), 132.7 (OCH\(_2\)CH=CH\(_2\)), 129.3, 128.4, 126.1, 125.4, 122.2 (C-Ar), 118.8 (OCH\(_2\)CH=CH\(_2\)), 101.5 (CHPh), 95.5 (C-1), 79.5, 74.0, 69.7, 68.4, 65.4, 60.9 (C-2, C-3, C-4, C-5, C-6, OCH\(_2\)CH=CH\(_2\)). MALDI TOF-MS: unstable. Anal Calcd for C\(_{24}\)H\(_{22}\)N\(_2\)O\(_{10}\): C, 57.83; H, 4.45; N, 5.62. Found: C, 57.70; H, 4.40; N, 5.56.

Second eluted compound 10 (299 mg, 62%) was recovered as a white powder. \( [\alpha]_D +60 \) (c 0.9; CH\(_2\)Cl\(_2\)); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 7.50-7.36 (m, 5H, H-Ar), 5.85 (m, 1H, OCH\(_2\)CH=CH\(_2\)), 5.61 (s, 1H, CHPh), 5.53 (d, 1H, \( J_{1,2} = 2.8 \) Hz, H-1), 5.31 (ddd, 1H, \( J_{\text{vic}} = 17.3 \) Hz, \( J_{1,1} = 3.1 \) Hz, \( J_{\text{gem}} = 1.3 \) Hz, \( \text{trans} \) OCH\(_2\)CH=CH\(_2\)), 5.22 (ddd, 1H, \( J_{\text{vic}} = 10.4 \) Hz, \( J_{1,1} = 2.4 \) Hz, \( J_{\text{gem}} = 1.3 \) Hz, \( \text{cis} \) OCH\(_2\)CH=CH\(_2\)), 4.94 (dd, 1H, \( J_{3,2} = 11.4 \) Hz, \( J_{3,4} = 10.0 \) Hz, H-3), 4.28 (m, 2H, H-6a, OCH\(_2\)HCH=CH\(_2\)), 4.07 (m, 2H, H-4, OCH\(_2\)HCH=CH\(_2\)), 3.99 (dd, 1H, \( J_{2,3} = 11.4 \) Hz, \( J_{2,1} = 2.8 \) Hz, H-2), 3.92 (m, 2H, H-5, H-6b); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta \) 150.4 (OCON), 150.0 (NCON), 136.4 (C\(_{\text{ipso}}\)), 132.3 (OCH\(_2\)CH=CH\(_2\)), 129.4, 128.4, 126.2 (C-Ar), 118.0 (OCH\(_2\)CH=CH\(_2\)), 101.4 (CHPh), 94.6 (C-1), 79.5, 74.0, 69.3, 68.4, 65.2, 61.0 (C-2, C-3, C-4, C-5, C-6, OCH\(_2\)CH=CH\(_2\)). MALDI TOF-MS: calced for
\[ C_{35}H_{36}N_2O_{13} \ (m/z), \ 692.22; \ \text{found}, \ 715.25 \ [M+Na]^+ \]. Anal Calcd for \( C_{35}H_{36}N_2O_{13} \): C, 60.69; H, 5.24; N, 4.04. Found: C, 60.49; H, 5.09; N 3.97.

**N,N-Bis(1-O-allyl-2,3-N,O-carbonyl-2-deoxy-\(\alpha\)-D-glucopyranos-2-yl)urea \ (14).**

Compound 10 (44.7 mg, 64.6 \(\mu\)mol) was dissolved in 4:1 v/v dioxane/water (1.2 mL) and then treated with (\(\pm\))-camphor-10-sulfonic acid (35.0 mg, 151 \(\mu\)mol). After 4 hours stirring at 60°C, silica gel (500 mg) was added and the mixture was evaporated. The residue was subjected to flash-chromatography (silica gel; 94:6 to 90:10 v/v chloroform/methanol) affording 7 (24.3 mg, 73%) as a white powder. [\(\alpha\)]\(D\) +134 (\(c\) 0.8; \(\text{H}_2\text{O}\)). \(^1\text{H NMR} \) (400 MHz, \(\text{D}_2\text{O})): \(\delta\) 5.86 (m, 1H, OCH\(_2\)CH=CH\(_2\)), 5.54 (d, 1H, \(J_{1,2}\) 2.9 Hz, H-1), 5.32 (dd, 1H, \(J_{\text{vic}}\) 17.3 Hz, \(J_{\text{gem}}\) 1.1 Hz, \(\text{trans}\) OCH\(_2\)CH=CH\(_2\)), 5.25 (dd, 1H, \(J_{\text{vic}}\) 10.5 Hz, \(J_{\text{gem}}\) 0.8 Hz, \(\text{cis}\) OCH\(_2\)CH=CH\(_2\)), 4.81 (dd, 1H, \(J_{3,2}\) 12.0 Hz, \(J_{3,4}\) 10.0 Hz, H-3), 4.30 (dd, 1H, \(J_{\text{gem}}\) 13.2 Hz, \(J_{\text{vic}}\) 5.0 Hz, OCH\(_2\)HCH=CH\(_2\)), 4.19 (dd, 1H, \(J_{2,3}\) 12.0 Hz, \(J_{2,1}\) 2.9 Hz, H-2), 4.11 (m, 2H, H-4, OCH\(_2\)HCH=CH\(_2\)), 3.86 (m, 2H, H-6a, H-6b), 3.73 (m, 1H, H-5); \(^{13}\text{C NMR} \) (50 MHz, \(\text{D}_2\text{O})): \(\delta\) 153.4 (OCON), 149.8 (NCON), 134.0 (OCH\(_2\)CH=CH\(_2\)), 118.8 (OCH\(_2\)CH=CH\(_2\)), 94.3 (C-1), 78.6 (C-3), 75.3 (C-5), 69.6 (OCH\(_2\)CH=CH\(_2\)), 67.9 (C-4), 60.6 (C-2), 60.4 (C-6). MALDI TOF-MS: calcd for \(C_{21}H_{26}N_2O_{13} \ (m/z)\), 516.16; found, 539.32 [M+Na]^+. Anal Calcd for \(C_{21}H_{26}N_2O_{13} \): C, 48.84; H, 5.46; N, 5.42. Found: C, 48.77; H, 5.28; N 5.35.

