UNIVERSITY OF NAPLES

"FEDERICO II"

FACULTY OF MATHEMATICAL, PHYSICAL AND NATURAL SCIENCES

PhD in Chemical Sciences

XXIII cycle (2007-2010)

"Synthetic Applications of Carbohydrates"

Dr. Luigi Cirillo

Supervisors

Prof. Michelangelo Parrilli **Dr. Emiliano Bedini**

Ph.D. Coordinator

Prof. Lucio Previtera

Assessor

Prof. Giovanni Palumbo

UNIVERSITÀ DI NAPOLI

"FEDERICO II"

FACOLTÀ DI SCIENZE MATEMATICHE, FISICHE E NATURALI

Dottoratodi Ricerca in Scienze Chimiche

XXIII ciclo (2007-2010)

"Applicazioni sintetiche di carboidrati"

Dott. Luigi Cirillo

Tutori

Prof. Michelangelo Parrilli **Dr. Emiliano Bedini**

Coordinatore **Relatore**

Prof. Lucio Previtera Prof. Giovanni Palumbo

INDEX

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 rule 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 "*gycocalyx*" [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

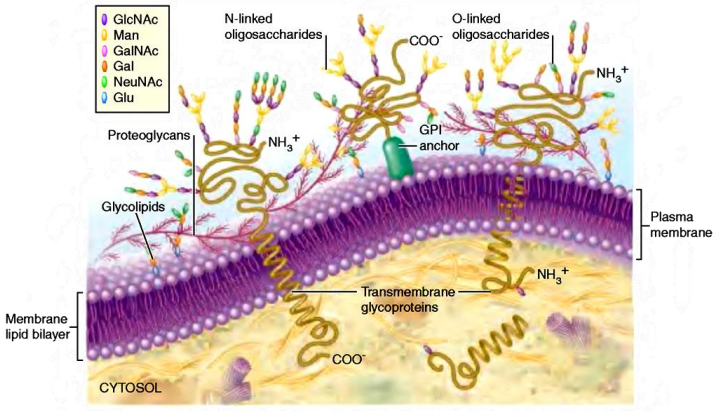


FIG. 1.1 The glycocalyx

[1] It is notable that even though the gycocalyx 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).

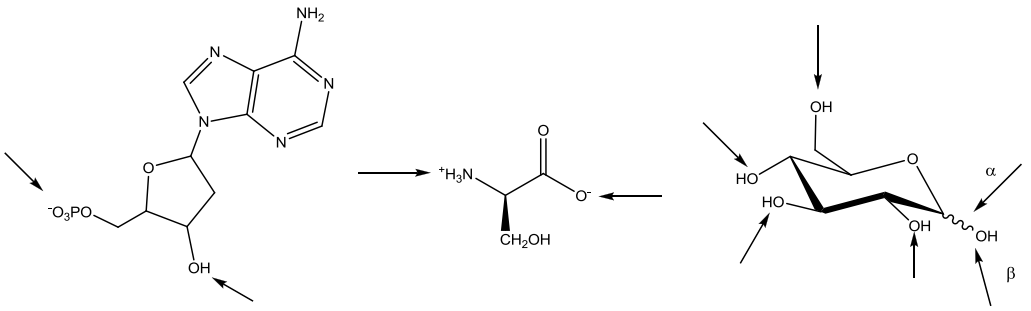


FIG. 1.2: Illustration of the chemical complexity of a monosaccharide unit compared to nucleotide and amino acid.

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 $^4C_1$ chair conformation ($^1C_4$ for L-sugars) is the most energetically preferred pyranose ring structure. [2] Some exceptions can occur, as in the case of L-iduronic acid in heparan and dermatan sulfate that assumes a skew-boat form $^2S_0$ to accommodate mechanical stress, conformational flexibility and elasticity. [3]

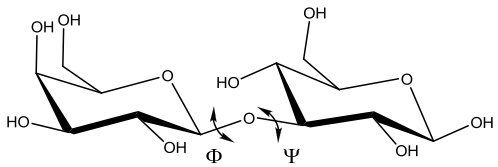


FIG. 1.3: Dihedral angles Φ and Ψ define the possible conformation of a disaccharide.

[2] a) Abeygunawardana, C.; Bush, C.A. *Adv. Biophys. Chem.* **1993**, *3*, 199; b) Brown, G.M., Levy, H.A. *Science* **1965**, *147*, 1038.
[3] a)Casu, B.; Petitou, M.; Provasoli, M.; Sinay, P. *Trends Biochem. Sci.* **1988**, *13*, 221; b) Marsalek, P.E.; Oberhauser, A.F.; Pang, Y.P.; Fernandez, J.M. *Nature* **1998**, *396*, 661.

However, the main contribution to the drfinition 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]

Lectin Source and lectin	*Abbreviations*	*Ligand(s)*
Plant		
Concavalin A	ConA	Manα1-OCH_3
Griffonia Simplicifolia lectin 4	GS4	Lewis b (Le^b) tetrasaccharide
Ricin		Gal(β1→4)Glc
Wheat germ agglutinin	WGA	Neu5Ac(α2→3)Gal(β1→4)Glc GlcNAc(β1→4)GlcNAc
Animal		
Galectin-1		Gal(β1→4)Glc
Mannose-binding protein A	MBP-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 pentasaccharide

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

[4] a) Bush, C.A.; Martin-Pastor, M.; Imberty, A. *Annu. Rev. Biophys. Biomol. Struct.* **1999**, *28*, 269; b) Imberty, A. *Curr. Opin. Struct. Biol.* **1997**, *7*, 617.

lectins. [5] 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 structures (Table 1.1). [6]

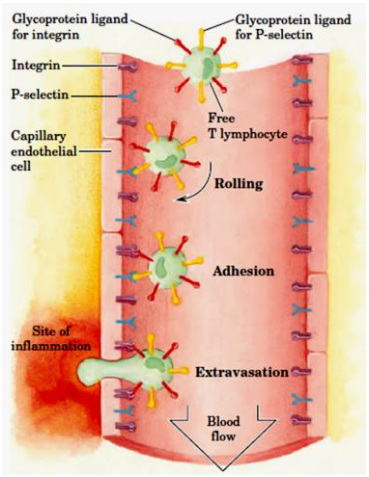


FIG. 1.4: Role of lectin-ligand interactions in lymphocyte movement

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). [7] 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. [8] 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

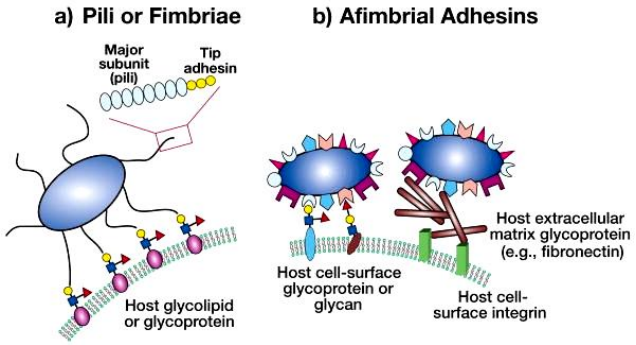


FIG. 1.5: Examples of mechanism of bacterial adherence

[5] Gabius, H.J.; Siebert, H.C.; André, S.; Jiménez-Barbero, J.; Rüdiger, H. *ChemBioChem* **2004**, *5*, 740.
[6] Weiss, W.I.; Drickamer, K. *Annu. Rev. Biochem.* **1996**, *65*, 441.
[7] Gabius, H.J., *Naturwissenschaften* **2000**, *87*, 108.
[8] Dustin, M.L.; Springer, T.A. *Annu. Rev. Immunol.* **1991**, *9*, 27-66.

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.[9] 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.

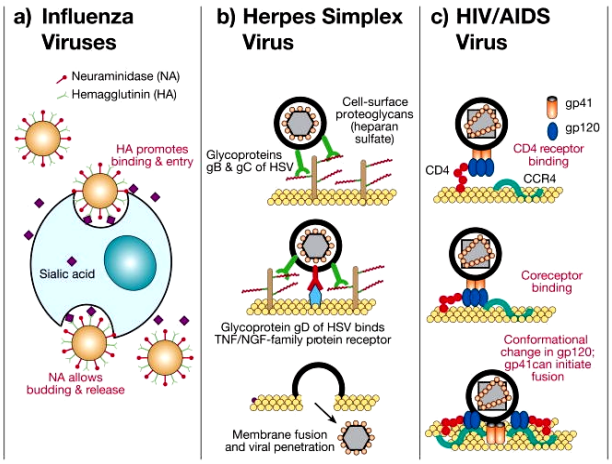


FIG. 1.6: Mechanism of viral entry into host cell

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). [10] 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). [11] 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)

[9] *Lehniger, Principles of Biochemistry,* 4th edn,; Nelson, D.L.; Cox, M.M., Editors. Worth Publisher, Inc.; New York, **2004**.

[10] *Essentials of Glycobiology*, 2nd edition.Varki A, Cummings RD, Esko JD, et al., editors. Cold Spring Harbor (NY), **2009**.

[11] a) Suzuki, Y.; Ito, T.; Suzuki, T.; Holland, J.; Robert, E.; Chambers, T.M.; Kiso, M.; Ishida, H.; Kawaoka, Y. *J. Virol.* **2000**, *74*, 11825-11831; b) Shinya, K.; Ebina, M.; Yamada, S.; Ona, M.; Kasai, N.; Kawaoka, Y. *Nature* **2006**, *440*, 435.

receptor family promotes membrane fusion (FIG. 1.6b). [12] 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.[13] (FIG. 1.6c).

[12] Lycke, E.; Johansson, M.; Svennerholm, B.; Lindahl, U. *J. Gen. Vir.* **1991**, *72*, 1131; b)Turner, A.; Bruun, B.; Minson, T.; Browne, H. *J. Virol.* **1998**, *72*, 873, c) Spear, P.G. *Cell Microbiol.* **2004**; *6*: 401–410.
[13] Ji, X.; Chen, Y.; Faro, J.; Gewurz, H.; Bremer, J.; Spear, G.T. *Curr Protein Pept Sci.* **2006**; *7*: 317–324.

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 specifity: 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

[14] Kiessling, L.L.; Pohl, N.L. *Curr. Biol.* **1996**, *3*, 71.
[15] Lundquist, J.J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555.

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. [16]

It has been largely shown [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.

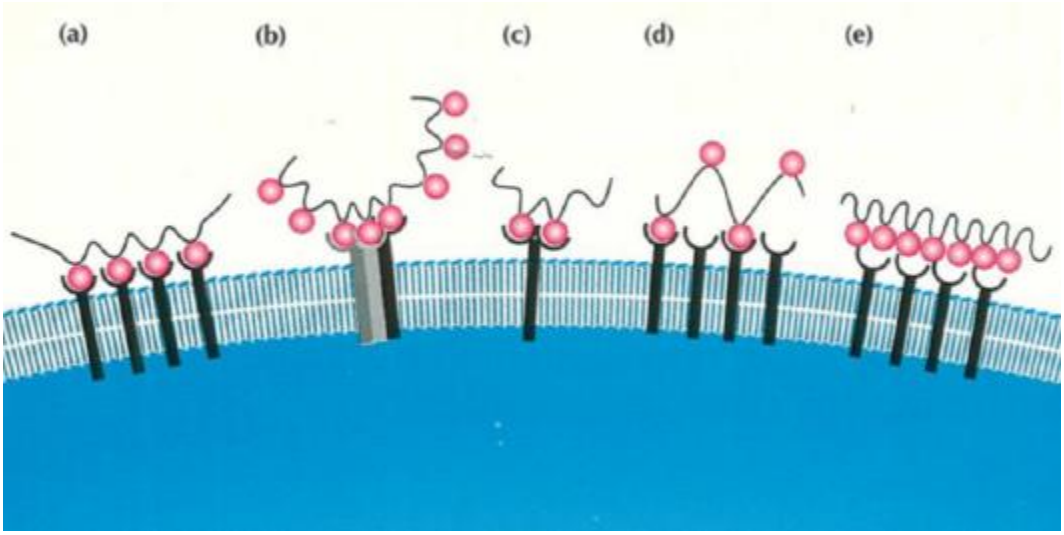


FIG.1.7: Specific recognition in multivalent interactions. Cells can use several strategies to bind to a multivalent ligand: (a) forming a cluster of many monovalent receptors on a small area of the cell surface, (b) using oligomeric receptors, or (c) using receptors with more than one saccharide binding type. Multivalent ligands with incompatible relative orientations (d) or spacing (e) of the saccharide units in the multivalent array will not bind tightly.

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). [18] On the other hand, multivalency can be used also for

[16] (a) Houseman, B.T.; Mrksich, M. *Top. Curr. Chem.* **2002**, *218*, 1; (b) Lee, R.T.; Lee, Y.C. *Glycoconjugate J.* **2000**, *17*, 543.
[17] Mammen, M.; Choi, S.K.; Whitesides, G.M. *Angew. Chem. Int. Ed.* **1998**, *37*, 2754.
[18] Indik, Z.K.; Park, J.C.; Hunter, A.D.; Schreiber, A.D. *Blood* **1995**, *86*, 4389, 4399.

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.

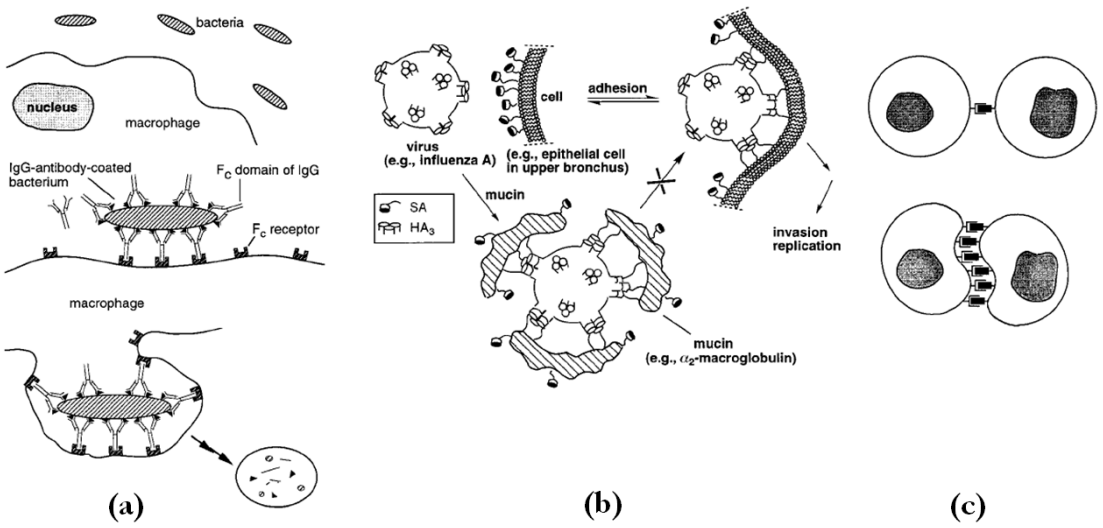


FIG. 1.8: Examples of multivalency in Nature: (a) A bacterium coated with IgG antibodies is eliminated from the circulation through phagocytosis by a macrophage. The multivalent binding of the antibody coated bacterium to multiple Fc receptors of a macrophage activates the phagocytotic process; (b) Prevention of the attachement of influenza virus to the surface of a cell due to the multivalent binding of mucins with the sialyl groups to hemagglutinins on the surface of virus; (c) Conformal contact of large biological surfaces by multivalent binding.

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

[19] Wolfenden, M.L.; Cloniger, M.J. *Bioconjugate Chem.* **2006**, *17*, 958.

co-workers. [20] Also Kihlberg and co-workers reported antiviral properties of a

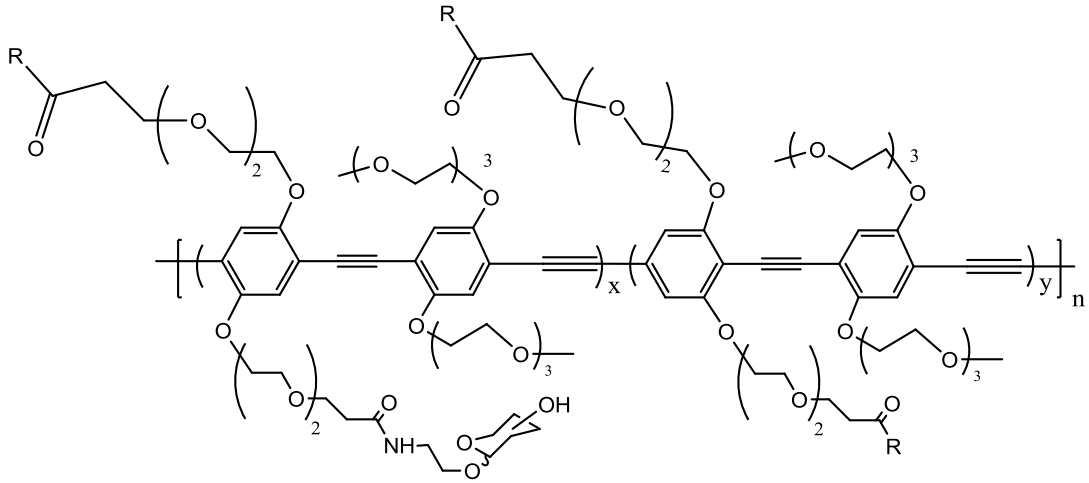


FIG. 1.9: Structure of the PPE polymer.

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. [21]

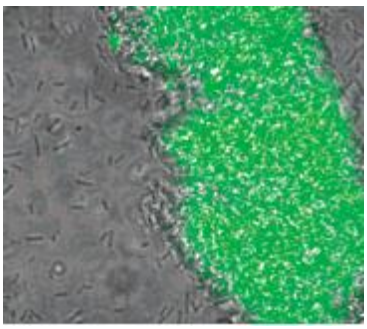


FIG. 1.10: A fluorescent bacterial aggregate due to multivalent interactions between the mannose-binding bacterial pili and the polymer.

Recently Disney and co-workers [22] 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

[20] Rojo, J.; Diaz, V.; de la Fuente, J.M.; Segura, I.; Barrientos, A.G.; Riese, H.H.; Bernad, A.; Penades, S. *ChemBioChem* **2004**, *5*, 291.
[21] Johnasson, S.M.C.; Arnberg, N.; Elofsson, M.; Wadell, G.; Kihlberg, H. *ChemBioChem* **2005**, *6*, 368.
[22] Disney, M.D.; Zheng, J.; Swager, T.M.; Seeberger, P.H. *J. Am. Chem. Soc.* **2004**, *126*, 13343.

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.[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.

[23] A review of some methods for pathogen detection : Willis, R.C. *Modern Drug Discovery*, **2004**, 36.

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. [24] 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.

[24] Information can be found in books on protecting groups: (a) Greene, T.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*, 4th ed., Wiley, New York, **2007**; (b) Kocienski, P. *Protecting Groups*, Georg Thieme Verlag, Stuttgart, **2000**, corrected edition.

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 continuosly 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

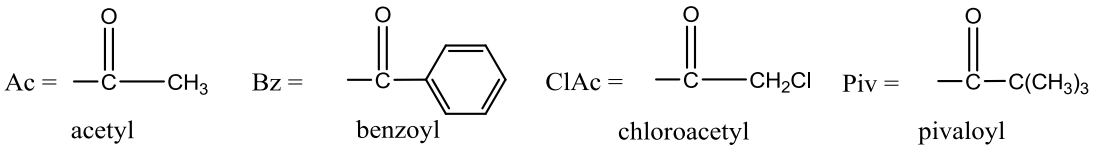


FIG. 1.11: Esters protecting groups in common use

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*-

[25] Ravidranathan Kartha, K.P.; Field, R.A. *Tetrahedron* **1997**, *53*, 11753.
[26] Haines, A. *Adv. Carbohydr. Chem. Biochem.* **1976**, *22*, 11.