**Allyl 4,6-\(\text{O}\)-benzylidene-2-deoxy-3-(\(p\)-nitro-phenoxycarbonyl)-2-(\(p\)-nitro-phenoxycarbonylamino)-\(\alpha\)-D-glucopyranoside \ (16).** Compound 8 (143 mg, 0.466 mmol) was treated as described above to give crude 3, that was then dissolved in DMF (3.3 mL) and treated at 5°C with DMAP (28.5 mg, 0.233 mmol). After 1 hour stirring at
5°C, the solution was diluted with CH₂Cl₂ (50 mL) and washed with 0.1 M HCl and water. The organic layer was collected, dried and concentrated. The residue was subjected to column chromatography (silica gel; 12:1 to 9:1 toluene-ethyl acetate) to give 16 (68.4 mg, 23%) as white amorphous crystals. [α]₀D = -15 (c 0.9; CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): δ 8.23 (d, 2H, J₃',2' = 9.1 Hz, 2 H-3' pNO₂-Ar), 7.49-7.39 (m, 5H, H-Ar benzylidene), 7.28 (d, 2H, J₂',3' = 9.1 Hz, 2 H-2' pNO₂-Ar), 7.27 (d, 2H, Jₓ,y = 9.1 Hz, 2 H-2' pNO₂-Ar), 5.94 (m, 1H, OCH₂C=CH₂), 5.61 (d, 1H, Jₓ,NH = 10.0 Hz, NH), 5.59 (s, 1H, C=CHPh), 5.37 (bd, 1H, Jvic = 17.2 Hz, trans OCH₂CH=CH₂), 5.32 (bd, 1H, Jvic = 10.6 Hz, cis OCH₂CH=CH₂), 5.27 (t, 1H, J₃,₂ = J₃,₄ = J₃,₄ = 9.9 Hz, H-3), 5.05 (d, 1H, Jₛ,Jₚ = 10.3 Hz, J₆a,J₅ = 4.8 Hz, H-6a), 4.28 (m, 2H, H-2, OCHHCH=CH₂), 4.09 (dd, 1H, Jvic = 12.6 Hz, Jvic = 6.5 Hz, OCHHCH=CH₂), 4.01 (dt, 1H, Jₛ,Jₚ = Jₛ,Jₚ = 10.3 Hz, Jₛ,Jₚ = 4.8 Hz, H-5), 3.88 (t, 1H, J₃,J₄ = J₃,J₄ = 10.3 Hz, H-4), 3.83 (t, 1H, Jvic,J₆ = 10.3 Hz, H-6b); ¹³C NMR (100 MHz, CDCl₃): δ 155.3, 155.2, 145.5, 144.8 (4 Cipso), 152.9, 152.5 (NCOO, OCOO), 136.6 (Cipso benzylidene), 132.7 (OCH₂CH=CH₂), 129.3-121.6 (C-Ar), 118.9 (OCH₂CH=CH₂), 101.8 (CHPh), 96.8 (C-1), 78.5, 76.3, 68.8, 68.6, 62.7, 54.2 (C-2, C-3, C-4, C-5, C-6, OCH₂CH=CH₂). MALDI TOF-MS: unstable. Anal Calcd for C₃₀H₂₇N₃O₁₃: C, 56.52; H, 4.27; N, 6.59. Found: C, 56.38; H, 4.19; N 6.50.

Allyl 4,6-O-benzylidene-2,3-N,O-carbonyl-2-deoxy-2-(p-nitrophenoxycarbonylamino)-α-D-galactopyranoside (24) and N,N'-Bis(1-O-allyl-4,6-O-benzylidene-2,3-N,O-carbonyl-2-deoxy-α-D-galactopyranos-2-yl)urea (25).

Compound 21 (235.2 mg, 0.766 mmol) was suspended in 2:1 v/v water/CH₃CN (3.67 mL) and then cooled to 40°C. NaHCO₃ (317 mg, 3.77 mmol) and then a solution of 4-
nitrophenyl chloroformate (750 mg, 3.97 mmol) in CH$_2$CN (1.656 mL) were added. After 50 min stirring at 0°C, the mixture was diluted with ethyl acetate (50 mL) and washed with 1M NaHCO$_3$. The organic layer was collected, dried over anhydrous Na$_2$SO$_4$ and concentrated. A portion of the crude gummy solid (168.0 mg) was dissolved in DMF (246 μL) and heated to 30°C. NaH (60% dispersion in oil, 28.4 mg, 0.71 mmol) was then added portionwise to avoid a sudden overheating. The yellow mixture was stirred for 45 min at 30°C, then cooled to 0°C and treated dropwise with some mL of water until production of gas ceased. The mixture was diluted with CH$_2$Cl$_2$ (30 mL) and washed with water. The organic layer was collected, dried and concentrated. The residue was subjected to flash-chromatography (silica gel; 5:1 to 1:5 hexane-ethyl acetate) to give, as first eluted compound, 24 (9.9 mg, 13%) as white amorphous crystals. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.47 (d, 2H, $J_{2',3'}$ 7.2 Hz, 2 H-3’ pNO$_2$-Ar), 7.51-7.38 (m, 7H, benzylidene, 2 H-2’ pNO$_2$-Ar), 5.87 (m, 1H, OCH$_2$CH=CH$_2$), 5.73 (d, 1H, $J_{1,2}$ 3 Hz, H-1), 5.67 (s, 1H, CHPh), 5.30 (dd, 1H, $J_{cis}$ 18.9 Hz, $J_{gem}$ 2.4 Hz trans OCH$_2$CH=CHH), 4.89 (dd, 1H, , $J_{2,3}$ 12 Hz, $J_{3,4}$ 3.2 Hz, H-3), 4.71 (m, 2H, H-2, H-4), 4.39-4.28 (m, 2H, OCHHCH=CH$_2$, H-6), 4.20-4.12 (m, 2H, H-6b, OCHHCH=CH$_2$), 3.76 (s, 1H, H-5);

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 154.1, 145.8 (2 C$_{ipso}$), 150.3, 148.9 (2 NCOO), 136.8 (C$_{ipso}$ benzylidene), 133.0 (OCH$_2$CH=CH$_2$), 129.3, 128.3, 126.1, 125.3, 122.1 (C-Ar), 118.4 (OCH$_2$CH=CH$_2$), 100.3 (CHPh), 96.1 (C-1), 79.6, 73.4, 71.58, 69.8, 68.4, 63.6, 55.4 (C-2, C-3, C-4, C-5, C-6, OCH$_2$CH=CH$_2$). MALDI TOF-MS: unstable. Anal Calcd for C$_{24}$H$_{22}$N$_2$O$_{10}$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.68; H, 4.33; N 5.23.

Second eluted compound 25 (41.4 mg, 78%) was recovered as a white powder. (400 MHz, CDCl$_3$): $\delta$ 7.56-7.18 (m, 5H, benzylidene, H-Ar), 5.87 (m, 1H, OCH$_2$CH=CH$_2$),
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A solution of compound 10 (45.6 mg, 65.9 μmol) in chloroform (1.5 mL) was treated
with water (110 μL), triethylamine (110 μL) and finally with dioxane (660 μL). After 3 days stirring at rt, the mixture was treated at 0°C with some drops of 0.1 M HCl, then quickly diluted with dichloromethane (40 mL) and washed with water. The organic layer was collected, dried over anhydrous Na₂SO₄ and concentrated to give a residue, that, after flash-chromatography (silica gel; 6:1 to 2:1 toluene-ethyl acetate), afforded 26a (35.6 mg, 81%) as a white powder. [α]D +81.4 (c 1.0; CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): δ 7.89 (d, 1H, J₁,NH 8.7 Hz, NH), 7.51-7.36 (m, 10H, H- Ar), 5.90 (m, 2H, 2 OCH₂CH=CH₂), 5.76 (d, 1H, J₁,₂ 2.8 Hz, H-1₃), 5.62 (s, 1H, CHPh), 5.56 (s, 1H, CHPh), 5.32 (dd, 1H, Jvic 17.2 Hz, J_gem 1.5 Hz, trans OCH₂CH=CH₂H), 5.29 (dd, 1H, Jvic 17.2 Hz, J_gem 1.5 Hz, cis OCH₂CH=CH₂H), 5.24 (dd, 1H, Jvic 10.3 Hz, J_gem 1.5 Hz, cis OCH₂CH=CH₂H), 5.22 (dd, 1H, Jvic 10.5 Hz, J_gem 1.5 Hz, cis OCH₂CH=CH₂H), 4.91 (d, 1H, J₁,₁ 3.8 Hz, H-1₄), 4.83 (dd, 1H, J₁,₂ 11.7 Hz, J₁,₄ 10.1 Hz, H-3₄), 4.26 (m, 4H, H-6ₐ), 3 OCH/HCH=CH₂), 4.16 (m, 2H, H-2ₐ, OCH/HCH=CH₂), 4.04 (m, 3H, H-5ₐ, H-5₋, H-6₄), 3.91 (m, 4H, H-2₉, H-3ₐ, H-4ₐ, H-4₉), 3.76 (d, 1H, J_gem=J₆,₅ 10.3 Hz, H-6bₐ), 3.59 (d, 1H, J_gem=J₆,₅ 9.9 Hz, H-6b₉); ¹³C NMR (100 MHz, CDCl₃): δ 154.9 (OCON), 137.0, 136.4 (2 Cipso), 133.2, 133.1 (2 OCH₂CH=CH₂), 129.3-126.1 (C-Ar), 118.4, 118.3 (2 OCH₂CH=CH₂), 102.0, 101.4 (2 CHPh), 96.9, 96.5 (2 C-1), 81.9, 79.8, 74.1, 70.5, 69.9, 68.9, 68.8, 68.5, 65.4, 62.6, 60.9 (C-2ₐ, C-3ₐ, C-3₋, C-4ₐ, C-4₋, C-5ₐ, C-5₋, C-6ₐ, C-6₋, 2 OCH₂CH=CH₂), 54.7 (C-2₉). MALDI TOF-MS: calcd for C₃₄H₃₈N₂O₁₂ (m/z), 666.24; found, 689.39 [M+Na]⁺. Anal Calcd for C₃₄H₃₈N₂O₁₂: C, 61.25; H, 5.75; N, 4.20. Found: C, 61.06; H, 5.57; N 4.11.