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 NH_3 but they can be also cleaved using acid conditions (MeOH/HCl). [27] The relative order of stability of the most common ester groups reflects both steric and electronic aspects and is as follows:

$$\text{t-BuCO} > \text{PhCO} > \text{MeCO} > \text{ClCH}_2\text{CO}$$

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) [27] and in basic conditions ($Mg(OMe)_2$/MeOH). [28]

As far as the cleavage of chloroacetate esters is concerned, several mild reagents have been reported such as 2-mercaptoethylamine, thiourea or hydrazinedithiocarbonate ($H_2NNHC(=S)SH$). [29]

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

[27] Byramova, N.E.; Ovchinnikov, M.V.; Backinowsky, L.V.; Kochetov, N.K. *Carbohydr. Res.* **1983**, *124*, C8.
[28] Josephson, S.; Bundle, D.R. *Can. J. Chem.* **1979**, *53*, 3073.
[29] (a) Kihlberg, J.; Frejol, T.; Jansson, K.; Magnusson, G. *Carbohydr. Res.* **1986**, *152*, 113; (b) Trumtel. M.; Tavecchia, P.; Veyrières, A.; Sinaÿ, P. *Carbohydr. Res.* **1989**, *191*, 29.

catalytic amount of acid (generally TfOH) with good yields. [30] . Benzyl ethers are usually

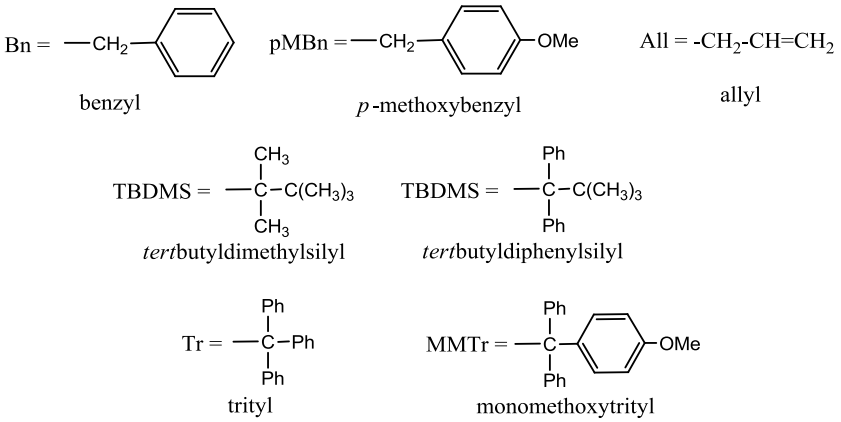


FIG.1. 12: Commonly used ether protecting groups

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. [31]

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

[30] Wessel, H.P.; Iversen, T.; Bundle, D.R. *J. Chem. Soc. Perkin Trans. 1* **1985**, 2247.
[31] Iseloh, U.; Dudkin, V.; Wang, Z.G.; Danishefsky, S. *Tetrahedron Lett.* **2002**, *43*, 7027.
[32] (a) Johansson, R.; Samuelsson, B. *J. Chem. Soc. Perkin Trans. 1* **1984**, 2371; (b) Nakajima, N.; Abe, R.; Yonemitsu, O. *Chem. Pharm. Bull.* **1988**, *36*, 4244.
[33] (a) Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 885; (b) Horita, K.; Yoshioka, T.; Tanaka, T.; Oikawa, Y.; Yonemitsu, O. *Tetrahedron* **1986**, *42*, 3021; (c) Garregg, P.J.; Olsson, L.; Oscarson, O. *J. Carbohydr. Chem.* **1993**, *12*, 955.

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). [34]

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.[35] Silyl ethers can be easily cleaved by treatment with fluoride ion (HF/acetonitrile; TBAF/THF).

Trityl 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

[34] Dahlén, A.; Sundgren, A,; Lahmann, M., Oscarson, S.; Hilmersson, G. *Org. Lett.* **2003**, *5*, 4058.
[35] Jones, S.S.; Reese, C.B. *J. Chem. Soc. Perkin Trans. 1* **1979**, 2762.

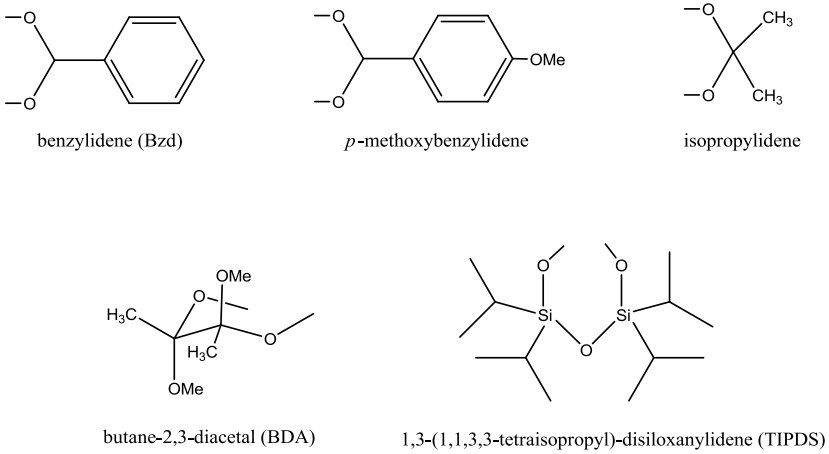


FIG. 1.13: Acetal and ketal protecting groups in common use.

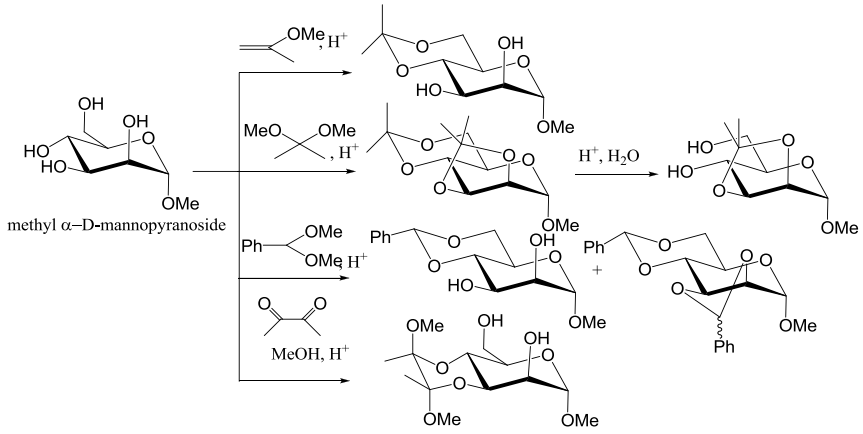


FIG. 1.14: Acetal and ketal formation on methyl α-D- mannopyranoside

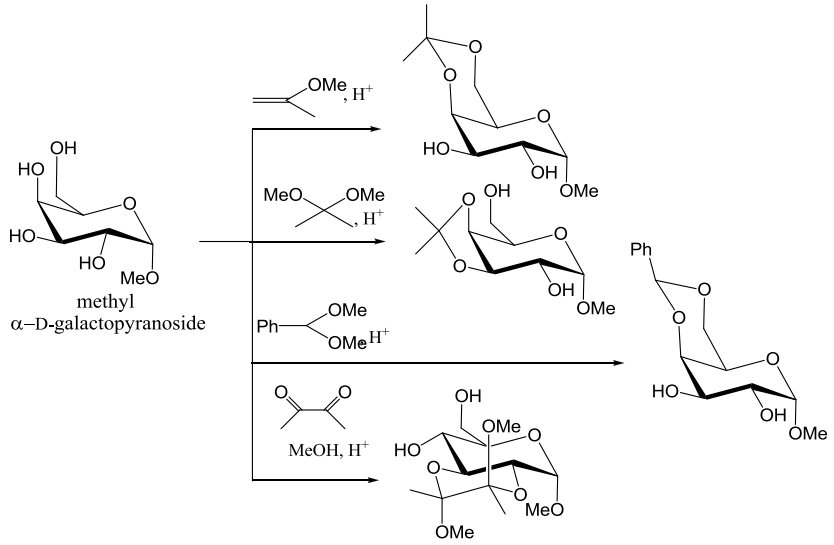


FIG. 1.15: Acetal and ketal formation on methyl α-D- galactopyranoside

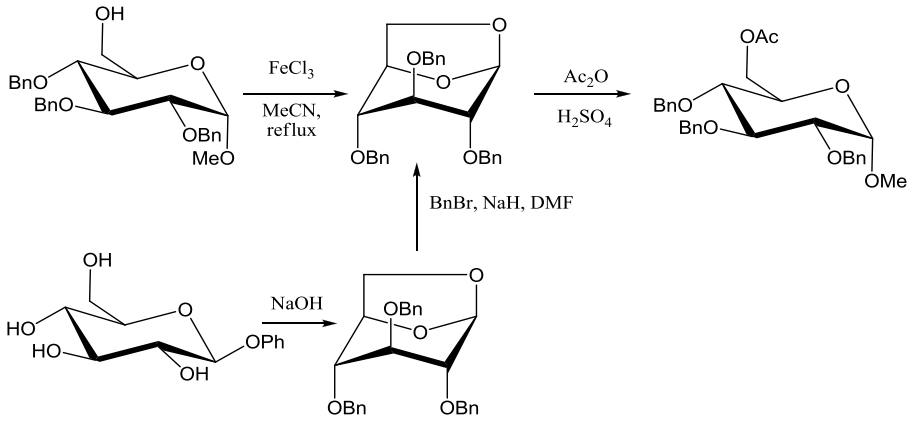


FIG. 1.16: Formation and opening of 1,6-anhydro derivative

stable as five-membered dioxolane rings formed on *cis*-diols. Examples of acetals formation and not selective deprotection are reported in FIG.1.14 and FIG.1.15An important alternative for benzylidene acetal is the more acid labile *p*-methoxybenzylidene acetal that can be cleaved selctively in the presence of the benzylidene one. In contrast to benzylidene, isopropylidene and *p*-methoxybenzylidene acetals, that exhibit all the same regioselectivity for vicinal *cis*-diols, the newly developed butane-2,3-diacetal (BDA) shows completely different regioselectivity providing simultaneous protection of *trans*-diols. [36]

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

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, [37] piperidine in THF).

[36] Ley, S.V.; Baeschlin, D.K.; Dixon, D.J.; Foster, A.C.; Ince, S.J.; Priepke, H.W.M.; Reynolds, D.J. *Chem. Rev.* **2001**, *101*, 53.

[37] (a) Excoffer, G.; Gagnaire, D.; Utille, J.P. *Carbohydr. Res.* **1975**, *39*, 368; (b) Nudelman, A.; Herzig, J.; Gottlieb, H.E.; Kerinan, E.; Sterling, J. *Carbohydr. Res.* **1987**, *162*, 145; (c) Gurjar, M.K.; Saha, U.K. Tetrahedron **1992**, *48*. 4039; (d) Nakano, T.; Ito, Y., Ogawa, T. *Carbohydr. Res.* **1993**, *243*, 43.

More specific anomeric 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 $BF_3 \cdot OEt_2$ 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 β-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.

[38] Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G.; Dahmen, J.; Noori, G.; Stenwall, K. *J. Org. Chem.* **1988**, *53*, 5629.
[39] Zhang, Z.; Magnusson, G. *Carbohydr. Res.* **1996**, *295*, 41.
[40] Cerny, M.; Stanek, J. *Adv. Carbohydr. Chem. Biochem.* **1977**, *34*, 23.
[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. [42]

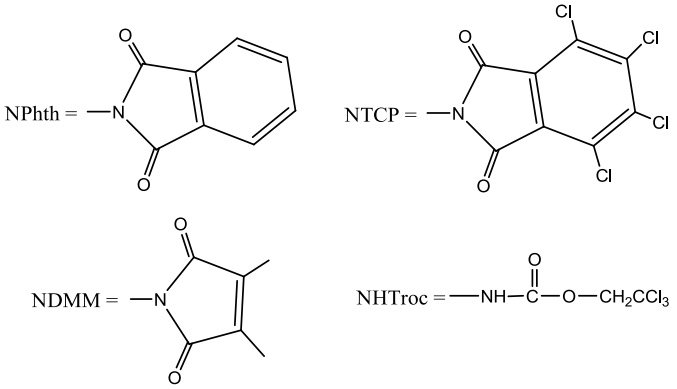


FIG. 1.17: Amino protecting groups in common use.

The first developed protecting group is the phthalimido that can be incorporated treating the substrate with phthalic anhydride in basic conditions. [43] 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 phtaloyl-based protecting groups such as the tetrachlorophtalimides (TCP) [44] and dimethylmaleoides (DMM) were developed: [45] they are formed in similar conditions but they can be removed under milder basic conditions. Another frequently

[42] (a) Banoub, J.; Boudlanger, P.; Lafont, D. *Chem. Rev.* **1992**, *92*, 1167; (b) Bongat, A.F.G.; Demchenko, A.V. *Carbohydr. Res.* **2007**, *342*, 364.
[43] Debenham, J.; Rodebaugh, R.; Fraser-Reid, B. *Liebigs. Annal.* **1997**, 791.
[44] Debenham, J.; Rodebaugh, R.; Roberts C.l.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117*, 3302.
[45] (a) Aly, M.R.E; Castro-Palomino, J.C.; Ibrahim, E.S.I.; El-Ashry, E.S.H.; Schmidt, R.R. *Eur. J. Org. Chem.* **1998**, *11*, 2305; (b) Aly, M.R.E; Ibrahim, E.S.I.; El-Ashry, E.S.H.; Schmidt, R.R. *Carbohydr. Res.* **2001**, *331*, 129.

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 Openig of the cyclic Acetals;*
- *Cyclic Orthoesters Opening.*

[46] Ellervik, U.; Magnusson, G. *Carbohydr. Res.* **1996**, *280*, 251.
[47] (a) Yan, R.B.; Yang, F.; Wu, Y.; Zhang, L.H.; Ye, X.S. *Tetrahedron Lett.* **2005**, *46*, 8993; (b) Titz, A.; Radic, Z.; Scwardt, O.; Ernst, B. *Tetrahedron Lett.* **2006**, *47*, 2383.
[48] Goddard-Borger, E. D.; Stick, R. V. *Org. Lett.*, **2007**, *9*, 3797.

- *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.

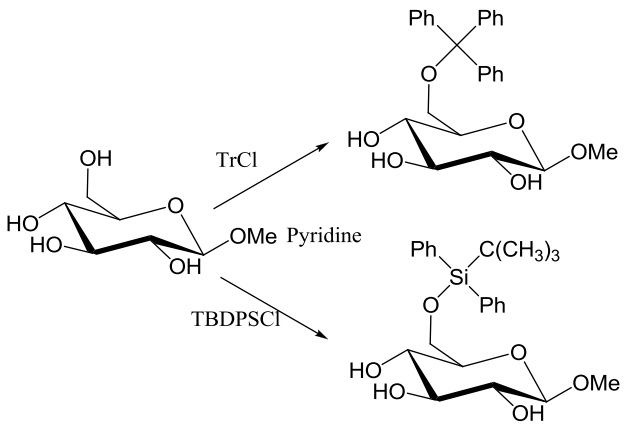


FIG. 1.18: Examples of selective protection of primary hydroxyl groups

Additionally, primary hydroxyl groups can be selectively benzoylated using special reagents as benzoyl cyanide/triethylamine [49] or 1-acyloxy-1*H*-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

[49] Rio, S.; Beau, J.M.; Jacquinet, J.C. *Carbohydr. Res.* **1994**, *255*, 103.
[50] Pelyvàs, I.F.; Lindhorst, T.K.; Streicher, H.; Thiem, J. *Synthesis* **1991**, 1015.
[51] Kadereit, D.; Waldmann, H. *Chem. Rev.* **2001**, *101*, 3367.

alkylation or acylation of saccharides possible. [52] 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. [53]

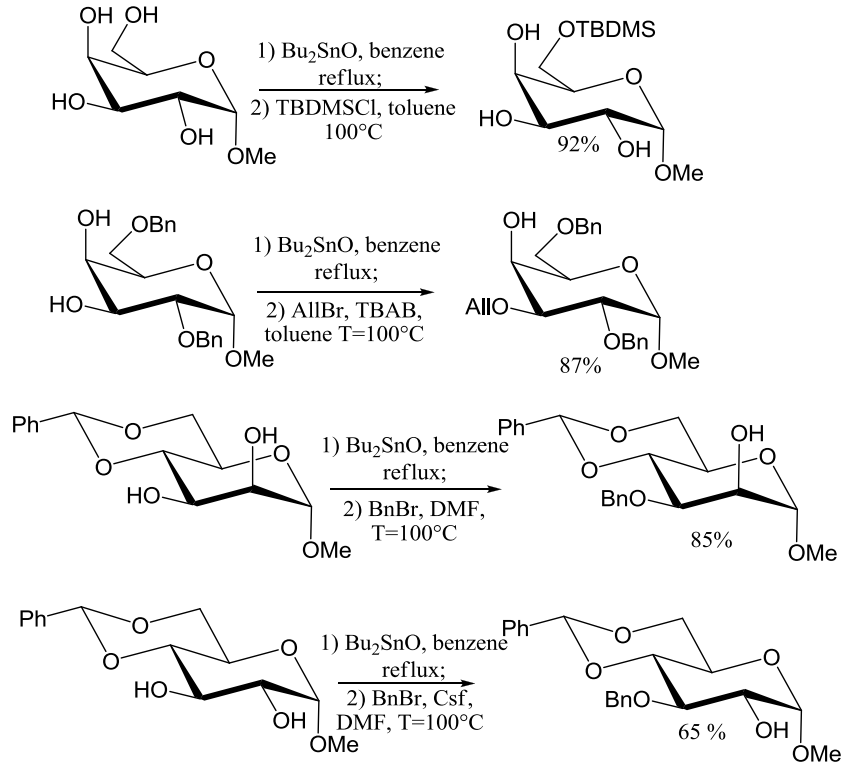


FIG. 1.19: Examples of stannyl activated regioselective protection

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

[52] Grindley, T.B. *Adv. Crbohydr. Chem. Biochem.* **1998**, *53*, 17.
[53] (a) David, S.; Pascard, C.; Cesaria, M. *J. Neouveau Chim.* **1979**, *3*, 63; (b) Cameron, T. S.; Bakshi, P. K.; Thangarasa, R.; Gridley, T. B. *Can. J. Chem.* **1992**, *70*, 1623; (c) Nagy, L.; Gyurcsik, B.; Burger, K.; Yamashita, S.; Yamaguchi, T.; Wakita, H.; Nomura, M. *Inorg. Chim. Acta* **1995**, *230*, 105, (d) Li, S.L.; Lan, Y.Q.; Ma, J.F.; Yang, J.; Zahng, M.; Su, Z.M. *Inorg. Chem.* **2008**, *47*, 2931.

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 [54] and so the methods is not very common (FIG.1.19).

- *Phase-Transfer Alkylation and Acylation:*

In this method a two-phase system (H_2O/CH_2Cl_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).

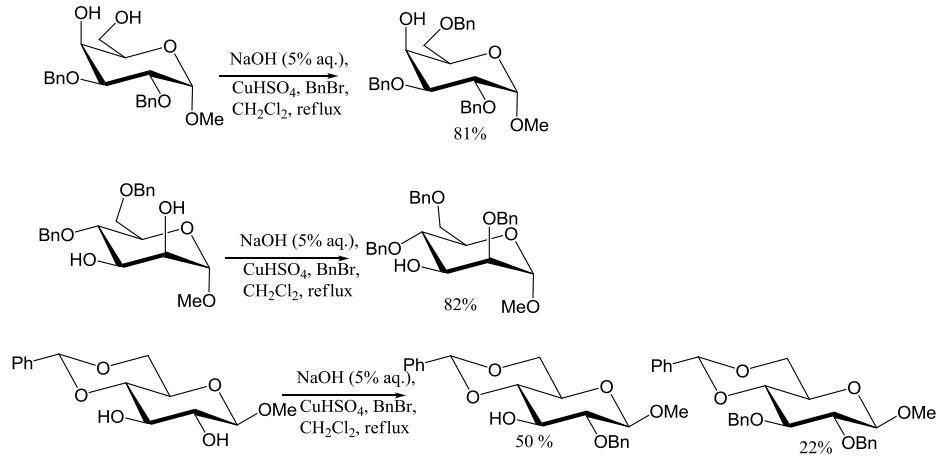


FIG. 1.20 Examples of phase-transfer benzylations

[54] Ysuda, Y.; Haque, M.E.; Yoshimoto, K. *Chem. Pharm. Bull.* **1983**, *31*, 1612.

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. [55]

- *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).

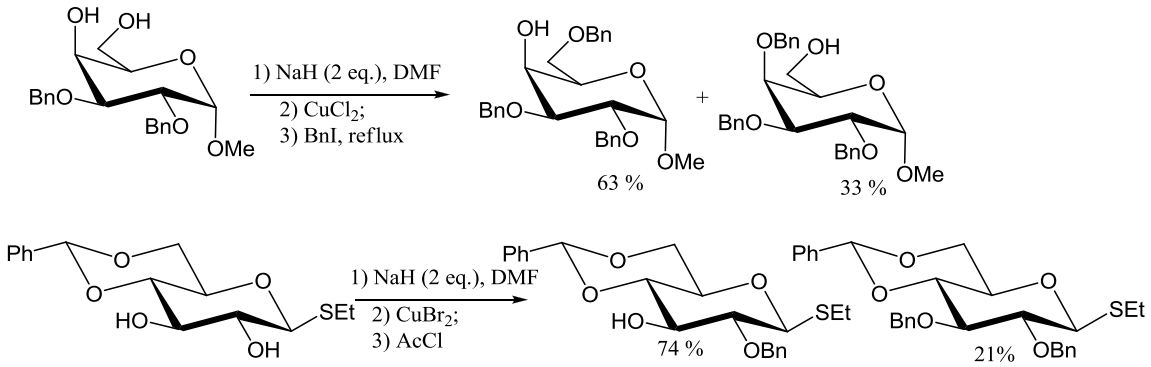


FIG. 1.21: Examples of regioselective protection from copper complexes

Using this technique both selective alkylations (allylation, benzylation) and acylations (acetylation, benzoylation and pivaloylation) are possible but often the outcomes depends on reaction conditions and on structural features. [56]

[55] Garegg, P.J.; Iversen, T.; Oscarson, S. *Carbohydr. Res.* **1976**, *53*, C-5.