N,N'-Bis(1-O-allyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranos-2-yl)urea (27a). A solution of compound 10 (172 mg, 0.25 mmol) in 3:1 v/v dioxane/water (8.0 mL)
was treated with triethylamine (1.0 mL). After 14 hours stirring at 80°C, silica gel (1.25 g) was added. The mixture was immediately cooled to rt and concentrated. Flash-chromatography (silica gel; 99:1 to 96:4 chloroform-methanol) afforded 27a (122 mg, 76%) as a white powder. [α]D +32 (c 0.5; CH2Cl2); 1H NMR (400 MHz, CDCl3): δ 7.50-7.34 (m, 5H, H-Ar), 5.91 (m, 1H, OCH2CH=CH2), 5.55 (s, 1H, CHPh), 5.32 (bd, 1H, Jvic 17.2 Hz, trans OCH2CH=CH2), 5.24 (bd, 1H, Jvic 10.4 Hz, cis OCH2CH=CH2), 4.88 (d, 1H, J1,2 3.0 Hz, H-1), 4.26 (dd, 1H, Jgem 9.9 Hz, J6a,5 4.5 Hz, H-6a), 4.20 (dd, 1H, Jgem 12.8 Hz, Jvic 5.2 Hz, OCHHCH=CH2), 3.93 (m, 2H, H-2, H-3), 3.83 (dt, 1H, J5,4=J5,6b 9.9 Hz, J5,6a 4.5 Hz, H-5), 3.74 (t, 1H, Jgem=J4,5 9.9 Hz, H-6b), 3.56 (t, 1H, J4,3=J4,5 9.9 Hz, H-4); 13C NMR (100 MHz, CDCl3): δ 159.1 (NCON), 137.2 (Cipso), 133.4 (OCH2CH=CH2), 129.1, 128.2, 126.4 (C-Ar), 118.2 (OCH2CH=CH2), 102.0 (CHPh), 97.5 (C-1), 82.2, 71.5, 68.9, 68.8, 62.6, 55.7 (C-2, C-3, C-4, C-5, C-6, OCH2CH=CH2). MALDI TOF-MS: calcd for C33H40N2O11 (m/z), 640.26; found, 641.39 [M+H]+. Anal Calcd for C33H40N2O11: C, 61.86; H, 6.29; N, 4.37. Found: C, 62.02; H, 6.40; N 4.45.

N-(1-O-allyl-4,6-O-benzylidene-2,3-N,O-carbonyl-2-deoxy-α-D-glucopyranos-2-yl)-N'-1-O-allyl-4,6-O-benzylidene-2-deoxy-3-methoxycarbonyl-α-D-glucopyranos-2-yl)-urea (26b) and N,N'-Bis(1-O-allyl-4,6-O-benzylidene-2-deoxy-3-methoxycarbonyl-α-D-glucopyranos-2-yl)-urea (27b). Compound 10 (32.3 mg, 46.7 µmol) was dissolved in CH2Cl2 (1.4 mL), cooled to -60°C and treated with a 1.5 M solution of NaOMe in MeOH (155 mL). After 40 min stirring at -60°C sat. NH4Cl (5 mL) was added. The mixture was immediately diluted with EtOAc (30 mL), heated to rt and washed with water. The organic layer was collected, dried over
anhydrous Na₂SO₄, filtered and concentrated to give a residue, that, after flash-
chromatography (silica gel; 7:1 to 5:1 toluene-ethyl acetate), afforded, as first eluted
compound, 26b (11.5 mg, 34%) as a white powder. [z]D +66 (¢ 0.5; CH₂Cl₂); ¹H NMR
(400 MHz, CDCl₃): δ 7.91 (d, 1H, J₆,₇ 9.5 Hz, NH), 7.49-7.34 (m, 10H, H-Ar), 5.90 (m,
2H, 2 OCH₂CH=CH₂), 5.75 (d, 1H, J₅₂ 2.8 Hz, H-1), 5.62 (s, 1H, CHPh), 5.52 (s, 1H,
CHPh), 5.33 (dd, 1H, Jvic 17.2 Hz, Jgem 1.5 Hz, trans OCH₂CH=CHH), 5.30 (dd, 1H, Jvic
17.2 Hz, Jgem 1.5 Hz, trans OCH₂CH=CHH), 5.23 (m, 3H, H-3b, 2 ¢äis OCH₂CH=CHH),
4.91 (d, 1H, J₁₂ 3.7 Hz, H-1b), 4.80 (dd, 1H, J₃₄ 11.7 Hz, J₁₂ 10.1 Hz, H-3a), 4.36-4.21
(m, 6H, H-2b, H-6a₁, H-6a₂, H-6b, H-6b₁, 2 OCH₂HCH=CH₂), 4.15 (dd, 1H, Jvic 12.8 Hz, Jvic
5.9 Hz, OCH₂HCH=CH₂), 4.05 (m, 2H, H-4p, OCH₂HCH=CH₂), 3.98 (td, 1H, J₅₆a = J₅₆b
10.3 Hz, J₅₆ 9.4 Hz, H-5b), 3.90 (d, 1H, J₃₄ 6.8 Hz, H-5a), 3.87 (dd, 1H, J₂₁ 11.7 Hz, J₂,₁
2.8 Hz, H-2a), 3.78 (t, 1H, J₆₅ = Jvic 10.3 Hz, H-6b), 3.76 (s, 3H, OCH₃), 3.74 (t, 1H,
J₄₅ = J₄,₅ 9.4 Hz, H-4b); ¹³C NMR (50 MHz, CDCl₃): δ 155.3, 154.7, 151.8 (COOCH₃,
NCON, OCON), 136.9, 136.4 (2 Cᵥvic), 133.1, 133.0 (2 OCH₂CH=CH₂), 129.3, 129.1,
128.3, 128.2, 126.2, 126.1 (C-Ar), 118.5, 118.3 (2 OCH₂CH=CH₂), 101.6, 101.4 (2
CHPh), 97.0, 96.3 (C-1, C-1b), 79.8, 79.2, 74.6, 74.0, 69.7, 69.0, 68.7, 68.5, 65.4, 62.9,
60.9, 55.2, 52.9 (C-2, C-2b, C-3, C-3b, C-4, C-4b, C-5, C-5b, C-6, C-6b, 2
OCH₂CH=CH₂, OCH₃). MALDI TOF-MS: calcd for C₃₆H₄₉N₂O₁₄ (m/z), 724.25;
C, 59.48; H, 5.47; N 3.80.