- *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. [57]

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. [58] 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 (Table1.2).

The same procedures (with slight modifications) have been used also for the regioselective opening of other types of acetal protecting groups such as *p*-methoxybenylidene 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.

---

[56] Osborn, H.M.I.; Brome, V.A.; Harwood, L.M.; Suthers, W.G. *Carbohydr. Res.* **2001**, *332*, 157.
[57] Gelas, J, *Adv. Carbohydr. Chem. Biochem.* **1981**, *39*, 71; Garegg, P.J. in *Preparative Carbohydrate Chemistry*, Hanessian, S, Ed., Marcel Dekker, New York, **1997**, p. 53.
[58] de Ninno, M.P.; Etienne, J.B.; Duplantier, K.C. *Tetrahedron Lett.* **1995**, *36*, 669.

Benzylidene acetals can also be opened under oxidative conditions to give benzoyl esters halogen derivatives(FIG.1.23a) (which are useful for the synthesis of deoxy-sugars). [59] or simply hydroxyl benzoates (FIG.1.23b). [60]

Lewis Acid / Proton Acid	*Reducing Agent*	*Solvent*	*Product*
Ph_2BBr	PhS or BH_3·THF	CH_2Cl_2	6-OH [61]
Bu_2BOTf	BH_3·THF	CH_2Cl_2	6-OH [62]
$AlCl_3$	Me_3NBH_3	$PhCH_3$ or CH_2Cl_2 THF	6-OH 4-OH [63]
BF_3·OEt_2	Me_2NHBH_3	CH_2Cl_2 CH_3CN	6-OH 4-OH [64]
HCl	$NaCNBH_3$	THF	4-OH [65]
CF_3COOH	Et_3SiH	CH_2Cl_2	6-OH [58]
CF_3SO_3H	$NaCNBH_3$	THF	4-OH [61]
$Cu(OTf)_2$	BH_3·THF	CH_2Cl_2	6-OH [66]

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

[59] (a) Chen, Y.; Wang, P. G. *Tetrahedron Lett.* **2001**, *42*, 4955;(b) Karimi, B.; Rajabi, J. *Synthesis* **2003**, 2373; (c) Adinolfi, M.; Barone, G.; Guariniello, L.; Iadonisi, A. *Tetrahedron Lett.* **1999**, *40*, 8439; (d) Ponminor Senthil, K.; Amit, B.; Sundarababu, B. *Angew. Chem., Int. Ed.* **2010**, *49*, 804; (e) Stevénin, A.; Boyer, F.D.; Beau, J.M. *J. Org. Chem.* **2010**, *75*, 1783.

[60] Deslongchamps, P.; Moreau, C.; Fréhel, D.; Chênevert, R. *Can. J. Chem.* **1975**, *53*, 1204.

[61] Guindon, Y.; Girard, Y.; Berthiaume, S.; Gorys, V.; Lemieux, R.; Yoakim, C. *Can. J. Chem.* **1990**, *68*, 897.

[62] Jiang, L.; Chan, T.H. *Tetrahedron Lett.* **1998**, *39*, 355.

[63] Ek, M.; Garegg, P.J.; Hultberg, H.; Oscarson, S. *J. Crobohydr. Chem.* **1983**, *2*, 305

[64] Oikawa, M.; Liu, W.C.; Nakai, Y.; Koshida, S.; Fukase, K.; Kusumoto, S. *Synlett* **1996**, 1179.

[65] Garegg, P.J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97.

[66] Shie, C.R.; Tzeng, Z.H.; Kulkarni, S.S.; Uang, B.J.; Hsu, C.Y.; Hung, S.C. *Angew. Chem. Int. Ed.* **2005**, *44*, 1665.

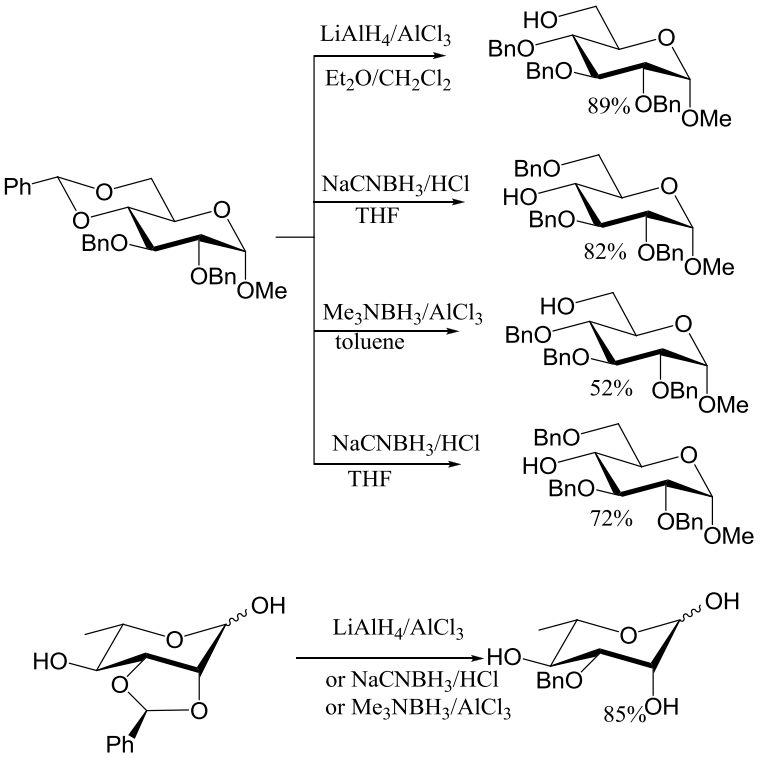


FIG. 1.22: Examples of regioselective reductive benzylidene opening

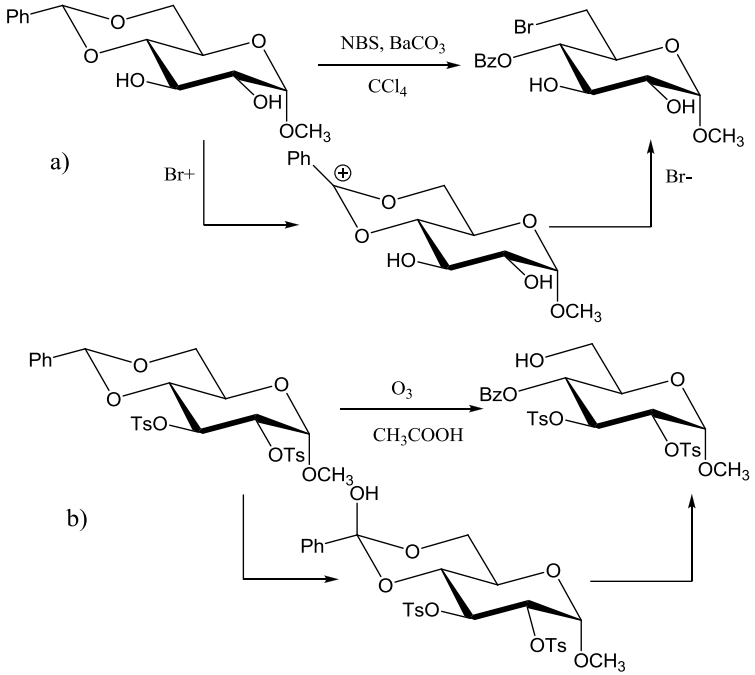


FIG. 1.23: Examples of oxidative opening of benzylidene acetals

- *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 advantage of the orthoester method is that they can be opened by mild hydrolysis to give the corresponding ester derivative exposing a free hydroxyl group. [67] 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).

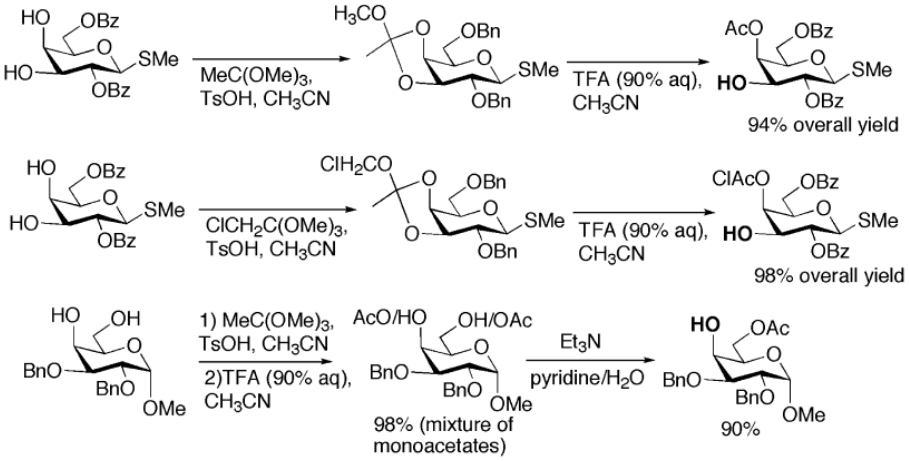


FIG. 1.24: Examples of orthoesters formation end openings.

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*. [68] When 2,3-*O*-

[67] Lemieux, R U, Driguez, H, *J. Am. Chem. Soc.* **1975**, *97*, 4069.
[68] Oscarson, S, Szönyi, M, *J. Carbohydr. Chem.* **1989**, *8*, 663.

orthoesters of thioglycosides are used, care must be taken to avoid participation of the sulfur affording the formation of 2-thiolated-*O*-alkyl glycosides. [69]

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 acetolysis (FIG. 1.25). [70]

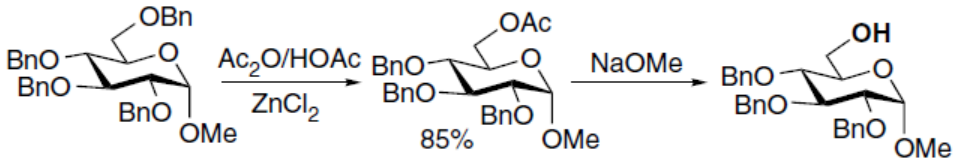


FIG. 1.25: Regioselective deprotection of aprimary hydroxyl group

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). [71]

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.

[69] (a) Pozsgay, V, A. *Carbohydr. Res.* **1992**, *235*, 295; (b) Auzanneau, F I, Bundle, D R, *Carbohydr. Res.* **1991**, *212*, 13.
[70] Yang, G.; Ding, X.; Kong, F. *Tetrahedron Lett.* **1990**, *31*, 1331.
[71] Boons, G.J.; Hale, K.J. in *Organic Synthesis with Carbohydrates*, Sheffield Academic Press **2000**, pp. 29.

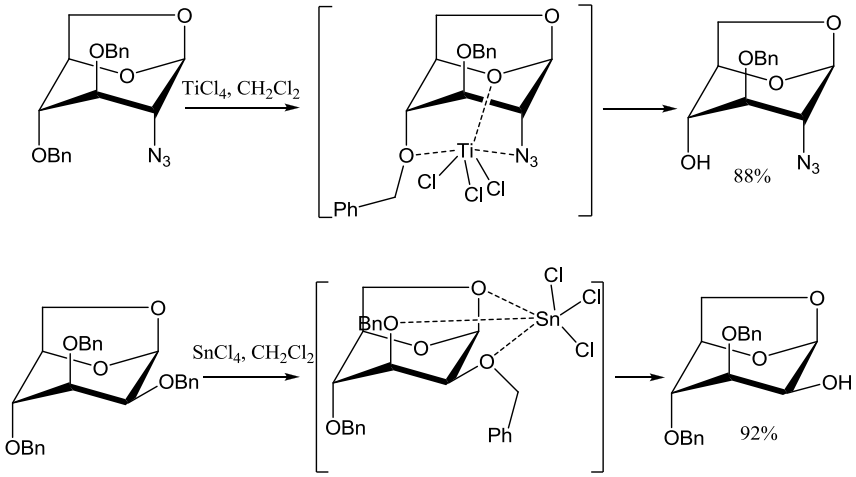


FIG. 1.26: Lewis acid catalyzed debenzylation.

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]

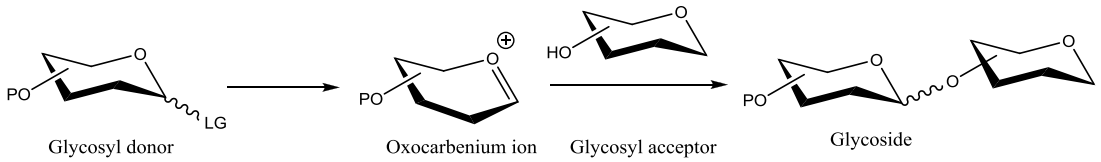


FIG. 1.27: Basic glycosylation rection mechanism

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

[72] Mydock, L.K.; Demchenko, A.V. *Org. Biomol.Chem.* **2010**, *8*, 497.
[73] Mootoo, R.U.; Konradsson, P.; Udodong, U.; Faser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 5583.

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

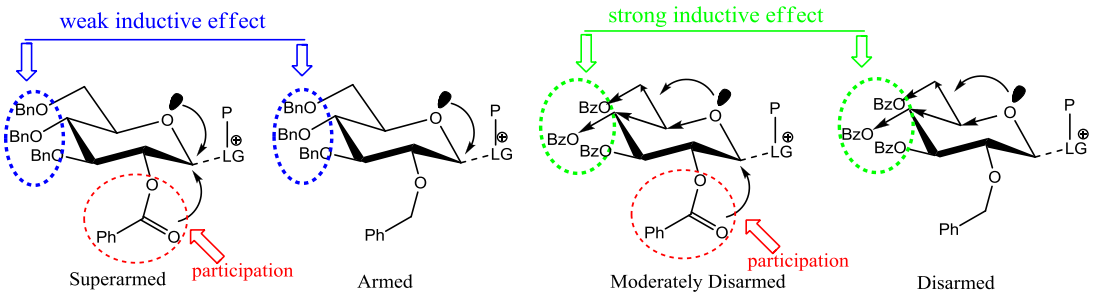


FIG. 1.28: Mixed protecting groups and O2/O-5 cooperative effect

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). [74] 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. [75] 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

[74] (a) Mydock, L.K.; Demchenko, A.V. *Org. Lett.* **2008**, *10*, 2107,(b)Mydock, L.K.; Demchenko, A.V. *Org. Lett.* **2008**, *10*, 2103; (c) Premathilake, H.D.; Mydock, L.K.; Demchenko, A.V. *J. Org. Chem.* **2010**, *75*, 1095.
[75] Crich, D.; Li, M. *Org. Lett.* **2007**, *9*, 4115.

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

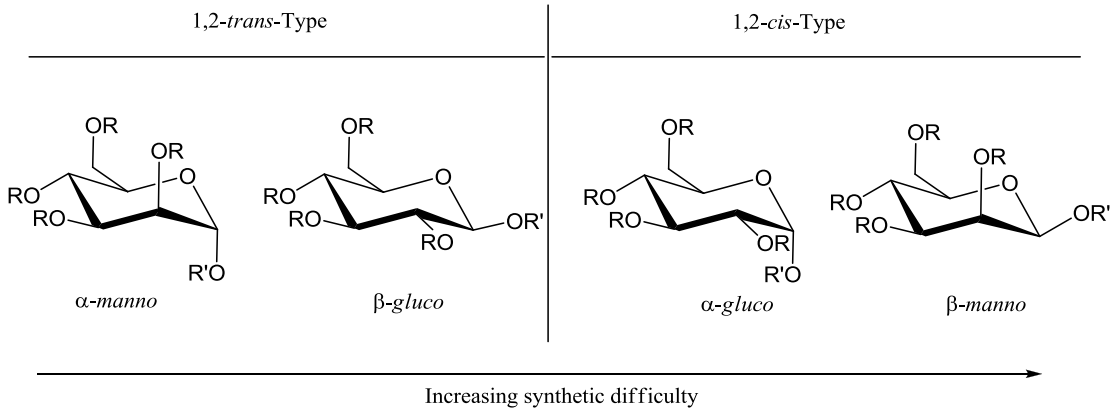
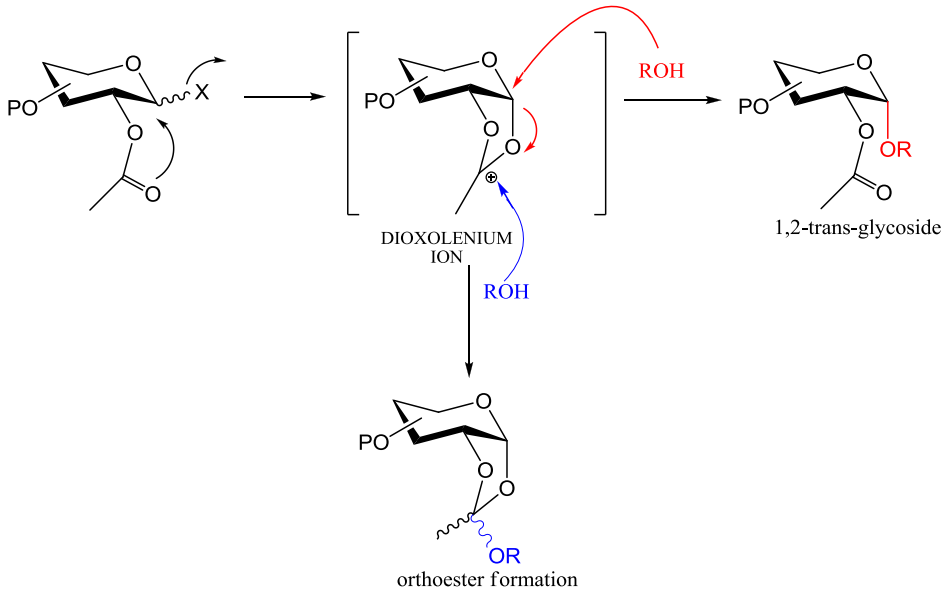


FIG. 1.29: Types of glycosidic bond.

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]

[76] Zeng, Y.; Ning, J.; Kong, F. *Carbohydr. Res.* **2003**, *338*, 307; (b) Zeng, Y.; Ning, J.; Kong, F. *Tetrahedron Lett.* **2002**, *43*, 3729; (c) Seeberger, P.H.; Eckhardt, M.; Gutteridge, C.E.; Danishefsky, S.J. *J. Am. Chem. Soc.* **1997**, *119*, 10064.



P= protecting group; X= leaving group; ROH= glycosyl acceptor

FIG. 1.30: Mechanism of the neighboring-group parteciaption.

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 methylsulfonylethoxycarbonyl (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-*cis* glycosides. This is the case of the chiral auxiliary groups developed by Boons and coworkers in 2005 for stereoselective glycosylation reactions. [82] The

[77] Yamada, T.; Takemura, K.; Yoshida, J.; Yamago, S. *Angew. Chem Int. Ed.* **2006**, *45*, 7575.
[78] Smoot, J.T.; Pornsuriyasak, P.; Demchenko, A.V. *Angew. Chem. Int. Ed.* **2005**, *44*, 7123.
[79] Yu, H.; Williams, D.L.; Ensley, H.E. *Tetrahedron Lett.* **2005**, *46*, 3417.
[80] Crich, D.; Cai, F. *Org. Lett.* **2007**, *9*, 1613.
[81] Ali, A.; van den Berg, R.; Overkleeft, H.S.; Filippov, D.V.; Van der Marel, G.A.; Codee, J. *Tetrahedron Lett.* **2009**, *50*, 2185.
[82] Kim, J.H.; Yang, H.; Boons, G.J. *Angew. Chem. Int. Ed.* **2005**, *44*, 947. For applications of this methodology, see: Kim, J.H.; Yang, H.; Boons, G.J. *J. Am. Chem. Soc.* **2005**, *127*, 12090.