Second eluted compound 26b (12.1 mg, 34%) was recovered as a white powder. [z]D
+84.1 (¢ 1.5; CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃): δ 7.46-7.35 (m, 5H, H-Ar), 5.87 (m,
1H, OCH₂CH=CH₂), 5.52 (s, 1H, CHPh), 5.30 (bd, 1H, Jvic 17.2 Hz, trans
OCH₂CH=CHH), 5.23 (bd, 1H, Jvic 10.3 Hz, ¢äis OCH₂CH=CHH), 5.09 (t, 1H, J₃₂ = J₃,₂
10.0 Hz, H-3), 4.90 (d, 1H, J_{1,2} 3.6 Hz, H-1), 4.85 (d, 1H, J_{H,NH} 9.6 Hz, NH), 4.27 (dd, 1H, J_{gem} 10.2 Hz, J_{6a,5} 4.8 Hz, H-6a), 4.18 (m, 2H, H-2, OCH\_CH=CH\_2), 4.00 (dd, 1H, J_{gem} 12.8 Hz, J_{6a,5} 9.9 Hz, J_{5,6a} 4.8 Hz, H-5), 3.75 (m, 5H, H-4, H-6b, OCH\_3); $^{13}$C NMR (100 MHz, CDCl\_3): $\delta$ 156.2, 155.9 (C\_OOC\_H\_3, N\_CON), 137.0 (C\_ipso), 133.2 (OCH\_2C\_H=CH\_2), 129.1, 128.3, 126.2 (C\_Ar), 118.2 (OCH\_2CH=CH\_2), 101.6 (CHPh), 97.6 (C-1), 79.1, 74.5, 68.8, 68.7, 62.9, 55.1, 53.4 (C-2, C-3, C-4, C-5, C-6, OCH\_2CH=CH\_2, OCH\_3). MALDI TOF-MS: calcd for C\_37H\_44N\_2O\_15 (m/z), 756.27; found, 779.38 [M+Na]^+. Anal Calcd for C\_35H\_36N\_2O\_13: C, 58.72; H, 5.86; N, 3.70. Found: C, 58.50; H, 5.68; N 3.65.

Alternatively, compound 27b could be obtained by dissolving 10 (42.4 mg, 61.3 μmol) in chloroform (1.4 mL) and then treating it with methanol (0.6 mL) and triethylamine (0.1 mL). The solution was stirred at rt for two days. The reaction was quenched by adding some drops of 0.1M HCl. The mixture was immediately diluted with CH\_2Cl\_2 (25 mL) and washed with water. The organic layer was collected, dried and concentrated. Column chromatography (silica gel; 7:1 to 5:1 toluene-ethyl acetate) on the residue afforded 27b (24.9 mg, 54%).

$^{N'}$-[3-O-(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-1-O-allyl-4,6-O-benzylidene-2-deoxy-β-D-glucopyranos-2-yl]-$^{N''}$-[1-O-allyl-4,6-O-benzylidene-2,3-β-N'-O-carbonyl-2-deoxy-α-D-glucopyranos-2-yl]-urea (29). A mixture of 26a (39.0 mg, 58.6 μmol) and 28 (70.8 mg, 117 μmol) was coevaporated three times with toluene, the residue was dried and then mixed with freshly activated AW-300 4Å molecular sieves, suspended under argon in 1:1 v/v CH\_2Cl\_2/THF (1.5 mL). The mixture was stirred at -30°C for 15 min. A 24.6 mg/mL solution of TMSOTf in CH\_2Cl\_2 (27.4 μL, 3.0 μmol)
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was then added. The mixture was stirred for 75 min at -30°C. Few drops of Et$_3$N were then added. The mixture was filtered over a Celite pad and concentrated. The residue was subjected to column chromatography (silica gel; 12:1 to 8:1 toluene/ethyl acetate) to give 29 (48.1 mg, 76%) as a colourless oil. [61] D +23.1 (c 1.7; CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$): δ 8.08 (d, 1H, $J_{H,NH}$ 10.0 Hz, NH), 7.39-7.25 (m, 25H, H-Ar), 5.86 (m, 1H, OCH$_2$CH=CH$_2$), 5.69 (m, 2H, H-1$_A$, OCH$_2$CH=CH$_2$), 5.54 (s, 1H, CHPh), 5.51 (s, 1H, CHPh), 5.35 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1$_C$), 5.31 (dd, 1H, $J_{vic}$ 17.2 Hz, $J_{gem}$ 1.5 Hz, trans OCH$_2$CH=CH$_2$), 5.22 (dd, 1H, $J_{vic}$ 10.5 Hz, $J_{gem}$ 1.5 Hz, cis OCH$_2$CH=CH$_2$), 5.09 (dd, 1H, $J_{vic}$ 17.0 Hz, $J_{gem}$ 1.5 Hz, trans OCH$_2$CH=CH$_2$), 5.06 (dd, 1H, $J_{vic}$ 10.5 Hz, $J_{gem}$ 1.5 Hz, cis OCH$_2$CH=CH$_2$), 4.89 (d, 1H, $J_{gem}$ 11.5 Hz, OCHHPh), 4.87 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1$_b$), 4.83 (d, 1H, $J_{gem}$ 11.7 Hz, OCHHPh), 4.73 (d, 1H, $J_{gem}$ 11.6 Hz, OCHHPh), 4.66 (d, 1H, $J_{gem}$ 11.7 Hz, OCHHPh), 4.65 (t, 1H, $J_{3,4}$=$J_{3,2}$ 10.0 Hz, H-3$_C$), 4.56 (d, 1H, $J_{gem}$ 11.6 Hz, OCHHPh), 4.55 (d, 1H, $J_{gem}$ 11.5 Hz, OCHHPh), 4.35 (dt, 1H, $J_{2,3}$=$J_{2,NH}$ 10.0 Hz, $J_{2,3}$ 3.5 Hz, H-2$_b$), 4.31-4.17 (m, 5H, H-2$_A$, H-6$_A$, H-6$_B$, OCH$_2$CH=CH$_2$), 4.12 (q, $J_{gem}$ 1.5 Hz, H-5$_B$), 4.05-3.72 (m, 11H, H-2$_C$, H-3$_B$, H-3$_C$, H-4$_A$, H-4$_B$, H-5$_A$, H-5$_B$, H-6$_A$, H-6$_B$, OCH$_2$CH=CH$_2$), 3.49 (d, 1H, $J_{4,5}$ 2.0 Hz, H-4$_C$), 0.88 (d, 3H, $J_{6,5}$ 6.4 Hz, H-6$_C$); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 154.9, 152.4 (OCON, NCON), 139.0, 138.6, 138.2, 137.3, 136.4 (3 C$_{qsp}$ N), 2 C$_{qsp}$ benzylidene), 133.4, 133.0 (2 OCH$_2$CH=CH$_2$), 129.3-125.9 (C-Ar), 118.0, 117.9 (2 OCH$_2$CH=CH$_2$), 101.6, 101.4 (2 CHPH), 97.2, 96.6, 96.5 (C-1$_A$, C-1$_B$, C-1$_C$), 80.6, 79.8, 79.4, 77.9, 76.0, 74.7, 73.6, 73.3, 72.2, 71.7, 69.1, 69.0, 68.9, 68.5, 66.7, 65.1, 62.9, 61.2, 55.0 (C-2$_A$, C-2$_B$, C-2$_C$, C-3$_A$, C-3$_B$, C-3$_C$, C-4$_A$, C-4$_B$, C-4$_C$, C-5$_A$, C-5$_B$, C-5$_C$, C-6$_A$, C-6$_B$, 3 OCH$_2$Ph, 2 OCH$_2$CH=CH$_2$), 16.3 (C-6$_C$). MALDI TOF-MS: calcd for C$_{29}$H$_{56}$N$_2$O$_{16}$ (m/z), 1082.44; found, 1105.21 [M+Na]$^+$. Anal Calcd for C$_{29}$H$_{56}$N$_2$O$_{16}$: C, 67.64; H, 6.14; N, 2.59. Found: C, 67.48; H, 6.00; N 2.50.
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$N'\{3-O(2,3,4-tri-O-benzyl-α-L-fucopyranosyl)-1-O-allyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranos-2-yl\}-N'(1-O-allyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranos-2-yl)-urea (30).$ A solution of compound 29 (36.6 mg, 33.8 μmol) in 3:1 v/v dioxane/water (2.1 mL) was treated with triethylamine (260 μL). After 30 hours stirring at 80°C, silica gel (600 mg) was added. The mixture was immediately cooled to rt and concentrated. Flash-chromatography (silica gel; 6:1 to 2:1 toluene/ethyl acetate) afforded 30 (20.8 mg, 58%) as white amorphous crystals. $[\alpha]_D -3 (c 0.7; CH_2Cl_2); ^1H NMR$ (400 MHz, CDCl$_3$): $\delta$ 7.40–7.27 (m, 25H, H-$\text{Ar}$), 5.88 (m, 1H, OCH$_2$C=CH$_2$), 5.78 (m, 1H, OCH$_2$CH=CH$_2$), 5.54 (s, 2H, 2 CHPh), 5.30 (dd, 1H, $J_{\text{vic}}$ 17.2 Hz, $J_{\text{gem}}$ 1.4 Hz, trans OCH$_2$C=CHH), 5.28 (d, 2H, $J_{\text{1,2}}$ 3.1 Hz, H-$1_A$, H-$1_B$), 5.23 (dd, 1H, $J_{\text{gem}}$ 10.4 Hz, $J_{\text{vic}}$ 1.4 Hz, cis OCH$_2$C=CHH), 5.28 (d, 2H, $J_{\text{1,2}}$ 3.1 Hz, H-$1_A$, H-$1_B$), 5.17 (dd, 1H, $J_{\text{vic}}$ 17.2 Hz, $J_{\text{gem}}$ 1.4 Hz, trans OCH$_2$C=CHH), 5.14 (dd, 1H, $J_{\text{vic}}$ 10.4 Hz, $J_{\text{gem}}$ 1.4 Hz, cis OCH$_2$C=CHH), 5.05 (bs, 2H, 2 NH), 5.01 (d, 1H, $J_{\text{1,2}}$ 3.8 Hz, H-$1_c$), 4.89 (d, 1H, $J_{\text{gem}}$ 11.5 Hz, OCHHPh), 4.82 (d, 1H, $J_{\text{gem}}$ 11.9 Hz, OCHHPh), 4.75 (s, 2H, OCH$_2$Ph), 4.65 (d, 1H, $J_{\text{gem}}$ 11.5 Hz, OCHHPh), 4.57 (d, 1H, $J_{\text{gem}}$ 11.9 Hz, OCHHPh), 4.28 (dd, 1H, $J_{\text{1,2}}$ 10.0 Hz, $J_{\text{2,3}}$ 3.5 Hz, H-$2_A$), 4.23 (dd, 1H, $J_{\text{1,2}}$ 10.0 Hz, $J_{\text{2,3}}$ 3.5 Hz, H-$2_B$), 4.16 (dd, 1H, $J_{\text{gem}}$ 13.4 Hz, $J_{\text{vic}}$ 6.8 Hz, OCHHCH=CH$_2$), 4.09-3.68 (m, 16H, H-$2_C$, H-$3_A$, H-$3_B$, H-$3_C$, H-$4_A$, H-$4_B$, H-$5_A$, H-$5_B$, H-$5_C$, H-$6a_A$, H-$6b_A$, H-$6a_B$, H-$6b_B$, 3 OCHHCH=CH$_2$), 3.53 (d, 1H, $J_{\text{a,d}}$ 2.0 Hz, H-$4_C$), 0.96 (d, 3H, $J_{\text{6,9}}$ 6.4 Hz, H-$6_c$); $^{13}$C NMR (50 MHz, CDCl$_3$): $\delta$ 158.9 (NCON), 138.7, 138.5, 137.6, 137.3, 137.1 (3 C$_{\text{sp3}}$ Bn), 2 C$_{\text{sp3}}$ benzylidene), 133.7, 133.2 (2 OCH$_2$CH=CH$_2$), 129.0-125.9 (C-Ar), 118.4, 117.9 (2 OCH$_2$CH=CH$_2$), 101.7, 101.1 (2 CHPh), 97.9, 97.4, 97.3 (C-$1_A$, C-$1_B$, C-$1_c$), 81.7, 81.5, 79.2, 79.1, 77.5, 74.9, 74.5, 73.7, 73.3, 71.8, 71.7, 68.8, 68.7, 68.6, 67.2, 63.0, 62.5, 55.6, 55.1 (C-$2_A$, C-$2_B$, C-$2_C$, C-$3_A$, C-$3_B$).
N-(1-O-allyl-3-O-α-L-fucopyranosyl-2-deoxy-α-D-glucopyranos-2-yl)-N’-(1-O-allyl-2-deoxy-α-D-glucopyranos-2-yl)-urea (31). A solution of 30 (10.0 mg, 9.5 μmol) in 9:1 v/v MeOH/HCOOH (500 μL) was treated with Pd/C (3 mg) under Ar atmosphere. After 1h in an ultrasound bath, the mixture was filtered over a Celite pad and concentrated. The residue was lyophilized to give pure 31 (4.2 mg, 72%) as a white foam [α]D +37 (c 0.3; H2O); 1H NMR (400 MHz, D2O): δ 5.04 (d, 1H, J1,2 3.9 Hz, H-1), 4.89 (d, 1H, J1,2 3.4 Hz, H-1), 4.82 (d, 1H, J1,2 3.6 Hz, H-1), 4.34 (q, 1H, J5,6 6.6 Hz, H-5C), 3.88-3.40 (m, 19H, H-2A, H-2B, H-2C, H-3A, H-3B, H-4A, H-4B, H-4C, H-5A, H-5B, H-6A, H-6B, 2 OCH2CH2CH3), 1.62 (m, 4H, 2 OCH2CH2CH3), 1.16 (d, 3H, J6,5 6.6 Hz, H-6C), 0.93 (t, 6H, Jvic 7.4 Hz, 2 OCH2CH2CH3); 13C NMR (100 MHz, D2O): δ 160.1 (NCON), 100.0, 98.3 (C-1A, C-1B, C-1C), 79.0, 72.6, 72.5, 70.9, 70.8, 70.7, 69.3, 68.9, 67.8, 61.4 (C-2C, C-3A, C-3B, C-3C, C-4A, C-4B, C-4C, C-5A, C-5B, C-5C, C-6A, C-6B, OCH2CH2CH3), 55.0, 54.7 (C-2A, C-2B), 22.8 (OCH2CH2CH3), 16.0 (C-6C), 10.7 (OCH2CH2CH3). MALDI TOF-MS: calcd for C25H46N2O15 (m/z), 614.29; found, 637.10 [M+Na]+. Anal Calcd for C25H46N2O15: C, 48.85; H, 7.54; N, 4.56. Found: C, 48.60; H, 7.78; N 4.39.