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 α-glucan pentasaccharide found in *A. carmichaeli*. [84]

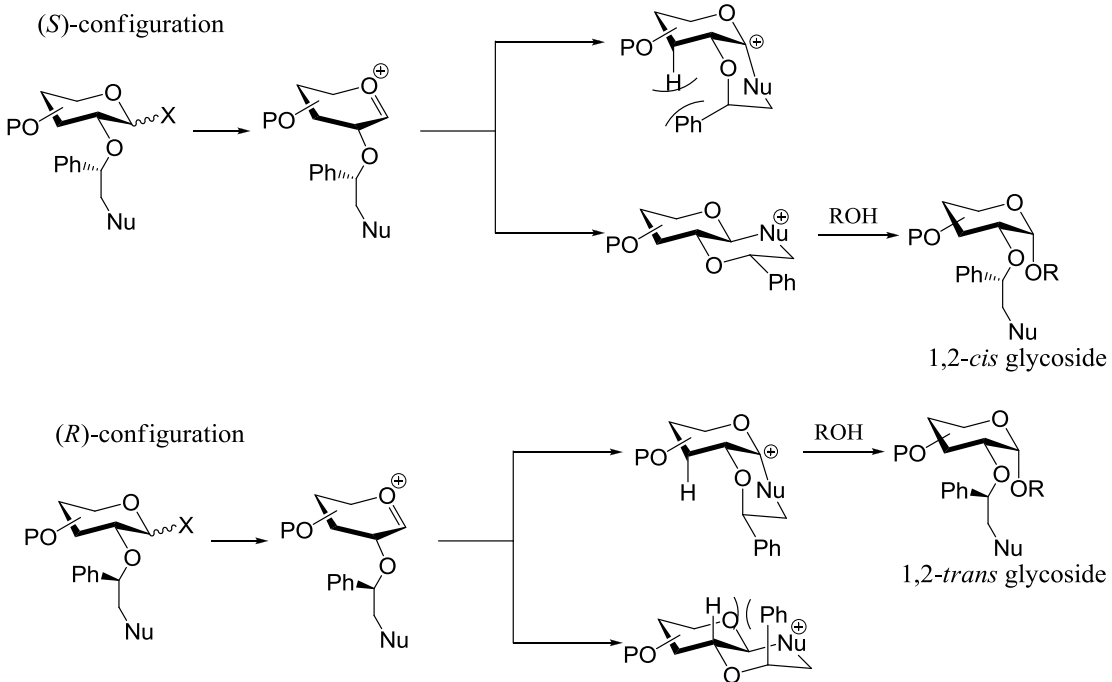


FIG. 1.31: Mechanism of chiral auxiliary group participating.

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

[83] Boltje, T.J.; Kim, J.; Park, J.; Boons, G.J. *Nat. Chem.* **2010**, *2*, 552.
[84] Zaho, C.; Li, M.; Luo, Y.; Wu, W. *Carbohydr. Res.* **2006**, *341*, 485.
[85] (a) Amin, M.N.; Ishiwata, A.; Ito, Y. *Tetrahedron*, **2007**, *63*, 8181; Park, J.; Boltje, T.; Boons, G.J. *Org. Lett.* **2008**, *10*, 4367; (b) Crich, D.; Hu, T.S.; Cai, F. *J. Org. Chem.* **2008**, *73*, 8942; (c) Baek, J.Y.; Lee, B.; Jo, M.G.; Kim. K.S. *J. Am. Chem. Soc.* **2009**, *131*, 17705.

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

When there is no a participating groups on the glycosyl donor, the anomeric 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 α-glycosides because of the preferential formation of the β-oxonium ion intermediate (for reverse anomeric effect) that reacts to form the α-glyoside.[86] Nitrile-type solvents have a well-known tendency to form β-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 α-face forming the kinetically controlled α-nitrilium-nitrile complex. This complex finally gives the β-glycoside after the attack of the glycosyl acceptor (in this case the equilibration process to the most thermodynamically stable β-nitrilium-

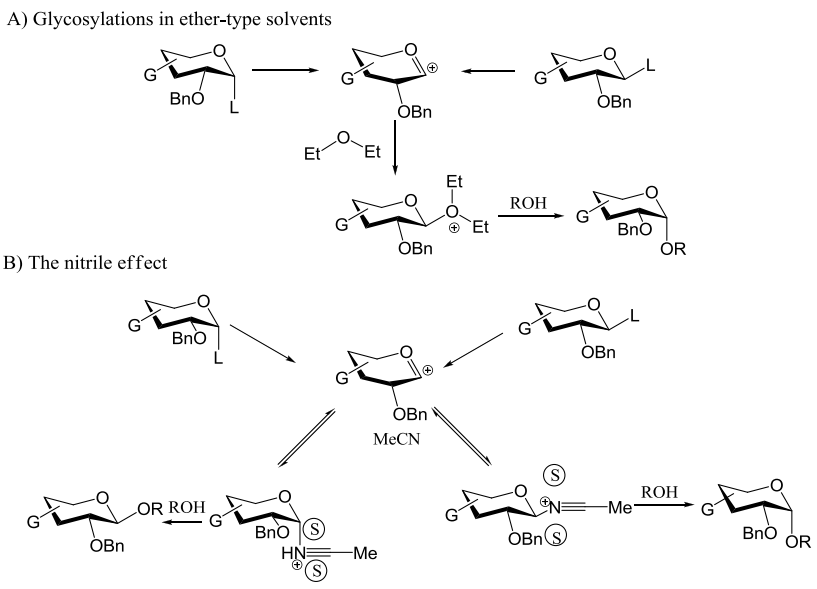


FIG. 1.32: Solvent effect in glycosylation reaction

[86] Lemieux, R. U. *Pure Appl. Chem.,* **1971***, 25*, 527.

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.

Glycosyl halides	*Thio derivatives*	*O-derivatives*	*Other derivatives*
Bromides and chlorides Fluorides Iodides	Thioglycosides Sulfoxides, sulfones	Imidates Esters *O*-Glycosides Hemiacetals 1,2-Anhydro derivatives Phosphites, phosphates Orthoesters Carbonates	Selenoglycosides Telluroglycosides

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-

[87] Vankar, D.; Vankar, P. S.; Behrendt, M.; Schmidt, R. R. *Tetrahedron* **1992**, *47*, 9985.
[88] For recent reviews see: (a) Zhu, X.; Schmidt, R.R. *Angew. Chem. Int. Ed.* **2009**, *48*, 1900; (b) Nicolaou, K.C.; Mitchell, H.J. *Angew. Chem. Int. Ed.* **2001**, *40*, 1576.

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 [89] reagent (Me_2N^+=CHClCl$^-$) 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_2CO_3 or Ag_2O, [90] silver imidazolate, [91] $Hg(CN)_2$ and $HgBr_2$, [92] HgO and $HgBr_2$ [93] and $AgClO_4$. [94] It is worth noting that besides heavy metals salts, glycosyl chlorides and bromides could be activated also with Lewis acids such as $SnCl_4$, $BF_3{\cdot}OEt_2$, [95] $ZnCl_2$, $ZnCl_2$ –TrCl, [96] $Sn(OTf)_2$, [97] $Cu(OTf)_2$ [98] or $InCl_3$. [99]

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 $AgClO_4/SnCl_2$. [100] Several methods for the activation of glycosyl fluorides have been reported [101] and

---

[89] Spohr, U.; Lemieux, R.U. *Carbohydr. Res.* **1988**, *174*, 211.
[90] (a) Koenigs, W; Knorr, E, *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 957; (b) Wulff, G, Röhle, G, *Angew. Chem. Int. Ed. Engl.* **1974**, *13*, 157; (c) Igarashi, K, *Adv. Carbohydr. Chem. Biochem.* **1977**, *34*, 243.
[91] Garegg, P. J.; Johansson, R.; Samuelsson, B. *Acta Chem. Scand. B* **1982**, *36*, 249.
[92] Helferich, B.; Berger, A. *Chem. Ber.* **1957**, *90*, 2492.
[93] Schroeder, L.R.; Green, J.W. *J. Chem. Soc. C* **1966**, 530.
[94] Bredereck, H.; Wagner, A.; Geissel, D.; Ott, H. *Chem. Ber.* **1962**, *95*, 3064.
[95] Ogawa, T.; Matsui, M., *Carbohydr. Res.* **1976**, *51*, C13.
[96] Higashi, K.; Nakayama, K.; Soga, T.; Shioya, E.; Uoto, K.; Kusama, T. *Chem. Pharm. Bull.* **1990**, *38*, 3280.
[97] (a) Lubineau, A.; Malleron, A. *Tetrahedron Lett.* **1985**, *26*, 1713; (b) Lubineau, A.; Le Gallic, J.; Malleron, A. *Tetrahedron Lett.* **1987**, *28*, 5041.
[98] Yamada, H.; Hayashi, T. *Carbohydr. Res.* **2002**, *337*, 581.
[99] Mukherjee, D.; Ray, P. K.; Chowdhury, U.S. *Tetrahedron* **2001**, *57*, 7701.
[100] Mukaiyama, T.; Murai, Y.; Shoda, S, *Chem. Lett.* **1981**, 431.
[101] Toshima, K. *Carbohydr. Res.* **2000**, *327*, 15-26.

now commonly used promoters are $BF_3{\cdot}OEt_2$, [102] Cp_2MCl_2-$AgClO_4$ (M=Hf, Zr), [103] and Cp_2HfCl_2-AgOTf. [104] 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) [105] or the reaction of a lactol with DAST or 2-fluoro-1-methylpyridinium *p*-toluensulfonate. [106]

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. [107]

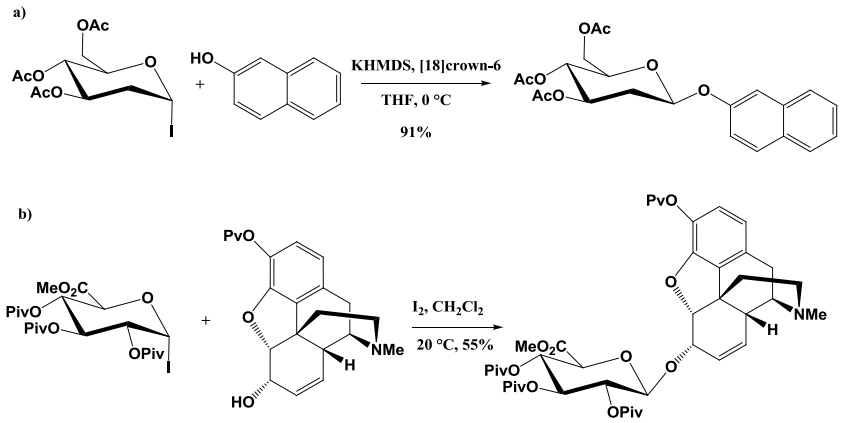


FIG. 1.34: Examples of glycoside synthesis with glycosyl iodides as donors.

Glycosylation reactions involving glycosyl iodides occurs usually with a S_N2 displacement of the anomeric iodide and this feature is of particular interest when there

---

[102] (a) Nicolaou, K.C.; Chucholowski, A.; Dolle, R.E.; Randall, J.L. *J. Chem. Soc., Chem. Commun.* **1984**, 1155; (b) Kunz, H.; Sager, W. *Helv. Chim. Acta* **1985**, *68*, 283.

[103] (a) Matsumoto, T.; Maeta, H.; Suzuki, K.; Tsuchihashi, G. *Tetrahedron Lett.* **1988**, *29*, 3567; (b) Suzuki, K.; Maeta, H.; Matsumoto, T.; Tsuchihashi, G. *Tetrahedron Lett.* **1988**, *29*, 3571; (c) Matsumoto, T.; Maeta, H.; Suzuki, K.; Tsuchihashi, G., *Tetrahedron Lett.* **1988**, *29*, 3575; (d) Matsumoto, T.; Katsuki, M.; Suzuki, K. *Chem. Lett.* **1989**, 437–440.

[104] (a) K. Suzuki, H. Maeta, T. Suzuki, T. Matsumoto, *Tetrahedron Lett.* **1989**, *30*, 6879; (b) Nicolaou, K.C.; Caulfield, T.J.; Kataoka, H.; Stylianides, N.A. *J. Am. Chem. Soc.* **1990**, *112*, 3693; (c) K.C. Nicolaou, K.C.; Hummel, C.W.; Iwabuchi, Y. *J. Am. Chem. Soc.* **1992**, *114*, 3126.

[105] Nicolaou, K.C.; Dolle, R.E..; Papahatjis, D.P.; Randall, J.L. *J. Am. Chem. Soc.* **1984**, *106*, 4189.

[106] Posner, G.H.; Haines, S:R: *Tetrahedron Lett.* **1985**, *26*, 5; (b) Rosenbrook, W.; Riley, D.A.; Lartley, P.A. *Tetrahedron Lett.* **1985**, *6*, 3.

[107] (a) Miquel, N.; Vignando, S.; Russo, G.; Lay, L. *Synlett* **2004**, *2*, 341; (b) van Well, R.M.; Kartha, K.P.R.; Field, R.A. *J. Carbohydr. Chem.* **2005**, *24*, 463; (c) Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 7863.

is not a participating protecting group at *C*-2 [108] 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-glucurunide, an important starting material for pharmacological evaluation. [109]

1.2.2.2.2 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.* $BF_3{\cdot}OEt_2$). [110] 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 [111] or per-*O*-acetylated glycosyl iodide). [112] This procedure has the advantage to avoid the use of thiols that are well-known smelly reactants.

[108] Lam, S.N.; Gervay-Hague, J. *Org. Lett.* **2003**, *5*, 4219.
[109] Bickley, J.; Cottrell, J.R.; Ferguson, J.R.; Field, R.A.; Harding, J.R.; Hughes, D.L.; Kartha, K.P.R.; Law, J.L.; Scheinmann, F.; Stachulski, A.V. *Chem. Comm.* **2003**, 1266.
[110] (a) Tai, C.A.; Kulkarni, S.S.; Hung, S.C. *J. Org.Chem.* **2003**, *68*, 8719, (b) Hasegawa, J.Y.; Hamada, M.; Miyamoto, T.; Nishide, K.; Kajimoto, T.; Uenishi, J.I.; Node, M. *Carbohydr. Res.* **2005**, *340*, 2360; (c) Kajimoto, T.; Ishioka, Y.; Katoh, T.; Node, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 57736.
[111] Ibatullin, F.M.; Selivanov, S.I.; Shavva, A.G. *Synthesis* **2001**, 419; (b) Ibatullin, F.M.; Shabalin, K.A.; Jänis, J.V.; Shavva, A.G. *Tetrahedron Lett.* **2003**, *44*, 7961.
[112] Valerio, S.; Iadonisi, A.; Adinolfi, M.; Ravidà. A. *J. Org. Chem.* **2007**, *72*, 6097.

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

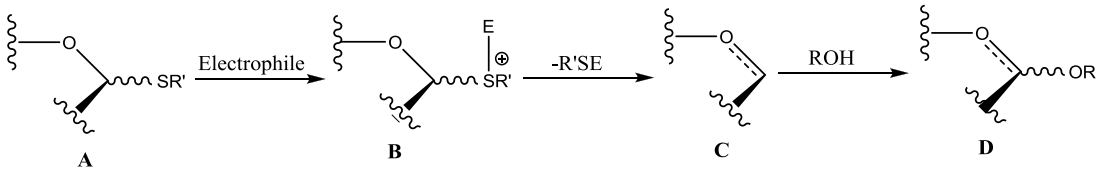


FIG. 1.35 Activation of thioglycosides by electrophilic agents.

group and the oxonium ion (**C**) is rapidily formed. [113]

The first method for the activation of thioglycosides was reported by van Boom and co-workers and is based on the use of a stoichiometric amount of *N*-iodocuccinimide (NIS) with a catalytic amount of triflic acid used as promoter. [114] Other *in situ* generating iodonium systems have been proposed (*e.g.* NIS/AgOTf) [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 $TrB(C_6F_5)_4$ as promoter systems. [116] Recently, also bromonium ion (generated *in situ* by the reaction of *N*-bromosuccinimide and a catalytic amount of $Bi(OTf)_3$) has been employed for the activation of thioglycosides. [117] Other activation methods are based nowadays on soft sulfur electrofile. Initially sulfonium or sulfenyl triflates (DMTST, MeSOTf and PhSOTf) were used but recently sulfenamide activators in combination with Lewis acids were proposed. [118] Sulfinates in combination with Tf_2O 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.
[114] Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331.
[115] Konradsson, P.; Udodong, U.E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313.
[116] Takeuchi, K.; Tamura, T.; Mukaiyama, T. *Chem. Lett.* **2000**, 124.
[117] Valerio, S.; Iadonisi, A.; Adinolfi, M.; Ravidà, A. *J. Org. Chem.* **2007**, *72*, 6097.
[118] (a) Jona, H.; Takeuchi, K.; Saitoh, T.; *Chem. Lett.* **2000**, 124; (b) Durón, S.G.; Polat, C.H.; Wong, C.H. *Org. Lett.* **2004**, *6*, 839.

activate thioglycosides also at low temperature. [119] Sulfinyl-systems mediated glycosylations have been used for the synthesis of numerous complex structures. [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_2O as promoter in the preactivation steps. [121]

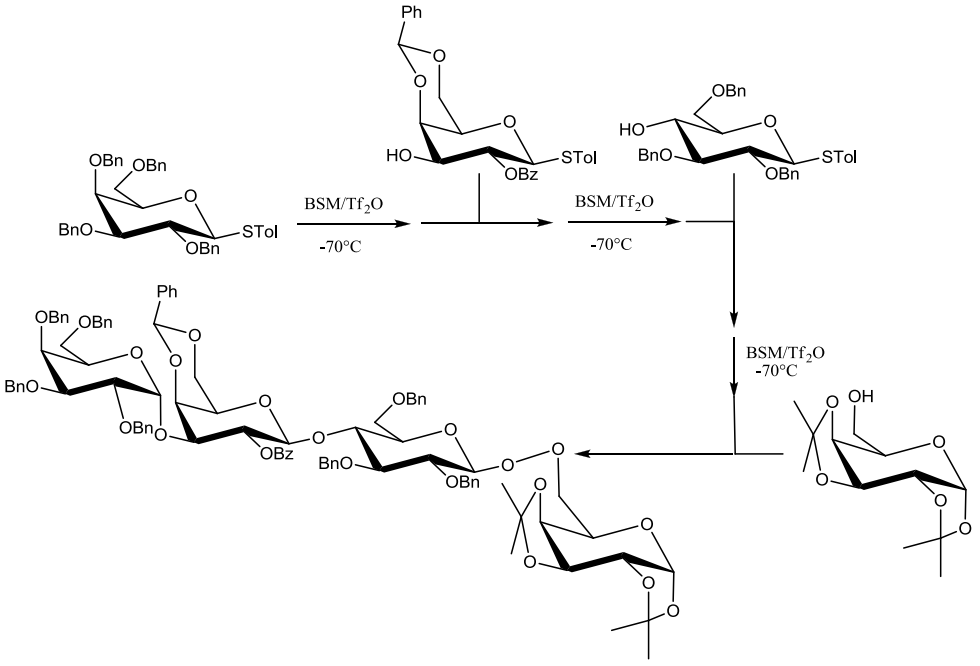


FIG. 1.36: Oligosaccharides synthesis with thioglycosides as donors.

[119] Codée, J.D.C., van den Bos, L.J.; Litjens, R.E.J.N.; Overkleeft, H.S.; van Boeckel, C.A.A.; van Boom, J.H.; van der Marel, G.A. *Tetrahedron* **2004**, *60*, 1057; (b) van den Bos, L.J.; Duivenvoorden, B.A.; de Konong, M.C.; Filippov, D.V.; Overkleeft, H.S.; van der Marel, G.A. *Eur. J. Org. Chem.* **2007**, 116.
[120] Crich, D.; Li, H. *J. Org. Chem.* **2002**, *67*, 4640.
[121] Wang, C.; Wang, H.; Huang, X.; Zhang, L.H.; Ye, X.S. *Synlett* **2006**, 2846.

1.2.2.2.3 O-Glycosyl Imidates

O-Glycosyl imidates (and in particular trichloracetimidates and more recently also *N*-phenyltrifluoroacetimidates) [122] are probably the most common glycosyl donors used in glycosylation reactions.

Glycosyl trichloroacetimidates are usually prepared by treatment with the electron-deficient trichloroacetonitrile (Cl_3CCN) that adds to the hydroxyl of lactols under basic conditions. In presence of a weak base (*e.g.* K_2CO_3), 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]

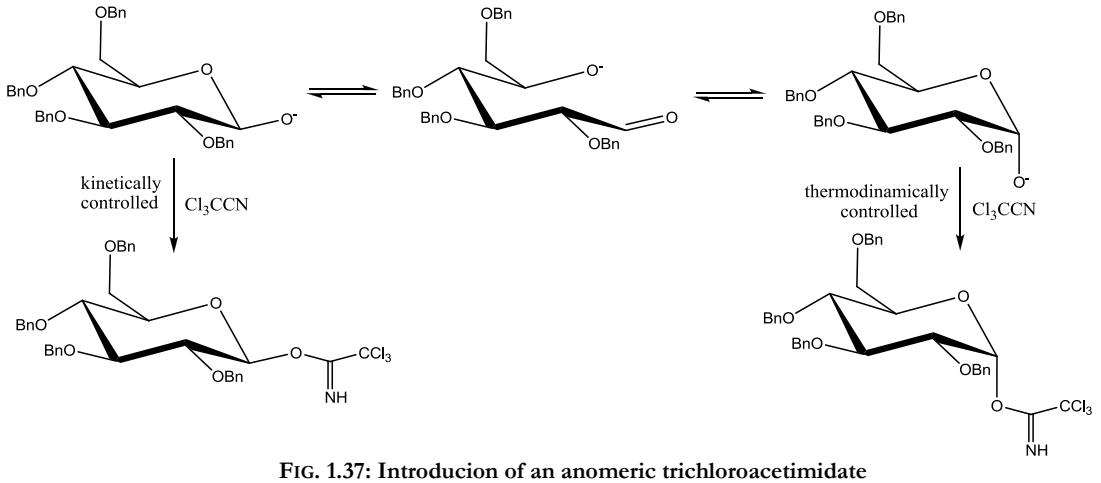


FIG. 1.37: Introducion of an anomeric trichloroacetimidate

Recently also polymer-supported DBU [124] and TBD (1,5,7-triazabicyclo[4.4.0]dec-5-ene) [125] have been employed for the synthesis of trichloroacetimidates.

The most common activation catalysts for trichloroacetimidates are TMSOTf [126] and $BF_3{\cdot}OEt_2$ (using the latter promoter at very low temperature and in absence of a

[122] Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405.
[123] Schmidt, R.R. in *Preparative Carbohydrate Chemistry* (Ed.; Hanessian S.) **1997**, Marcel Dekker, INC.; p. 283.
[124] Ohashi, I.; Lear, M.J.; Yoshimura, F.; Hirama, M. *Org. Lett.* **2004**, *6*, 719.
[125] Oikawa, M.; Tanaka, N.; Fukuda, N.; Kusumoto, S. *Tetrahedron Lett.* **2004**, *45*, 4039.

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). [127] 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, [128] pyridinium *p*-toluensulfonate (PPTS), [129] and $ZnBr_2$). [130]

Various metal triflates ($Sm(OTf)_3$, [131] $Sn(OTf)_2$, [132] $Yb(OTf)_3$, [133] and $Cu(OTf)_2$ [134]) 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. [135] More recently, silica supported perchloric acid ($HClO_4$-SiO_2), [136] $HB(C_6F_5)_4$, [137] I_2/Et_3SiH system [138] and acid washed molecular sieves [139] were used as efficient promoters in various glycosylation reactions with trichloroacetimidates as glycosyl donors.

---

[126] (a) Schmidt, R.R.; Grundler, G. *Angew. Chem. Int. Ed.* **1982**, *21*, 781; (b) Grundler, G; Schmidt, R.R. *Liebigs Ann. Chem.* **1984**, *2*, 1826.
[127] Schmidt, R.R.; Michel, J. *Angew. Chem. Int. Ed.* **1980**, *19*, 731
[128] Fügedi, P. *J. Carbohydr. Chem.* **1987**, *6*, 377; (b) Fügedi, P.; Nánási, P.; Szejtli, J. *Carbohydr. Res.* **1988**, *175*, 173.
[129] (a) Nicolaou, K.C.; Daines, R.A.; Chakraborty, T.K.; Ogawa, Y. *J. Am. Chem. Soc.* **1987**, *109*, 2821; (b) Nicolaou, K.C.; Daines, R.A.; Ogawa, Y.; Chakraborty, T.K. *J. Am. Chem. Soc.* **1988**, 110, 1988.
[130] Urban, F.J.; Moore, B.S.; Breitenbach, R. *Tetrahedron Lett.* **1990**, *31*, 4421.
[131] Adinolfi, M.; Barone, G.; Guariniello, L.; Iadonisi, A. *Tetrahedron Lett.* **2000**, *41*, 9005.
[132] (a) Castro-Palomino, J.C.; Schmidt, R.R. *Tetrahedron Lett.* **1995**, *36*, 5343; (b) Bartek, J.; Müller, R.; Kosma, P. *Carbohydr. Res.* **1998**, *308*, 259.
[133] Adinolfi, M.; Barone, G.; Iadonisi, A.; Mangoni, L.; Schiattarella, M. *Tetrahedron Lett.* **2001**, *42*, 5967.
[134] Yamada, H.; Hayashi, T. *Carbohydr. Res.* **2002**, *337*, 581.
[135] Wei, G.; Gu, G.; Du, Y. *J. Carbohydr. Chem.* **2003**, *22*, 385.
[136] Du, Y.; Wei, G.; Cheng, S.; Hua, Y.; Linhardt, R.J. *Tetrahedron Lett.* **2006**, *47*, 307.
[137] Jona, H.; Mandai, H.; Mukaiyama, T. *Chem. Lett.* **2001**, 426.
[138] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Synlett.* **2002**, 269.
[139] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Org. Lett.* **2003**, *5*, 987.

Besides the introduction of new promoters, a new technique in the glycosylation reaction is the so called *inverse procedure* (FIG. 1.38). [140] 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). [141] In the inverse procedure it is thought that acceptor molecules aggregate around the catalyst and the intramolecular glycosylation takes place.

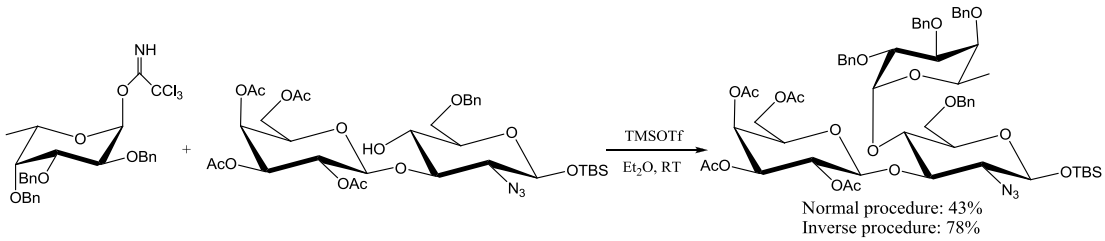


FIG. 1.38:Yield difference between normal and inverse procedure.

Glycosyl trichloroacetimidates have been largely used up to now in the synthesis of a large numbers of biological relevant molecules such as calicheamicin, [142] eleutherobin, [143] glycosphingolipids [144] and vancomycin. [145]

Glycosyl *N*-phenyltrifluoroacetimidates (PTFAI) are generally prepared treating an anomeric hemiacetal with *N*-phenyltrifluoroacetyl chloride with a base (K_2CO_3, [146] DBU, [147] DIPEA, [148] NaH [149] or Cs_2CO_3) [150] in CH_2Cl_2 or THF (FIG. 1.39). This type of

[140] Schmidt, R.R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353.
[141] (a) Ferguson, J.R.; Harding, J.R.; Lumbard, K.W.; Scheinmann, F.; Stachulski, A.V. *Tetrahedron Lett.* **2000**, *41*, 389; (b) de Paz, J.L.; Ojeda, R.; Reichardt, N.; Martìn-Lomas, M. *Eur. J. Org. Chem.* **2003**, 3308; (c) Fürstner, A.; Jeanjean, F.; Razon, P.; Wirtz, R.; Mynott, R. *Chem. Eur. J.* **2003**, *9*, 320.
[142] Halcomb, R.L; Boyer, S.H.; Danishesky , S.J. *Angew. Chem. Int. Ed.* **1992**, *31*, 338; (b) Nicolau, K.C.; Schreiner, E.P.; Iwabuchi, Y.; Suzuki, T. *Angew. Chem. Int. Ed.* **1992**, *31*, 340.
[143] (a) Nicolaou, K.C.; van Delft, F.L.; Ohshima, T.; Vourloumis, D.; Xu, J.; Hosokawa, S.; Pfefferkorn, S.; Kim, S.; Li, T. *Angew. Chem. Int. Ed.* **1997**, *36*, 2520; (b) Nicolaou, K.C.; Ohshima, T.; Hosokawa, S.; van Delft, F.L.; Vourloumis, D.; Xu, J.; Pfefferkorn, S.; Kim, S. *J. Am. Chem. Soc.* **1998**, *120*, 8674.
[144] Toepfer, A.; Schmidt, R.R. *Tetrahedron Lett.* **1992**, *33*, 5161; (b) Mayer, T.G.; Kratzer, B.; Schmidt, R.R. *Angew. Chem. Int. Ed.* **1994**, *33*, 2177.
[145] Nicolaou, K.C.; Mitchell, H.J.; van Delft, F.L.; Rübsam, F.; Rodriguez, R.M. *Angew. Chem. Int. Ed.* **1998**, *37*, 1871.
[146] Yu, B.; Tao, H. *J. Org. Chem.* **2002**, *67*, 9099.
[147] Doi, T.; Kinbara, A.; Inoue, H.; Takahashi, T. *Chem.-Asian J.* **2007**, *2*, 188.
[148] Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.*. **2002**, *43*, 5573.
[149] Comegna, D.; Bedini, E.; di Nola, A.; Iadonisi, A.; Parrilli, M. *Carbohydr. Res.* **2007**, *342*, 1021.
[150] Tanaka, H.; Iwata, Y.; Takahashi, D.; Adachi, M.; Takahashi, T. *J. Am. Chem. Soc.* **2005**, *127*, 1630.

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]

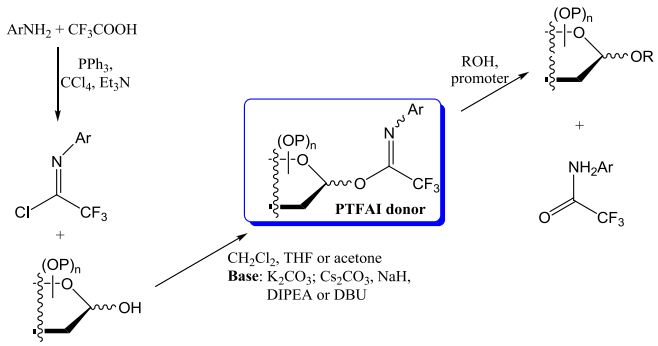


FIG. 1.39: Preparation of glycosyl PTFAI donors.

The great diffusion of this type of glycosyl donors can be explained in term of a greater stability respect to the trichloracetimidates [152] and of a minimization of the by-products often encountered with trichloroacetimidates 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

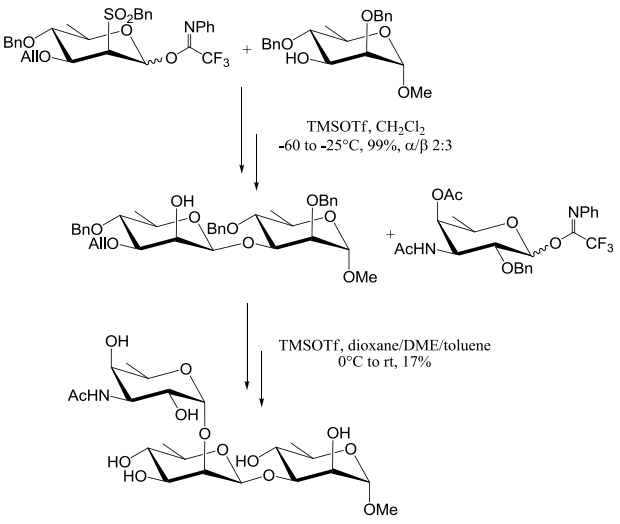


FIG. 1.40: Synthesis of *O*-antigen trisaccaride from *Xanthomonas Campestris*

[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.
[153] Tanaka, S.I.; Takashina, M.; Tokimoto, H.; Fujimoto, Y.; Tanake, K.; Fukase, K. *Synlett* **2005**, 2325.

polysaccharide fragment of *Bacillus anthracis*. [154] PTFAI donors has been extremely useful in glycosylation rections involving deoxysugars [150] as, for example, in the synthesis of the *O*-antigen from *Xanthomonas campestris* pv *campestris* (FIG. 1.40). [155] 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.

[154] Vasan, M.; Rauvolfova, M.; Wolfert, M.A.; Christine, L.; Kannenberg, E.L.; Quinn, C.P.; Carlson, R.W.; Boons, G.J. *ChemBioChem* **2008**, *9*, 1716.
[155] Comegna, D.; Bedini, E.; Parrilli, M. *Tetrahedron* **2008**, *64*, 3381.

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;
2) Cirillo, L.; Di Nola, A.; Bedini, E.; Parrilli, M. "The role of sugar configuration in the acetolysis of 6-deoxyhexose methyl glycosides"; *Carbohydr. Res.* **2009**, 344, 2406-2411;

3) Cirillo, L.; Bedini, E.; Molinaro, A.; Parrilli, M. "Synthesis of a β-GlcN-(1→4)-MurNAc building block *en route* to *N*-deacetylated peptidoglycan fragments"; *Tetrahedron Lett.* **2010**, *51*, 1117;

4) Cirillo, L.; Silipo, A.; Bedini, E.; Parrilli, M. "A urea-linked glucosamine dimer as building block for the synthesis of linear and cyclic neosaccharides"; *Eur. J. Org. Chem.* **2010**, 4062;

5) Bedini, E.; Cirillo, L.; Cumpstey, I.; Parrilli, M. "Acetolysis of 6-deoxysugars governed by armed-disarmed effect"; *Carbohydrate Chem.: Proven Methods*, **2011**, *1*, (Ed: Kovac, P.; Taylor and Francis, Boca Raton, FL, USA) in press.

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. [156] 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. [157] The first step is the activation of endocyclic or exocyclic anomeric oxygen toward glycoside cleavage promoted by acetylium ion, which is formed from Ac_2O 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

[156] *The Polysaccharides*; Aspinall, G.O., Ed.; Academic Press: London, United Kingdom, 1982; Vol.1, pp 64-66.

[157] (a) Guthrie, R. D.; McCarthy, J. F. Adv. *Carbohydr. Chem. Biochem.* **1967**, *22*, 11; (b) Lichtenthaler, F. W.; Bambach, G. *Carbohydr. Res.* **1979**, *68*, 305; (c) Dasgupta, F.; Singh, P. P.; Srivastava, H. C. *Ind. J. Chem.* **1988**, *27B*, 527; (d) Kanie, O.; Takeda, T.; Ogihara, Y. *Carbohydr. Res.* **1990**, *197*, 289; (e) McPhail, D. R.; Lee, J. R.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1992**, *114*, 1905; (f) Kaczmarek, J.; Preyss, M.; Lönnberg, H.; Szafranek, J. *Carbohydr. Res.* **1995**,*279*, 107; (g) Miljkovic´ , M.; Yeagley, D.; Deslongchamps, P.; Dory, Y. L. *J. Org. Chem.* **1997**, *62*, 7597; (h) Kaczmarek, J.; Kaczyński, Z.; Trumpakaj, Z.; Szafranek, J.; Bogalecka, M.; Lönnberg, H. *Carbohydr. Res.* **2000**, *325*, 16; (i) Forsman, J. J.; Wärnå, J.; Murzin, D. Y.; Leino, R. *Carbohydr. Res.* **2009**, *344*, 1102.

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]

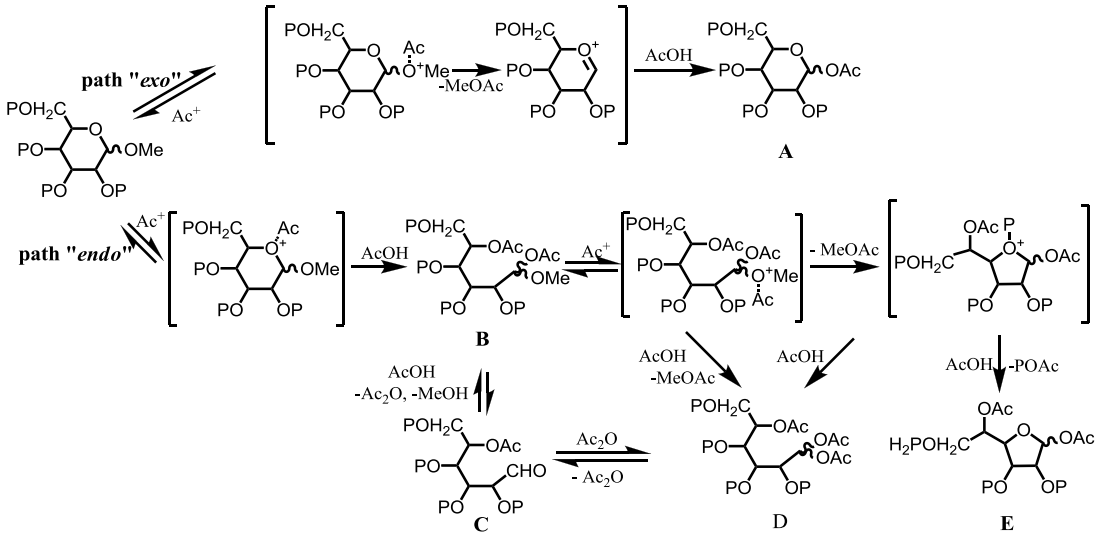


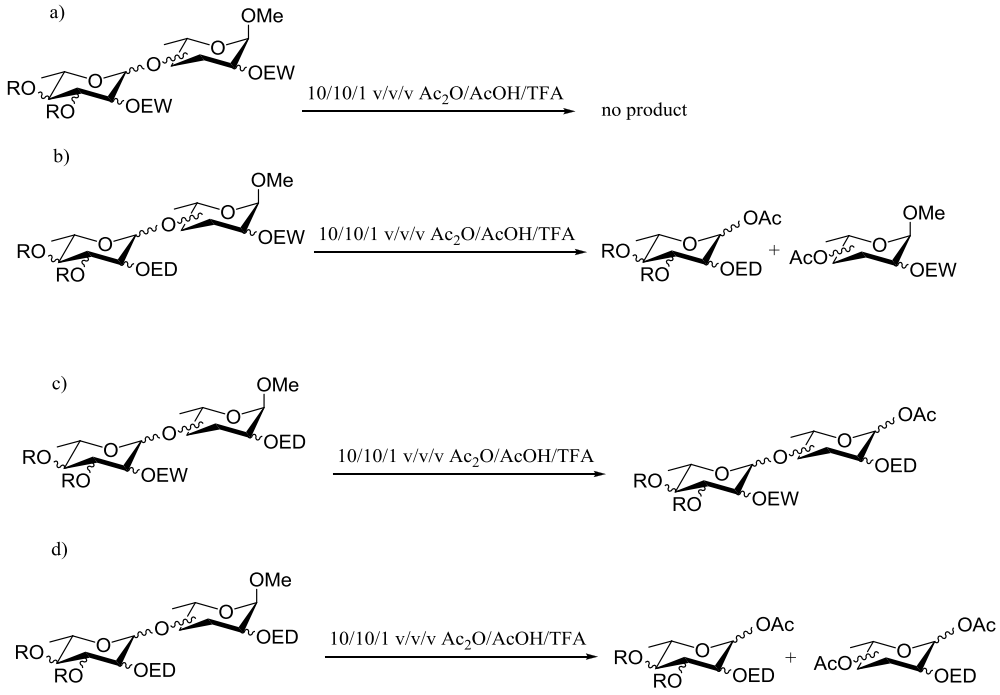
FIG. 2.1: *Endo*- versus *exo*-activation in acetolysis reactions

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

[158] (a) Angibeaud, P.; Bosso, C.; Utille, J.-P. *Carbohydr. Res.* **1990**, *198*, 403; (b) Wang, L.-X.; Lee, Y. C. J. *Chem. Soc., Perkin 1* **1996**, 581; (c) Bozó, É.; Boros, S.; Kuszmann, J. *Carbohydr. Res.* **2001**, *332*, 325; (d) Wu, Q. P.; Zhou, M.-X.; Xi, X.-D.; Song, D.; Wang, Y.; Liu, H.-X.; Li, Y.-Z.; Zhang, Q.-S. *Tetrahedron Lett.* **2008**, *49*, 2714.

[159] (a) Paulsen, H.; Lorentzen, J.P. *Carbohydr. Res.* **1987**, *165*, 207; (b) Banaszek, A.; Ciunik, Z. *Tetrahedron Lett.* **1997**, *38*, 273; (c) Banaszek, A. *Carbohydr. Res.* **1998**, *306*, 379.

carbohydrates in O-antigen lipopolysaccharides, [160] glycoproteins, glycolipids, and in other glycoconjugates such as cardioglycosides, natural antibiotics and anticancer agents. [161] Recently, it has been reported [162] that the outcome of acetolysis reaction with 10/10/1 (v/v/v) Ac_2O/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.



ED= electron donating group; **EW**= electron withdrawing group.

FIG. 2.2: Acetolysis reactions governed by arming/disarming effect.

[160] P.E. Jansson in *Endotoxin in Health and Disease* (Eds.: H. Brade, D.C. Morrison, S. Vogel), Marcel Dekker, New York, **1999**, p.155.
[161] He, X.M.; Liu, H.W. *Annu. Rev. Biochem.* **2002**, *71*, 701
[162] Bedini, E.; Comegna, D.; di Nola, A.; Parrilli, M. *Tetrahedron Lett.* **2008**, *49*, 2546.

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_2O/AcOH [164] (10 instead of 20 eq. of $ZnCl_2$, 5°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).

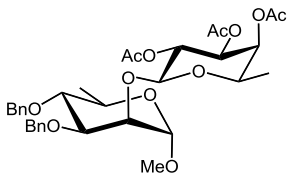
In next sections it will be therefore discussed the possibility to obtain kinetically controlled **B**-, **C**-, **D**-, **E**-like disaccharide products and which role the configuration of the sugar has in the outcome of the acetolysis reactions.