1-O-Allyl-4,6-O-benzylidene cyclic pseudodisaccharide (32). Compound 26a (36.8 mg, 55.2 μmol) was dissolved in DMF (2.0 mL) under Ar atmosphere. The solution was
treated with DBU (10.2 μL, 68.4 μmol) and then heated to 50°C. After overnight stirring, it was cooled to rt, diluted with ethyl acetate (20 mL) and washed with 0.1 M HCl and then water. The organic layer was collected, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated to give a residue, that, after flash-chromatography (silica gel; 6:1 to 2:1 toluene-ethyl acetate), afforded 32 (15.7 mg, 43%) as a white powder. [α]$_D$ +32 (c 0.9; CH$_2$Cl$_2$); $^1$H NMR (400 MHz, CDCl$_3$): δ 7.49-7.33 (m, 5H, H-Ar), 5.85 (m, 1H, OCH$_2$CH=CH$_2$), 4.92 (d, 1H, H-3, OCH$_2$CH=CH$_2$), 4.29 (dd, 1H, J$_{N,N}$ 9.8 Hz, NH), 4.20 (dd, 1H, J$_{gem}$ 12.5 Hz, J$_{vic}$ 5.4 Hz, OCH=CH$_2$), 4.07 (dt, 1H, J$_{2,3}$=J$_{N,N}$ 9.8 Hz, J$_{2,3}$ 3.8 Hz, H-2), 3.99 (dd, 1H, J$_{gem}$ 12.5 Hz, J$_{vic}$ 5.4 Hz, OCHHCH=CH$_2$), 3.94 (ddd, 1H, J$_{5,6b}$ 10.3 Hz, J$_{5,6}$ 9.6 Hz, J$_{5,6a}$ 4.8 Hz, H-5), 3.77 (t, 1H, J$_{gem}$=J$_{6b,5}$ 10.3 Hz, H-6b), 3.73 (t, 1H, J$_{4,3}$=J$_{4,5}$ 9.6 Hz, H-4); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 155.5 (OCON), 136.8 (C$_{pap}$), 132.7 (OCH$_2$CH=CH$_2$), 129.2, 128.2, 126.3 (C-Ar), 119.2 (OCH$_2$CH=CH$_2$), 101.8 (CH$_2$), 96.6 (C-1), 78.3, 75.1, 68.9, 68.8, 63.2, 56.4 (C-2, C-3, C-4, C-5, C-6, OCH$_2$CH=CH$_2$). MALDI TOF-MS: calcd for C$_{34}$H$_{38}$N$_2$O$_4$: 666.24; found, 689.37 [M+Na]$^+$. Anal Calcd for C$_{34}$H$_{38}$N$_2$O$_4$: C, 61.25; H, 5.75; N, 4.20. Found: C, 61.08; H, 5.66; N 4.11.