2.2 Results and Discussion

In order to investigate the role of sugar configuration in driving the acetolysisreaction, 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

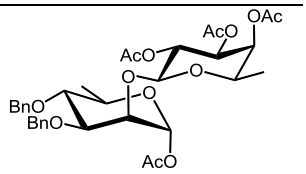
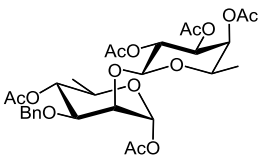
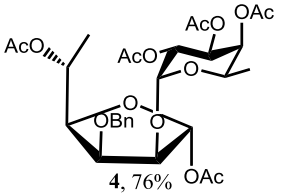
[163] Lam, S.N.; Gervay-Hague, J. *Carbohydr. Res.* **2002**, *337*, 1953.
[164] Yang, G.; Ding, X.; Kong, F. *Tetrahedron Lett.* **1997**, *38*, 6725.
[165] Valero, S.; Iadonisi, A.; Adinolfi, M.; Ravidà, A. *J. Org. Chem.* **2007**, *72*, 6097.
[166] Maddali, U.B.; Ray, A.K.; Roy, N. *Carbohydr. Res.* **1990**, *208*, 59.
[167] Smith III, A.B.; Rivero, R.A.; Hale, K.J.; Vaccaro, H.A. *J. Am. Chem. Soc.* **1991**, *113*, 2092.

Table 2.1: Screening of acetolysis conditions on model compound 1: [a] Reaction conditions: *A*: 10/10/1 (v/v/v) Ac_2O/AcOH/TFA, 70°C; *B*: 10/10/1 (v/v/v) Ac_2O/AcOH/TFA, 5°C; *C*: 100/1 (v/v) Ac_2O/H_2SO_4, 0°C; *D*: $ZnCl_2$ (10 eq), 2:1 (v/v) Ac_2O/AcOH, 5°C. [b] Isolated yield.



1

Model disaccharide

Entry	*Protocol* [a]	*Product (% Yield)*[b]
1	***A***	 **2**, 95%.
2	***B***	*No reaction*
3	***C***	 **3**, 25%.
4	***D***	 **4**, 76%

phase transfer benzylation of diol **16**).[169] Glycosylations under the NIS/TfOH protocol proceeded in moderate to excellent yields to give the disaccharide methyl glycosides **18**-**24**. Disaccharides **25** and **26** were obtained by de-*O*-benzensulfonylation of **18** [6] with sodium amide and subsequent acetylation or benzoylation of the resulting alcohol **19**. It is worth noting that all the disaccharides were synthesized with a disarming ester protecting group at the 2-positions of the non-pseudo-reducing unit in order to avoid the acetolytic cleavage of glycosidic linkage.

[168] Yang, C.; Cao, L.; Kong, F. *J. Carbohydr. Chem.* **1992**, *11*, 379.
[169] Liptàk, A.; Nánási, P.; Neszmélyi, A.; Wagner, H. *Tetrahedron* **1980**, *36*, 1261.

The obtained products were then subjected to acetolysis reaction by the $ZnCl_2$ protocol described above as well as with the 10/10/1 (v/v/v) Ac_2O/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

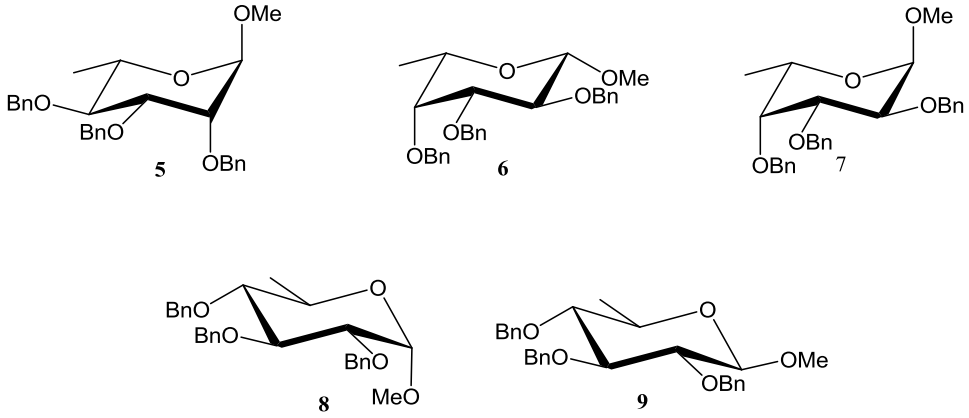


FIG. 2.3: 6-deoxymethyl glycosides used in acetolysis reaction

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 $ZnCl_2$ in 2:1 (v/v) Ac_2O/AcOH was strictly dependent on the sugar configuration. In fact on *rhamno*-configured units, acetolysis with $ZnCl_2$ 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

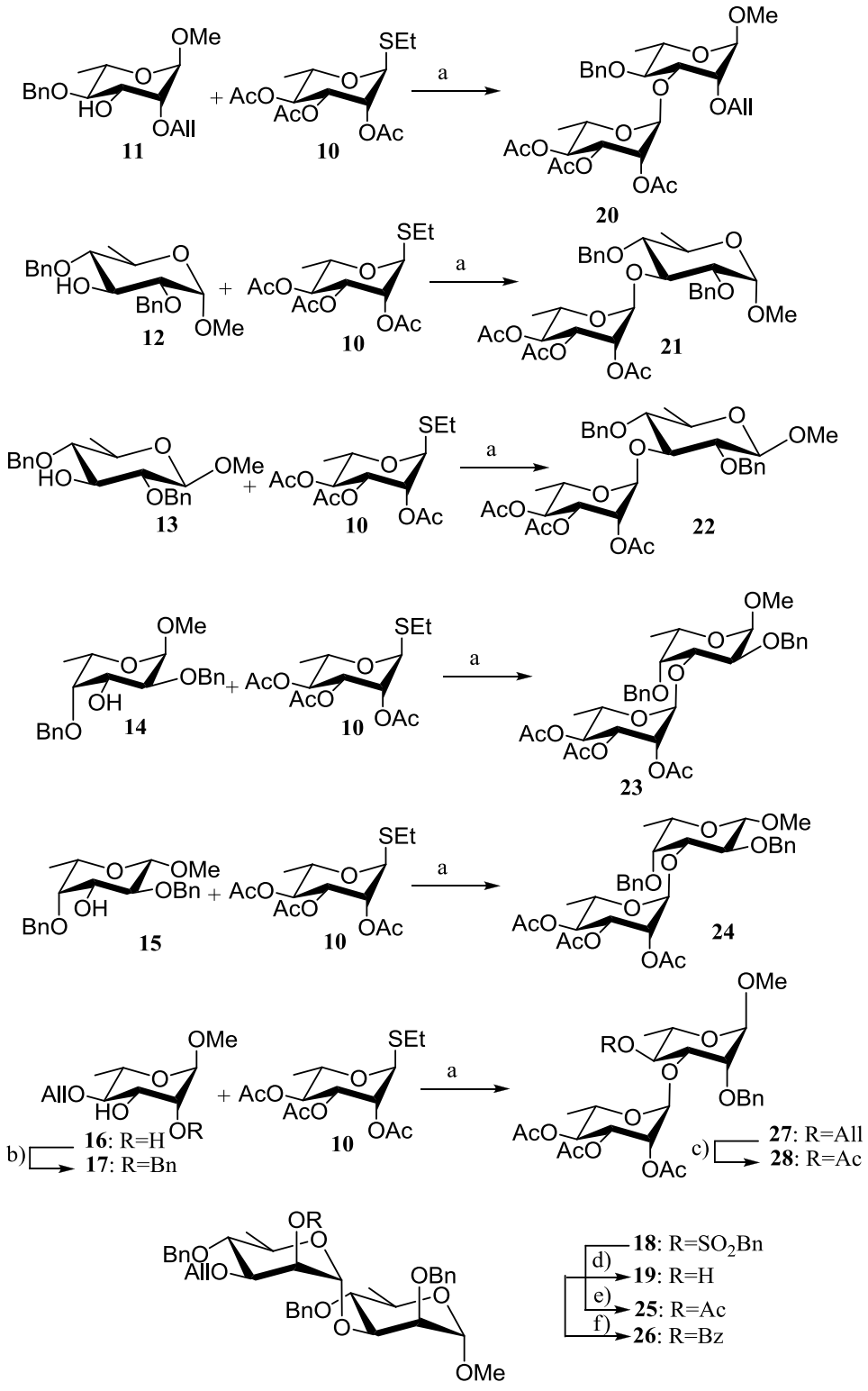


FIG. 2.4: Synthesis of a set of 6-deoxysugar disaccharide methyl glycosides. Reagents and conditions: (a) NIS, TfOH, AW-300 MS (4Å), CH_2Cl_2, -20°C, **20**: 49%; **21**: 83%; **22**: 82%, **23**: 87%; **24**: 88%; **25**: 40%; BnBr, KOH, TBAI, toluene, room temp. 72%; (c) i. $PdCl_2$, 1:1 (v/v) CH_2Cl_2/MeOH, room temp.; ii. Ac_2O, py, room temp. 75% (after two steps); (d) $NaNH_2$, DMF, 70°C, 64%; (e) Ac_2O, 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 4_A-*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*-4_A-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 Zn^{2+}) should be taken in account.

In contrast to *rhamno*-configured species, all the experiments with *fuco*-configured species (**6**, **7**, **23** and **24**) with $ZnCl_2$ 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 $ZnCl_2$ 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 $ZnCl_2$ protocol toward am *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 $n \rightarrow \sigma^*$ donation to the C_1-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-*quinovo*-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 [170] 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; [2g] 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. [171] Interestingly, the analogies of the results on mono- and disaccharides [172] 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.

[170] Jensen, H.H.; Lyngbye, L.; Bols, M. *Angew. Chem. Int. Ed.* **2001**, *40*, 3447.
[171] McDonnell, C.; López, O.; Murphy, P.; Fernández Bolaños, J.G.; Hazell, R.; Bols, M. *J. Am. Chem. Soc.* **2004**, *126*, 12374.
[172] Bedini, E.; Cirillo. L.; Cumpstey, I.; Parrilli, M. In *Carbohydrate Chemistry: Proven Methods*; Kovac, P. Ed.; Taylor & Francis: Boca Raton, FL, USA, in press.

Table 2.2: Acetolysis of 6-deoxysugar *rhamno* configured methyl glycosides: [a] Isolated yield; [b] Anomeric ratio measured by ^{1}H NMR spectroscopy; [c] Anomeric ratio measured by isolation of the two anomers.

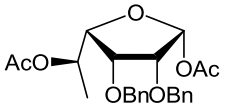
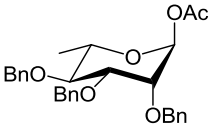
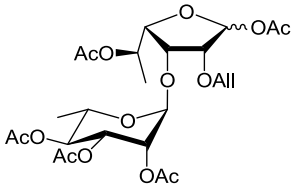
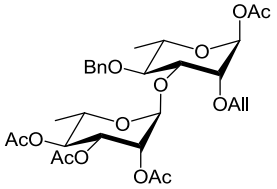
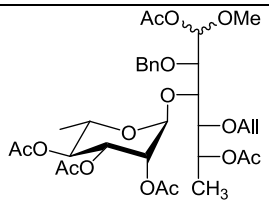
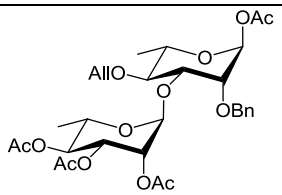
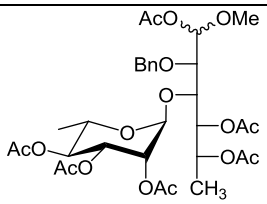
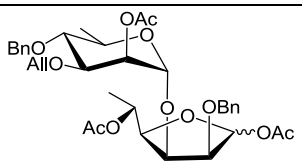
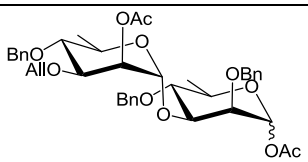
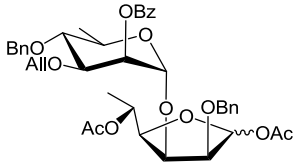
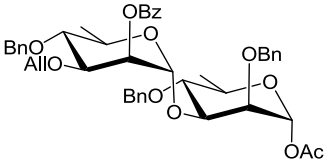
Entry	***Methyl Disaccaride***	***Products (% yield)*** [a]	
		***Protocol A*: $ZnCl_2$ (10eq.), Ac_2O, 5°C**	***Protocol B*: 1:1:0.1 v/v/v Ac_2O/AcOH/TFA**
1	**5**	 **29**, 63%; **30** 22% [b]	 **30**, 62%
2	**20**	 **31**, 64%; α/β =2.:1 [b]	 **32**, 55%
3	**27**	 **33**, 77%; *dr*=6:1 [b]	 **34**, 73%
4	**28**	 **35**, 66%; *dr*=1.3:1 [b]	**Not determined**
5	**25**	 **36**, 71% [c]	 **37**, 74%; α/β =2.5:1
6	**26**	 **38**, 62%	 **39**, 75%; α/β =2:1[b]

Table 2.3: Acetolysis of 6-deoxysugar *fuco* configured methyl glycosides: [a] Isolated yeld; [b] Anomeric ratio measured by ^{1}H NMR spectroscopy.

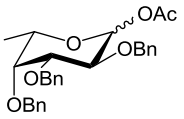
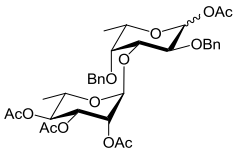
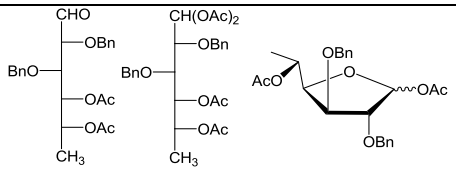
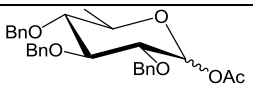
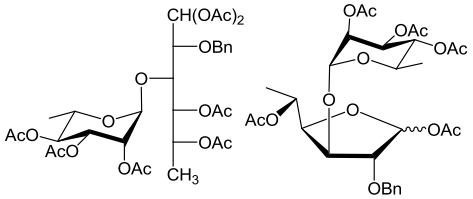
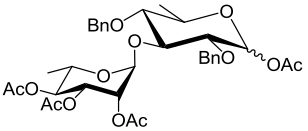
Entry	***Methyl Disaccaride***	***Products (% yield)*** [a]	
		***Protocol A*: $ZnCl_2$ (10eq.), Ac_2O, 5°C**	***Protocol B*: 1:1:0.1 v/v/v Ac_2O/AcOH/TFA**
1	**6**	 **40**, 83%; α/β =3:1 [b]	**40**, 72%; α/β =5:1 [b]
2	**7**	**40**, 99%; α/β =4:1 [b]	**40**, 98%; α/β =4:1 [b]
3	**24**	 **41**, 73%; α/β =3:1 [b]	**41**, 83%; α/β =3:1 [b]
4	**23**	**41**, 82%; α/β =6:1 [b]	**41**, 87%; α/β =6:1 [b]

Table 2.4 Acetolysis of 6-deoxysugar *quinovo* configured methyl glycosides: [a] Isolated yeld; [b] Anomeric ratio measured by ^{1}H NMR spectroscopy.

Entry	***Methyl Disaccaride***	***Products (% yield)*** [a]	
		***Protocol A*: $ZnCl_2$ (10eq.), Ac_2O, 5°C**	***Protocol B*: 1:1:0.1 v/v/v Ac_2O/AcOH/TFA**
1	**9**	 **42**,15% **43**,23% **44**, 24%; α/β =2:1 [b]	 **45**, 59%; α/β =3.5:1 [b]
2	**8**	**45**, 64%; α/β =2:1 [b]	**45**, 72%; α/β =3:1 [b]
3	**22**	 **46**, 43%; **47**, 41%; α/β =4:1 [b]	 **48**, 56%; α/β =4.5:1 [b]
4	**21**	**48**, 96%; α/β =6:1 [b]	**48**, 96%; α/β =6:1 [b]

2.3 Conclusions

In conclusion it has been demonstrated that under Ac_2O/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 $ZnCl_2$ 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: ^{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), or Varian INOVA 500 (^{1}H: 500 MHz; ^{13}C:125 MHz) instruments in $CDCl_3$ ($CHCl_3$ as internal standard: ^{1}H: C*H*Cl_3 at δ=7.26 ppm; ^{13}C: *C*DCl_3 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 CH_3CN 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_3CN/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_2SO_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-α-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_2SO_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. $[\alpha]_D = -9$ (c = 0.8, CH_2Cl_2). 1H NMR (200 MHz, $CDCl_3$): δ = 7.34 (m, 5 H, Ar-H), 5.93 (m, 1 H, $OCH_2CH{=}CH_2$), 5.26 (dq, J_{vic}=17.4, J_{gem}=$J_{\text{long-range}}$=1.4 Hz, 1 H, *trans* $OCH_2CH{=}CHH$), 5.13 (d, J_{vic}=10.2, J_{gem}=$J_{\text{long-range}}$=1.4 Hz, 1 H, *cis* $OCH_2CH{=}CHH$), 4.73 (dt, J_{gem}=11.6 Hz, 1 H, $OCHHPh$), 4.68 (d, $J_{1,2}$=1.4 Hz, 1 H, 1-H), 4.58 (d, J_{gem}=11.6 Hz, 1 H, $OCHHPh$), 4.33 (ddt, J_{gem}=12.4, J_{vic}=5.6, $J_{\text{long-range}}$=1.4 Hz, 1 H, $OCHHCH{=}CH_2$), 4.13 (ddt, J_{gem}=12.4, J_{vic}=5.6, $J_{\text{long-range}}$=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, $J_{5,4}$ =9.2, $J_{5,6}$=6.2 Hz, 1 H, 5-H), 3.31 (s, 3 H, OCH_3), 3.18 (t, $J_{4,3}$=$J_{4,5}$ = 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): δ = 137.7 (C_{ipso}-Bn), 135.0 ($OCH_2CH{=}CH_2$), 128.5–127.8 (C-Ar), 116.8 ($OCH_2CH{=}CH_2$), 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_2Ph, $OCH_2CH{=}CH_2$), 54.6 (OCH_3), 18.0 (C-6) ppm. MS (MALDITOF): calcd. for C17H24O5 $[M]^+$ 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 CH_2Cl_2 (3.0 mL). A 0.57 M solution of TfOH in CH_2Cl_2 (100 μL, 57 μmol) was added. After 1 h stirring at –20 °C, the mixture was diluted with CH_2Cl_2 and washed with 1:1 (v/v) 1 M $NaHCO_3$/10% $Na_2S_2O_3$. The organic layer was collected, dried with anhydrous Na_2SO_4, 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): $[\alpha]_D$=–50.6 (*c* = 1.8, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): *δ* = 7.30 (m, 5 H, Ar-H), 5.96 (m, 1 H, OCH_2CH=CH_2), 5.39–5.26 (m, 3 H, 2_B-H, 3_B-H, *trans* OCH_2CH=C*H*H), 5.23 (d, J_{vic} = 10.4 Hz, 1 H, *cis* OCH_2CH=C*H*H), 5.04 (t, $J_{4,3}$ = $J_{4,5}$ = 9.5Hz, 1 H, 4_B-H), 5.03 (s, 1 H, 1_B-H), 4.80 (d, J_{gem} = 11.0 Hz, 1 H, OC*H*HPh), 4.65 (s, 1 H, 1_A-H), 4.62 (d, J_{gem} = 11.0 Hz, 1 H, OC*H*HPh), 4.22 (dd, J_{gem} =13.0, J_{vic} = 5.6 Hz, 1 H, OC*H*HCH=CH_2), 4.07 (dd, J_{gem} = 13.0, J_{vic} = 5.6 Hz, OC*H*HCH=CH_2), 4.04–3.97 (m, 2 H, 3_A-H, 5_B-H), 3.65–3.56 (m, 3 H, 2_A-H, 4_A-H, 5_A-H), 3.32 (s, 3 H, OCH_3), 2.06, 2.05, 1.97 (3 s, 9 H, 3 $COCH_3$), 1.30 (d, $J_{6,5}$ = 5.7 Hz, 3 H, 6_A-H), 1.21 (d, $J_{6,5}$ = 6.3Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): *δ* = 169.9–169.8 (3 CO), 138.1 (C_{ipso}-Bn), 134.9 (OCH_2CH=CH_2), 128.3–127.5 (C-Ar), 117.4 (OCH_2CH=CH_2), 99.1, 98.4 (C-1_A, C-1_B), 80.6, 78.3, 77.7, 75.3, 72.0, 71.1, 69.8, 69.1, 67.9, 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, OCH_2Ph, OCH_2CH=CH_2), 54.6 (OCH_3), 20.8–20.7 (3 CH_3CO), 17.9, 17.4 (C-6_A, C-6_B) ppm. MS (MALDI-TOF): calcd. For $C_{29}H_{40}O_{12}$ $[M]^+$

580.25; found 603.00 $[M + Na]^+$. $C_{29}H_{40}O_{12}$ (580.62): calcd. C 59.99, H 6.94; found C 60.09, H 6.99.

Methyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-D-quinovopyranoside (21): $[\alpha]_D$ = –17.3 (*c* = 1.8, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.34–7.26 (m, 10 H, Ar-H), 5.38–5.27 (m, 3 H, 1_B-H, 2_B-H, 3_B-H), 4.97 (t, $J_{4,3} = J_{4,5}$ = 10.0 Hz, 1 H, 4_B-H), 4.84 (d, J_{gem} = 11.2 Hz, 1 H, OC*H*HPh), 4.68 (d, J_{gem} = 12.0 Hz, 1 H, OC*H*HPh), 4.61 (d, J_{gem} = 11.2 Hz, 1 H, OC*H*HPh), 4.55 (d, J_{gem} = 12.0 Hz, 1 H, OC*H*HPh), 4.45 (d, $J_{1,2}$ = 3.4 Hz, 1 H, 1_A-H), 4.11 (t, $J_{3,4} = J_{3,2}$ = 9.4 Hz, 1 H, 3_A-H), 4.02 (dq, $J_{5,4}$ = 10.0, $J_{5,6}$ = 6.2 Hz, 1 H, 5_B-H), 3.76 (dq, $J_{5,4}$ = 9.4, $J_{5,6}$ = 6.4 Hz, 1 H, 5_A-H), 3.51 (dd, $J_{2,3}$ = 9.4, $J_{2,1}$ = 3.4Hz, 1 H, 2_A-H), 3.30 (s, 3 H, OCH_3), 3.09 (t, $J_{4,3} = J_{4,5}$ = 9.4 Hz, 1 H, 4_A-H), 2.07, 1.99, 1.91 (3 s, 9 H, 3 CH_3CO), 1.27 (d, $J_{6,5}$ = 6.4 Hz, 3 H, 6_A-H), 0.88 (d, $J_{6,5}$ = 6.2Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ= 170.1, 169.9, 169.8 (3 CO), 137.9, 137.6 (2 C_{ipso}-Bn), 128.4–127.2 (C-Ar), 97.6, 97.1 (C-1_A, C-1_B), 82.4, 81.0, 75.6, 74.9, 72.7, 70.9, 69.7, 69.2, 66.5, 66.1 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, 2 O*C*H_2Ph), 54.9 (OCH_3), 20.7–20.6 (3 *C*H_3CO), 17.8, 16.9 C-6_A, C-6_B) ppm. MS (MALDI-TOF): calcd. for $C_{33}H_{42}O_{12}$ $[M]^+$630.27; found 653.11 $[M + Na]^+$. $C_{33}H_{42}O_{12}$ (630.68): calcd. C62.85, H 6.71; found C 62.59, H 6.59.

Methyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzyl-β-D-quinovopyranoside (22): $[\alpha]_D$ = –50.3 (*c* = 3.0, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.33–7.26 (m, 10 H, Ar-H), 5.38 (d, $J_{1,2}$ = 1.0 Hz, 1 H, 1_B-H), 5.32–5.23 (m, 2 H, 2_B-H, 3_B-H), 4.98–4.81 (m, 3 H, 4_B-H, 2 OC*H*HPh), 4.70 (d, J_{gem} = 11.4 Hz, 1 H, OC*H*HPh), 4.56 (d, J_{gem} = 11.8 Hz, 1 H, OC*H*HPh), 4.27 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1_A-H),

4.03 (dq, $J_{5,4}$ = 10.0, $J_{5,6}$ = 6.2 Hz, 1 H, 5_BH), 3.83 (t, $J_{3,4}$ = $J_{3,2}$ = 9.2Hz, 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_3CO), 1.37 (d, $J_{6,5}$ = 6.0 Hz, 3 H, 6_A-H), 0.87 (d, $J_{6,5}$ = 6.2 Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 170.1, 169.8, 169.7 (3 CO), 138.0, 137.7 (2 C_{ipso}-Bn), 128.2–127.3 (C-Ar), 104.2, 97.2 (C-1_A, C-1_B), 82.9, 82.0, 76.3, 75.2, 74.3, 71.1, 70.8, 69.4, 69.1, 66.1 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, 2 O*C*H_2Ph), 56.9 (OCH_3), 20.7–20.6 (3 *C*H_3CO), 17.8, 16.9 (C-6_A, C-6_B) 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-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-D-fucopyranoside (23): $[\alpha]_D$ = –71.0 (c = 2.1, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.43–7.28 (m, 10 H, Ar-H), 5.46 (dd, $J_{3,4}$ = 9.9, $J_{3,4}$ = 3.3 Hz, 1 H, 3_B-H), 5.18 (dd, $J_{2,3}$ = 3.3, $J_{2,1}$ = 1.8 Hz, 1 H, 2_B-H), 5.08 (t, $J_{4,3}$ = $J_{4,5}$ = 9.9 Hz, 1 H, 4_B-H), 5.01 (s, 1 H, 1_B-H), 4.90 (d, J_{gem} = 11.7 Hz, 1 H, OC*H*HPh), 4.79 (d, J_{gem} = 12.0 Hz, 1 H, OC*H*HPh), 4.67 (m, 2 H, 1_A-H, OC*H*HPh), 4.59 (d, J_{gem} = 12.0 Hz, 1 H, OC*H*HPh), 4.20 (dq, $J_{5,4}$ = 9.9, $J_{5,6}$ = 6.0 Hz, 1 H, 5_B-H), 4.15 (dd, $J_{3,2}$ = 10.2, $J_{3,4}$ = 2.7Hz, 1 H, 3_A-H), 3.98 (dd, $J_{2,3}$ = 10.2, $J_{2,1}$ = 3.6Hz, 1 H, 2_A-H), 3.84 (q, $J_{5,6}$ = 6.0 Hz, 1 H, 5_A-H), 3.68 (d, $J_{3,4}$ = 2.7 Hz, 1 H, 4_A-H), 3.35 (s, 3 H, OCH_3), 2.16, 2.02, 2.00 (3 s, 9 H, 3 CH_3CO), 1.16 (d, $J_{6,5}$ = 6.0Hz, 3 H, 6_A-H), 1.12 (d, $J_{6,5}$ = 6.0Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 170.4, 170.0, 169.9 (3 CO), 138.3, 138.2 (2 C_{ipso}-Bn), 129.0–127.7 (C-Ar), 98.4, 93.9 (C-1_A, C-1_B), 75.5, 74.6, 74.5, 74.4, 73.3, 71.1, 70.3, 68.9, 66.6, 65.8 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, 2 O*C*H_2Ph), 55.3 (OCH_3), 20.9, 20.7, 20.6 (3 *C*H_3CO), 17.5, 16.7

($C\text{-}6_A$, $C\text{-}6_B$) ppm. MS (MALDI-TOF): calcd. for $C_{33}H_{42}O_{12}$ $[M]^+$ 630.27; found 653.01 $[M + Na]^+$. $C_{33}H_{42}O_{12}$ (630.68): calcd. C 62.85, H 6.71; found C 62.82, H 6.72.

Methyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzyl-β-D-fucopyranoside (24): $[\alpha]_D$ = –61.0 (c = 2.0, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.42–7.27 (m, 10 H, Ar-H), 5.40 (dd, $J_{3,4}$ = 9.9, $J_{3,4}$ = 3.3 Hz, 1 H, 3_B-H), 5.18 (dd, $J_{2,3}$ = 3.3, $J_{2,1}$ = 1.8 Hz, 1 H, 2_B-H), 5.03 (t, $J_{4,3} = J_{4,5}$ = 9.9 Hz, 1 H, 4_B-H), 4.96 (s, 1 H, 1_B-H), 4.93 (d, J_{gem} = 10.5 Hz, 1 H, OC*H*HPh), 4.91 (d, J_{gem} = 11.4 Hz, 1 H, OC*H*HPh), 4.71 (d, J_{gem} = 10.5 Hz, 1 H, OC*H*HPh), 4.60 (d, J_{gem} = 11.4 Hz, 1 H, OC*H*HPh), 4.26 (d, $J_{1,2}$ = 7.2 Hz, 1 H, 1_A-H), 4.16 (dq, $J_{5,4}$ = 9.9, $J_{5,6}$ = 6.0 Hz, 1 H, 5_BH), 3.74 (m, 2 H, 2_A-H, 3_A-H), 3.56 (s, 1 H, 4_A-H), 3.55 (s, 3 H, OCH_3), 3.48 (q, $J_{5,6}$ = 6.3 Hz, 1 H, 5_A-H), 2.15, 2.01, 2.00 (3 s, 9 H, 3 CH_3CO), 1.23 (d, $J_{6,5}$ = 6.3 Hz, 3 H, 6_A-H), 0.96 (d, $J_{6,5}$ = 6.0Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 170.2, 169.9, 169.7 (3 CO), 138.6, 138.2 (2 C_{ipso}-Bn), 129.0–127.4 (C-Ar), 105.0, 94.0 ($C\text{-}1_A$, $C\text{-}1_B$), 77.7, 77.3, 75.3, 75.1, 75.0, 71.6, 70.4, 70.2, 69.0, 66.4 ($C\text{-}2_A$, $C\text{-}2_B$, $C\text{-}3_A$, $C\text{-}3_B$, $C\text{-}4_A$, $C\text{-}4_B$, $C\text{-}5_A$, $C\text{-}5_B$, 2 O*C*H_2Ph), 56.9 (OCH_3), 20.9–20.8 (3 *C*H_3CO), 17.2, 17.0 ($C\text{-}6_A$, $C\text{-}6_B$) ppm. MS (MALDI-TOF): calcd. for $C_{33}H_{42}O_{12}$ $[M]^+$ 630.27; found 653.15 $[M + Na]^+$. $C_{33}H_{42}O_{12}$ (630.68): calcd. C 62.85, H6.71; found C 62.75, H 6.62.

Methyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-allyl-2-*O*-benzyl-α-L-rhamnopyranoside (27): $[\alpha]_D$ = –40.1 (c = 2.0, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.41–7.28 (m, 5 H, Ar-H), 5.87 (m, 1 H, OCH_2C*H*=CH_2), 5.34 (m, 2 H, 1_B-H, 3_B-H), 5.24 (d, J_{vic} = 16.8 Hz, 1 H, OCH_2CH=C*H*H), 5.10 (d, J_{vic} = 9.9 Hz, 1 H, OCH_2CH=C*H*H), 5.01 (m, 2 H, 2_B-H, 4_B-H), 4.75 (d, J_{gem} = 12.3 Hz, 1 H, OC*H*HPh),

4.66 (br. s, 1 H, 1_A-H), 4.64 (d, J_{gem} = 12.3 Hz, 1 H, OC*H*HPh), 4.25 (dd, J_{gem} = 12.2, J_{vic} = 5.7 Hz, 1 H, OC*H*HCH=CH_2), 4.10 (dd, J_{gem} = 12.4, J_{vic} = 5.7 Hz, 1 H, OC*H*HCH=CH_2), 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_B-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, OCH_3), 2.10, 2.02, 1.97 (3 s, 9 H, 3 CH_3CO), 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. ^{13}C NMR ($CDCl_3$,75 MHz): δ = 169.7 (3 CO), 138.0 (C_{ipso}-Bn), 134.6(OCH_2CH=CH_2), 128.3–127.5 (C-Ar), 116.7 (OCH_2CH=CH_2), 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, OCH_2Ph, OCH_2CH=CH_2), 54.6 (OCH_3), 20.7–20.6 (3 CH_3CO), 17.8, 17.4 (C-6_A, C-6_B) ppm. MS (MALDI-TOF): calcd. For $C_{29}H_{40}O_{12}$ $[M]^+$ 580.25; found 603.18 $[M + Na]^+$. $C_{29}H_{40}O_{12}$ (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) CH_2Cl_2/MeOH (0.6 mL) and then treated with $PdCl_2$ (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 CH_2Cl_2, and washed with water. The organic phase was collected, dried with anhydrous Na_2SO_4, filtered, and concentrated. The residue was dissolved in 1:1 (v/v) Ac_2O/py (1.0 mL) and stirred at room temp. overnight. The solution was then concentrated. The residue was diluted with CH_2Cl_2 and the resulting solution washed with 1 M HCl. The organic phase was collected, dried with anhydrous Na_2SO_4, 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. $[\alpha]_D$ = –34.7 (*c* = 1.1, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.37 (m, 5 H, Ar-H), 5.30 (dd, $J_{3,4}$ = 10.0, $J_{3,2}$ = 3.2 Hz, 1 H, 3_B-H), 5.21 (t, $J_{4,3}$ = $J_{4,5}$ = 9.8 Hz, 1 H, 4_A-H), 5.12 (dd, $J_{2,3}$ = 3.2, $J_{2,1}$ = 2.0Hz, 1 H, 2_B-H), 5.02 (t, $J_{4,3}$ = $J_{4,5}$ = 10.0 Hz, 1 H, 4_B-H), 4.89 (d, $J_{1,2}$ = 1.6 Hz, 1 H, 1_B-H), 4.74–4.68 (m, 3 H, 1_A-H, OC*H*$_2$Ph), 4.00 (dd, $J_{3,4}$ = 9.8, $J_{3,2}$ = 3.2Hz, 1 H, 3_A-H), 3.82 (dq, $J_{5,4}$ = 9.8, $J_{5,6}$ = 6.2 Hz, 1 H, 5_A-H), 3.70 (m, 2 H, 2_A-H, 5_B-H), 3.33 (s, 3 H, OCH_3), 2.13, 2.12, 2.03, 1.99 (4 s, 12 H, 4 CH_3CO), 1.20 (d, $J_{6,5}$ = 6.2 Hz, 3 H, 6_B-H), 1.07 (d, $J_{6,5}$ = 6.2 Hz, 3 H, 6_A-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 170.2, 170.1, 170.0, 169.6 (4 CO), 138.0 (C_{ipso}-Bn), 128.5–127.6 (C-Ar), 98.6, 98.5 (C-1_A, C-1_B), 76.9, 72.7, 72.6, 71.2, 71.1, 70.2, 68.6, 66.9, 66.8 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, O*C*H$_2$Ph), 54.9 (OCH_3), 20.9– 20.7 (4 *C*H_3CO), 17.6, 17.4 (C-6_A, C-6_B) ppm. MS (MALDI-TOF): calcd. for $C_{28}H_{38}O_{13}$ $[M]^+$ 582.23; found 605.09 $[M + Na]^+$. $C_{28}H_{38}O_{13}$ (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 $NaNH_2$ (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 $NaHCO_3$ and brine. The organic layer was collected, dried with anhydrous Na2SO4, filtered, and concentrated. The residue waspurified by chromatography (8 to 16% ethyl acetate in toluene) to afford **19** (129 mg, 64%) as a yellowish oil. $[\alpha]_D$ = +26.5 (*c* = 2.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.19 (m, 15 H, Ar-H), 5.91 (m, 1 H, OCH_2CH=CH_2), 5.26 (dd, J_{vic} = 17.2, J_{gem} = 1.6 Hz, 1 H,

trans $OCH_2CH=CHH$), 5.16–5.13 (m, 2 H, *cis* $OCH_2CH=CHH$, 1_B-H), 4.87 (d, J_{gem} = 11.0 Hz, 1 H, $OCHHPh$), 4.78 (d, J_{gem} = 11.0 Hz, 1 H, $OCHHPh$), 4.70 (s, 2 H, OCH_2Ph), 4.64 (s, 1 H, 1_A-H), 4.63 (d, J_{gem} = 11.0 Hz, 1 H, $OCHHPh$), 4.62 (d, J_{gem} = 11.6 Hz, 1 H, $OCHHPh$), 4.07 (dd, J_{gem} = 11.8, J_{vic} = 6.6Hz, 1 H, $OCHHCH=CH_2$), 4.05 (dd, J_{gem} = 11.8, J_{vic} = 6.6 Hz, 1 H, $OCHHCH=CH_2$), 4.00 (d, $J_{2,1}$ = 1.3 Hz, 1 H, 2_B-H), 3.80 (m, 1 H, 5_B-H), 3.75–3.61 (m, 3 H, 3_A-H, 3_B-H, 2_A-H), 3.66 (m, 1 H, 5_A-H), 3.58 (t, $J_{4,5}$ = $J_{4,3}$ = 9.3 Hz, 1 H, 4_B-H), 3.42 (t, $J_{4,5}$ = $J_{4,3}$ = 9.3 Hz, 1 H, 4_A-H), 3.31 (s, 3 H, OCH_3), 1.32 (d, $J_{6,5}$ = 5.8 Hz, 3 H, 6_A-H), 1.25 (d, $J_{6,5}$ = 6.0 Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 138.7, 138.2, 138.1 (3 C_{ipso}-Bn), 134.6 ($OCH_2CH=CH_2$), 128.4–127.8 (C-Ar), 117.2 ($OCH_2CH=CH_2$), 100.8, 98.6 (C-1_A, C-1_B), 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-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, 3 OCH_2Ph, $OCH_2CH=CH_2$), 54.9 (OCH_3), 18.2 (C-6_A, C-6_B) ppm. MS (MALDI-TOF): calcd. for $C_{37}H_{46}O_9$ $[M]^+$ 634.31; found 657.33 $[M + Na]^+$. $C_{37}H_{46}O_9$ (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 Ac_2O/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 CH_2Cl_2 and washed with water. The organic layer was collected, dried with anhydrous Na_2SO_4, 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. $[\alpha]_D$ = +28.2 (c = 0.9, CH_2Cl_2). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.41–7.26 (m, 15 H, Ar-H), 5.87 (m, 1 H, $OCH_2CH=CH_2$), 5.44 (br. s, 1 H, 2_B-H), 5.25 (d, J_{vic} = 17.4 Hz, 1 H, *trans* $OCH_2CH=CHH$), 5.13–5.10 (m, 2 H,

cis $OCH_2CH=CHH$, 1_A-H), 4.94 (d, J_{gem} = 11.0 Hz, 1 H, OC*H*HPh), 4.83 (d, J_{gem} = 11.0 Hz, 1 H, OC*H*HPh), 4.72 (s, 2 H, OCH_2Ph), 4.66–4.61 (m, 3 H, 2 OC*H*HPh, 1_B-H), 4.16–4.06 (m, 2 H, OC*H*HCH=CH_2, 3_A-H), 3.98 (dd, J_{gem} = 12.0, J_{vic} = 5.4 Hz, 1 H, OC*H*HCH=CH_2), 3.88 (dd, $J_{3,2}$ = 3.3, $J_{3,4}$ = 9.3Hz, 1 H, 3_B-H), 3.85 (m, 1 H, 5_B-H), 3.71 (br. s, 1 H, 2_A-H), 3.65 (t, $J_{4,5}$ = *J*4,3 = 9.0 Hz, 1 H, 4_B-H), 3.41 (m, 1 H, 5_A-H), 3.36 (t, $J_{4,5}$ = $J_{4,3}$ = 9.6 Hz, 1 H, 4_A-H), 3.31 (s, 3 H, OCH_3), 2.09 (s, 3 H, $COCH_3$), 1.32 (d, $J_{6,5}$ = 5.4 Hz, 3 H, 6_A-H), 1.29 (d, $J_{6,5}$ = 5.7 Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 169.9 (CO), 138.8, 138.1, 138.0 (3 C_{ipso}-Bn), 134.6 ($OCH_2CH=CH_2$), 128.4–127.5 (CAr), 117.1 ($OCH_2CH=CH_2$), 99.2, 98.5 (C-1_A, C-1_B), 81.0, 80.9, 79.9, 77.7, 77.4, 77.0, 75.2, 72.8, 70.6, 69.1, 68.2, 67.9 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, 3 O*C*H_2Ph, O*C*H_2CH=CH_2), 54.6 (OCH_3), 20.9 (*C*H_3CO), 18.0, 17.9 (C-6_A, C-6_B) ppm. MS (MALDI-TOF): calcd. for $C_{39}H_{48}O_{10}$ $[M]^+$ 676.32; found 699.29 $[M + Na]^+$. $C_{39}H_{48}O_{10}$ (676.79): calcd. C 69.21, H 7.15; found C 69.15, H 7.09.