1-O-Allyl cyclic pseudodisaccharide (33). Compound 32 (36.0 mg, 54.1 μmol) was dissolved in 4:1 v/v dioxane/water (1.0 mL) and then treated with (±)-camphor-10-sulfonic acid (31.1 mg, 134 μmol). After 4 hours stirring at 60°C, silica gel (500 mg) was added and the mixture was evaporated. The residue was subjected to flash-chromatography (silica gel; 95:5 to 86:14 v/v chloroform/methanol) affording 33 (25.9 mg, 98%) as a white powder. [α]$_D$ +104 (c 0.9; D$_2$O); $^1$H NMR (400 MHz, D$_2$O): δ 6.00
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(m, 1H, OCH\_2CH=CH\_2), 5.38 (d, 1H, J\_vic 17.2 Hz, trans OCH\_2CH=CH\_2), 5.28 (d, 1H, J\_1,2 3.7 Hz, cis OCH\_2CH=CH\_2), 5.07 (d, 1H, J\_1,2 3.7 Hz, H-1), 4.87 (dd, 1H, J\_3,2 10.0 Hz, J\_3,4 9.2 Hz, H-3), 4.25 (dd, 1H, J\_gem 12.7 Hz, J\_vic 5.5 Hz, OCH\_2HCH=CH\_2), 4.15 (dd, 1H, J\_2,3 10.0 Hz, J\_2,1 3.7 Hz, H-2), 4.09 (dd, 1H, J\_gem 12.7 Hz, J\_vic 5.5 Hz, OCH\_2HCH=CH\_2), 3.91-3.73 (m, 4H, H-4, H-5, H-6a, H-6b); \(^{13}\)C NMR (50 MHz, D\_2O): \(\delta\) 158.6 (OCON), 134.0 (OCH\_2CH=CH\_2), 119.1 (OCH\_2CH=CH\_2), 96.4 (C-1), 79.6, 72.4, 69.2, 67.3, 60.8, 55.8 (C-2, C-3, C-4, C-5, C-6, OCH\_2CH=CH\_2). MALDI TOF-MS: calcd for C\(_{20}\)H\(_{30}\)N\(_2\)O\(_12\) (m/z), 490.18; found, 513.00 [M+Na]\(^+\). Anal Calcd for C\(_{20}\)H\(_{30}\)N\(_2\)O\(_12\): C, 48.98; H, 6.17; N, 5.71. Found: C, 48.78; H, 6.04; N 5.61.

**N,N-Bis(1-O-allyl-4,6-O-benzylidene-2-deoxy-\(\alpha\)-D-galactopyranos-2-yl)urea (34).**

A solution of compound 25 (74.8 mg, 0.25 mmol) in 3:1 v/v dioxane/water (3.6 mL) was treated with triethylamine (450 \(\mu\)L). After 14 hours stirring at 80°C, silica gel (1.25 g) was added. The mixture was immediately cooled to rt and concentrated. Flash chromatography (silica gel; 99:1 to 98:2 chloroform-methanol) afforded 34 (44.2 mg, 63.9%) as a white powder. \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.50-7.31 (m, 5H, H-Ar), 5.83 (m, 1H, OCH\_2CH=CH\_2), 5.37 (s, 1H, CHPh), 5.28 (bd, 1H, NH), 5.23 (bd, 1H, J\_vic 17.3 Hz, trans OCH\_2CH=CH\_2), 5.10(bd, 1H, J\_vic 10.3 Hz, cis OCH\_2CH=CH\_2), 4.94 (d, 1H, J\_1,2 3.2 Hz, H-1), 4.18 (bd, 1H, J\_gem 12.3 Hz, H-6a), 4.20 (dd, 1H, J\_gem 12.8 Hz, J\_vic 5.2 Hz, OCH\_2HCH=CH\_2), 4.11 (dd, 1H, J\_gem 12.9 Hz, J\_vic 4.9 Hz), 4.13-4.08 (m, 2H, H-2, OCH\_2HCH=CH\_2), 3.98-3.94 (m, 2H, H-6b, OCH\_2HCH=CH\_2), 3.83 (bd, 1H, H-3), 3.58 (s, 1H, H-5); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 159.5 (NCON), 137.8 (C\(_{\text{ipso}}\)), 133.6 (OCH\_2CH=CH\_2), 128.9, 128.0, 126.4 (C-Ar), 117.6 (OCH\_2CH=CH\_2), 101.1 (CHPh), 97.9 (C-1), 75.63, 69.2, 69.0, 68.5, 63.0, 51.4 (C-2, C-3, C-4, C-5, C-6, OCH\_2CH=CH\_2).
MALDI TOF-MS: calcd for C$_{33}$H$_{40}$N$_{11}$O$_{11}$ (m/z), 640.26; found, 641.44 [M+H]$^+$. Anal Calcd for C$_{33}$H$_{40}$N$_{11}$O$_{11}$: C, 61.86; H, 6.29; N, 4.37. Found: C, 62.12; H, 6.36; N 4.48.