Methyl 3-*O*-Allyl-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranosyl- (1→3)-2,4-di-*O*-benzyl-α-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 Na_2SO_4, filtered, concentrated, and purified by chromatography (6 to 9% ethyl acetate in toluene) to give **25** (83.0 mg, 83%) as a colorless oil. $[\alpha]_D$ = –0.8 (*c* = 0.9, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.06–7.24 (m, 20 H, Ar-H), 5.88 (m, 1 H, $OCH_2CH=CH_2$), 5.71 (dd, $J_{2,1}$ = 1.6, $J_{2,3}$ = 3.0 Hz, 1 H, 2_B-H), 5.24 (dd, J_{vic} = 15.6, J_{gem} = 1.8 Hz, 1 H, *trans* $OCH_2CH=CHH$), 5.26 (br. s, 1 H, 1_B-H), 5.09 (dd, J_{vic} = 10.2, J_{gem} = 1.8 Hz, 1 H, *cis*

$OCH_2CH=CHH$), 4.98 (d, J_{gem} = 11.0 Hz, 1 H, $OCHHPh$), 4.92 (d, J_{gem} = 11.0 Hz, 1 H, $OCHHPh$), 4.77 (s, 2 H, $OCHHPh$), 4.67–4.62 (m, 3 H, 2 $OCHHPh$, 1_A-H), 4.22 (dd, J_{gem} = 11.5, J_{vic} = 5.2 Hz, 1 H, $OCHHCH=CH_2$), 4.16–3.88 (m, 4 H, $OCHHCH=CH_2$, 3_A-H, 5_A-H, 5_B-H), 3.77 (dd, $J_{2,1}$ = 2.0, $J_{2,3}$ = 3.0Hz, 1 H, 2_A-H), 3.71–3.67 (m, 2 H, 3_B-H, 4_A-H), 3.54 (t, $J_{4,5}$ = $J_{4,3}$ = 9.2 Hz, 1 H, 4_B-H), 3.33 (s, 3 H, OCH_3), 1.36–1.30 (m, 6 H, 6_A-H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 165.4 (CO), 138.7, 138.1, 138.0 (3 C_{ipso}-Bn), 134.7 ($OCH_2CH=CH_2$), 133.0 (C_{ipso}-Bz), 129.8–127.5 (C-Ar), 117.0 ($OCH_2CH=CH_2$), 99.2, 98.6 (C-1_A, C-1_B), 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-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, 3 OCH_2Ph, $OCH_2CH=CH_2$), 54.6 (OCH_3), 18.2, 17.9 (C-6_A, C- 6_B) ppm. MS (MALDI-TOF): calcd. for $C_{44}H_{50}O_{10}$ $[M]^+$ 738.34; found 761.39 $[M + Na]^+$. $C_{44}H_{50}O_{10}$ (738.86): calcd. C 71.52, H 6.82; found C 71.45, H 6.77.

Typical Acetolysis Procedure ($ZnCl_2$ Protocol): Methyl glycoside (66 μmol) was dissolved in a 2:1 (v/v) Ac_2O/AcOH mixture (1.0 mL), cooled to 5 °C, and then treated with freshly fused $ZnCl_2$ (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 CH_2Cl_2 and washed with 1 M $NaHCO_3$ and then collected, dried with anhydrous Na_2SO_4, 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) Ac_2O/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 CH_2Cl_2 and washed with 1 M $NaHCO_3$. The organic layer was collected, dried with anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by chromatography (ethyl acetate in toluene).

1,5-di-*O*-acetyl-2,3-di-*O*-benzyl-α-L-rhamnofuranose (29): $[\alpha]_D$ -11.8 (*c* 1.6; CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35-7.30 (m, 10H, H-Ar), 6.29 (d, 1H, $J_{1,2}$ 2.3 Hz, H-1), 5.27 (quintet, 1H, $J_{5,6}$=$J_{5,4}$ 6.0 Hz, H-5), 4.71 (d, 1H, J_{gem} 1.6 Hz, OC*H*HPh), 4.68 (d, 1H, J_{gem} 11.6 Hz, OC*H*HPh), 4.63 (d, 1H, J_{gem} 11.6 Hz, OC*H*HPh), 4.50 (d, 1H, J_{gem} 11.6 Hz, OC*H*HPh), 4.23 (m, 2H, H-3, H-4), 4.02 (dd, 1H, $J_{2,3}$ 4.4 Hz, $J_{2,1}$=2.3 Hz, H-2), 2.07, 1.92 (2s, 6H, 2 CH_3CO), 1.32 (d, 3H, $J_{6,5}$ 6.4 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 169.8 (2 CO), 137.7, 137.4 (2 C_{ipso}-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 O*C*H_2Ph), 21.2 (2 *C*H_3CO), 16.1 (C-6). MALDI TOF-MS: calcd for $C_{24}H_{28}O_7$ (m/z), 428.18; found, 450.99 $[M+Na]^+$. Anal Calcd for $C_{24}H_{28}O_7$: C, 67.28; H, 6.59. Found: C, 67.46; H, 6.50.

Acetyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-5-*O*-acetyl-2-*O*-allyl-α-L-rhamnofuranoside (31): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.27 (d, $J_{1,2}$ = 4.5 Hz, 1 H_β, $1_{A\text{-}\beta}$-H), 6.19 (d, $J_{1,2}$ = 2.9Hz, 1 H_α, $1_{A\text{-}\alpha}$-H), 5.87 (m, 1 H_β + 1 H_α, OCH_2CH=CH_2), 5.39 (dd, $J3_{,4}$ = 9.9, $J_{3,2}$ = 3.6 Hz, 1 H_α, $3_{B\text{-}\beta}$-H), 5.32 (dd, J_{vic} = 17.2, J_{gem} = 1.5 Hz, 1 H_α + 1 H_β, *trans* OCH_2CH=C*H*H), 5.26–5.18 (m, 3H_α + 2H_β, *cis* OCH_2CH=C*H*H, $2_{B\text{-}\alpha}$-H, $2_{B\text{-}\beta}$-H, $3_{B\text{-}\alpha}$-H), 5.18–5.01 (m, 2H_α + 2H_β, $4_{B\text{-}\alpha}$-H, $4_{B\text{-}\beta}$-H, $5_{A\text{-}\alpha}$-H, $5_{A\text{-}\beta}$-H), 4.78 (d, $J1_{,2}$ = 1.5 Hz, 1 H_α, $1_{B\text{-}\alpha}$-H), 4.74 (d, $J_{1,2}$ = 1.7 Hz, 1 H_β, $1_{B\text{-}\beta}$-H), 4.46 (dq, $J_{5,4}$ = 9.9, $J_{5,6}$ = 6.2 Hz, 1 H_β, $5_{B\text{-}\beta}$-H), 4.35 (t, $J_{3,2}$ = $J_{3,4}$ = 4.9 Hz, 1 H_α, $3_{A\text{-}\alpha}$-H), 4.26–4.19 (m, 2 H_α + 1 H_β, $3_{A\text{-}\beta}$-H, $4_{A\text{-}\alpha}$-H,

$5_{B\text{-}\alpha}$-H), 4.13–4.03 (m, 2 H_α + 2 H_β, OC*H*$_2$CH=CH$_2$), 3.99 (dd, $J_{2,3}$ = 5.9, $J_{2,1}$ = 4.5 Hz, 1 H_β, $2_{A\text{-}\beta}$-H), 3.94 (dd, $J_{2,3}$ = 4.6, $J_{2,1}$ = 2.9 Hz, 1 H_α, $2_{A\text{-}\alpha}$-H), 3.91 (t, $J_{4,3}$ = $J_{4,5}$ = 5.9 Hz, 1 H_β, $4_{A\text{-}\beta}$-H), 2.18 (s, 3 H_β, CH_3CO), 2.13 (2 s, 3 H_α + 3 H_β, CH_3CO), 2.08 (s, 3 H_α, CH_3CO), 2.04 (2 s, 6 H_α, CH_3CO), 2.03 (s, 3 H_β, CH_3CO), 2.01 (s, 3 H_β, CH_3CO), 1.99 (s, 3 H_β, CH_3CO), 1.98 (s, 3 H_α, CH_3CO), 1.35 (d, $J_{6,5}$ = 6.2 Hz, 3 H_β, $6_{A\text{-}\beta}$-H), 1.31 (d, $J_{6,5}$ = 6.3 Hz, 3 H_α, $6_{A\text{-}\alpha}$-H), 1.17 (d, $J_{6,5}$ = 6.2 Hz, 3 H_α, $6_{B\text{-}\alpha}$-H), 1.16 (d, $J_{6,5}$ = 6.2 Hz, 3 H_β, $6_{B\text{-}\beta}$-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 170.1–169.6 (5 CO_α + 5 CO_β), 133.8 (α OCH$_2$*C*H=CH$_2$), 133.6 (β OCH$_2$*C*H=CH$_2$), 118.4 (α OCH$_2$CH=*C*H$_2$), 117.7 (β OCH$_2$CH=*C*H$_2$), 99.5, 98.4 (C-$1_{A\text{-}\alpha}$, C-$1_{B\text{-}\alpha}$), 98.8, 93.0 (C-$1_{A\text{-}\beta}$, C-$1_{B\text{-}\beta}$), 81.7, 81.0, 78.6, 71.7, 71.0, 69.9, 68.8, 68.7, 67.0 (α C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, O*C*H$_2$CH=CH$_2$), 82.8, 78.2, 72.4, 72.1, 71.5, 70.6, 68.8, 68.7, 66.7 (β C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, O*C*H$_2$CH=CH$_2$), 21.3–20.7 (5 *C*H$_3$CO$_\alpha$ + 5 *C*H$_3$CO$_\beta$), 17.6, 16.3 (C-$6_{A\text{-}\alpha}$, C-$6_{B\text{-}\alpha}$), 17.5, 16.7 (C-$6_{A\text{-}\beta}$, C-$6_{B\text{-}\beta}$) ppm. MS (MALDI-TOF): calcd. for $C_{25}H_{36}O_{14}$ $[M]^+$ 560.21; found 582.90 $[M + Na]^+$. $C_{25}H_{36}O_{14}$ (560.55): calcd. C 53.57, H 6.47; found C 53.70, H 6.40.

Acetyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-allyl-4-*O*-benzyl-α-L-rhamnopyranoside (32): $[\alpha]_D$ = –39.2 (*c* = 1.4, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.32 (m, 5 H, Ar-H), 6.09 (d, $J_{1,2}$ = 2.1 Hz, 1 H, 1_A-H), 5.98 (m, 1 H, OCH$_2$C*H*=CH$_2$), 5.40–5.34 (m, 3 H, 2_B-H, 3_B-H, *trans* OCH$_2$CH=C*H*H), 5.26 (dd, J_{vic} = 10.5, J_{gem} = 1.2 Hz, 1 H, *cis* OCH$_2$CH=C*H*H), 5.08 (t, $J_{4,3}$ = $J_{4,5}$ = 9.9 Hz, 1 H, 4_B-H), 5.05 (br. s, 1 H, 1_B-H), 4.84 (d, J_{gem} = 11.1 Hz, 1 H, OC*H*HPh), 4.64 (d, J_{gem} = 11.1 Hz, 1 H, OC*H*HPh), 4.27 (dd, J_{gem} = 12.9, J_{vic} = 5.4 Hz, 1 H, OC*H*HCH=CH$_2$), 4.12–3.98 (m, 3 H, 3_A-H, 5_B-H, OC*H*HCH=CH$_2$), 3.77 (dq, $J_{5,4}$ = 9.6, $J_{5,6}$ = 6.3 Hz, 1 H, 5_A-H), 3.67 (dd, $J_{2,3}$ = 3.0, $J_{2,1}$

= 2.1 Hz, 1 H, 2_A-H), 3.63 (t, $J_{4,3} = J_{4,5}$ = 9.6 Hz, 1 H, 4_A-H), 2.09, 2.07, 2.06, 1.99 (4 s, 12 H, 4 CH_3CO), 1.31 (d, $J_{6,5}$ = 6.3 Hz, 3 H, 6_A-H), 1.20 (d, $J_{6,5}$ = 6.3 Hz, 3 H, 6_BH) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 170.0, 169.9, 169.8, 169.1 (4 CO), 137.8 (C_{ipso}-Bn), 134.3 ($OCH_2CH=CH_2$), 128.4–127.8 (C-Ar), 117.9 ($OCH_2CH=CH_2$), 99.4 ($C\text{-}1_B$), 91.2 ($C\text{-}1_A$), 79.7, 78.5, 76.7, 75.6, 71.8, 70.9, 70.6, 69.8, 69.0, 66.9 ($C\text{-}2_A$, $C\text{-}2_B$, $C\text{-}3_A$, $C\text{-}3_B$, $C\text{-}4_A$, $C\text{-}4_B$, $C\text{-}5_A$, $C\text{-}5_B$, OCH_2Ph, $OCH_2CH=CH_2$), 21.0–20.8 (4 CH_3CO), 18.0, 17.5 ($C\text{-}6_A$, $C\text{-}6_B$) ppm. MS (MALDITOF): 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): ^{1}H NMR (500 MHz, $CDCl_3$) (major diastereoisomer): δ = 7.32 (m, 5 H, Ar-H), 5.99 (d, $J_{1,2}$ = 7.0 Hz, 1 H, 1_A-H), 5.85 (m, 1 H, $OCH_2CH=CH_2$), 5.35 (dd, $J_{2,3}$ = 3.0, $J_{2,1}$ = 1.5 Hz, 1 H, 2_B-H), 5.24 (dd, $J_{3,4}$ = 10.2, $J_{3,2}$ = 3.0 Hz, 1 H, 3_B-H), 5.19 (d, J_{vic} = 17.5 Hz, 1 H, *trans* $OCH_2CH=CHH$), 5.08 (m, 2 H, 1_B-H, 4_B-H), 5.04 (d, J_{vic} = 10.5 Hz, 1 H, *trans* $OCH_2CH=CHH$), 4.81 (dq, $J_{5,6}$ = 6.6, $J_{5,4}$ = 1.8 Hz, 1 H, 5_A-H), 4.62 (s, 2 H, OCH_2Ph), 4.15 (m, 3 H, $OCH_2CH=CH_2$, 5_B-H), 3.80 (m, 2 H, 3_A-H, 4_A-H), 3.52 (m, 4 H, 2_A-H, OCH_3), 2.12 (s, 6 H, 2 CH_3CO), 2.07 (s, 3 H, CH_3CO), 2.02 (s, 3 H, CH_3CO), 1.97 (s, 3 H, CH_3CO), 1.04 (d, $J_{6,5}$ = 6.2 Hz, 3 H, 6_B-H), 1.02 (d, $J_{6,5}$ = 6.6 Hz, 3 H, 5_A-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) (major diastereoisomer): δ = 170.5, 170.2, 169.9, 169.8, 169.7 (5 CO), 137.5 (C_{ipso}-Bn), 134.8 ($OCH_2CH=CH_2$), 128.5–127.9 (C-Ar), 116.5 ($OCH_2CH=CH_2$), 98.7, 98.0 ($C\text{-}1_A$, $C\text{-}1_B$), 80.6, 78.8, 77.7, 73.7, 73.5, 71.0, 70.8, 69.8, 69.6, 67.1 ($C\text{-}2_A$, $C\text{-}2_B$, $C\text{-}3_A$, $C\text{-}3_B$, $C\text{-}4_A$, $C\text{-}4_B$, $C\text{-}5_A$, $C\text{-}5_B$, OCH_2Ph, $OCH_2CH=CH_2$), 57.7 (OCH_3), 21.3, 21.2, 20.9, 20.8, 20.7 (5 CH_3CO), 17.4, 13.7 ($C\text{-}6_A$, $C\text{-}6_B$) ppm. MS (MALDI-TOF): calcd. for $C_{33}H_{46}O_{15}$ $[M]^+$

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): $[\alpha]_D$ = –32 (*c* = 0.9, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.39 (m, 5 H, Ar-H), 6.15 (d, $J_{1,2}$ = 2.0 Hz, 1 H, 1_A-H), 5.89 (m, 1 H, $OCH_2CH{=}CH_2$), 5.34–5.03 (m, 6 H, 1_B-H, 2_B-H, 3_B-H, 4_B-H, $OCH_2CH{=}CH_2$), 4.82 (d, J_{gem} = 12.0 Hz, 1 H, OC*H*HPh), 4.62 (d, J_{gem} = 12.0 Hz, 1 H, OC*H*HPh), 4 . 3 0 (dd , J_{gem} = 1 2 . 8 , J_{vic} = 5. 6 Hz, 1 H, OC*H*HCH=CH_2), 4.16 (dd, J_{gem} = 12.8, J_{vic} = 5.6 Hz, 1 H, OC*H*HCH=CH_2), 3.95 (dd, $J_{3,4}$ = 9.4, $J_{3,2}$ = 3.2 Hz, 1 H, 3_A-H), 3.79–3.67 (m, 3 H, 2_A-H, 5_A-H, 5_B-H), 3.54 (t, $J_{4,3}$ = $J_{4,5}$ = 9.4 Hz, 1 H, 4_A-H), 2.13, 2.08, 2.04, 2.00 (4 s, 12 H, 4 CH_3CO), 1.31 (d, $J_{6,5}$ = 6.2 Hz, 3 H, 6_A-H), 1.07 (d, $J_{6,5}$ = 6.2 Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 169.8–169.7 (4 CO), 137.5 (C_{ipso}-Bn), 134.4 ($OCH_2CH{=}CH_2$) , 128.5–127.7 (C-Ar) , 117.2 ($OCH_2CH{=}CH_2$), 99.5 (C-1_B), 91.2 (C-1_A), 79.7, 78.4, 77.2, 74.4, 72.3, 71.0, 70.9, 69.9, 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, OCH_2Ph, $OCH_2CH{=}CH_2$), 20.8–20.7 (4 CH_3CO), 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 631.11 $[M + Na]^+$. $C_{30}H_{40}O_{13}$ (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): ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.41–7.33 (m, 5 H' + 5H", Ar-H), 6.00 (d, $J_{1,2}$ = 5.0 Hz, 1 H", $1_{A"}$-H), 5.98 (d, $J_{1,2}$ = 6.0 Hz, 1 H', $1_{A'}$-H), 5.45 (m, 1 H' + 1 H", $4_{A'}$-H, $4_{A"}$-H), 5.25 (m, 1 H' + 1 H', $3_{B'}$-H, $3_{B"}$-H), 5.15 (dd, $J_{2,3}$ = 3.0, $J_{2,1}$ = 1.8 Hz, 1 H", $2_{B"}$-H), 5.13 (dd, $J_{2,3}$ = 3.0, $J_{2,1}$ = 1.8 Hz, 1 H', $2_{B'}$-H), 5.06 (t, $J_{4,3}$ =

$J_{4,5}$ = 10.0 Hz, 1 H' + 1 H", $4_{B'}$-H, $4_{B"}$-H), 4.96 (m, 1 H' + 1 H", $5_{A'}$- H, $5_{A"}$-H), 4.91 (d, $J_{1,2}$ = 1.8 Hz, 1 H', $1_{B'}$-H), 4.86 (d, $J_{1,2}$ = 1.8 Hz, 1 H", $1_{B"}$-H), 4.78 (d, J_{gem} = 12.0 Hz, 1 H', OC*H*HPh'), 4.69–4.61 (m, 2 H' + 1H", OC*H*HPh', OC*H*HPh"), 4.08 (dq, $J_{5,4}$ = 10.0, $J_{5,6}$ = 6.2 Hz, 1 H' + 1 H", $5_{B'}$-H, $5_{B"}$-H), 3.93 (dd, $J_{3,4}$ = 8.0, $J_{3,2}$ = 1.8 Hz, 1 H', $3_{A'}$-H), 3.89 (dd, $J_{3,4}$ = 8.0, $J_{3,2}$ = 3.0 Hz, 1 H", $3_{A"}$-H), 3.66 (dd, $J_{2,1}$ = 5.0, $J_{2,3}$ = 3.0 Hz, 1 H", $2_{A"}$-H), 3.57 (dd, $J_{2,1}$ = 6.0, $J_{2,3}$ = 1.8 Hz, 1 H', $2_{A'}$-H), 3.53 (s, 3 H', OCH_3'), 3.52 (s, 3 H", OCH_3"), 2.17 (s, 3 H", CH_3CO"), 2.14 (s, 3 H', CH_3CO'), 2.13 (s, 3 H", CH_3CO"), 2.11 (s, 3 H', CH_3CO'), 2.07 (s, 3 H', CH_3CO'), 2.06 (s, 3 H' + 3 H", CH_3CO', CH_3CO"), 2.05 (s, 3 H", 3 CH_3CO"), 1.97 (s, 6 H' + 6 H", 2 CH_3CO', 2 CH_3CO"), 1.11 (d, $J_{6,5}$ = 6.2 Hz, 3 H", $6_{B"}$-H), 1.05 (d, $J_{6,5}$ = 6.2 Hz, 3 H', $6_{B'}$-H), 1.03 (d, $J_{6,5}$ = 6.6 Hz, 3 H", $6_{A"}$-H), 1.00 (d, $J_{6,5}$ = 6.6 Hz, 3 H', $6_{A'}$-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 170.4–169.7 (5 CO', 5 CO"), 137.6 (C_{ipso}-Bn', C_{ipso}-Bn"), 128.5–127.8 (C-Ar), 98.8, 97.1 (C-$1_{A"}$, C-$1_{B"}$), 98.5, 97.7 (C-$1_{A'}$, C-$1_{B'}$), 79.1, 76.8, 73.6, 73.1, 71.0, 70.1, 68.9, 68.8, 67.2 (C-$2_{A'}$, C-$2_{B'}$, C-$3_{A'}$, C-$3_{B'}$, C-$4_{A'}$, C-$4_{B'}$, C-$5_{A'}$, C-$5_{B'}$, O*C*H_2Ph'), 79.1, 77.2, 73.9, 72.9, 71.1, 70.0, 68.9, 68.8, 67.2 (C-$2_{A"}$, C-$2_{B"}$, C-$3_{A"}$, C-$3_{B"}$, C-$4_{A"}$, C-$4_{B"}$, C-$5_{A"}$, C-$5_{B"}$, O*C*H_2Ph"), 57.9 (OCH_3'), 53.4 (OCH_3"), 21.2–20.7 (5 *C*H_3CO', 5 CH_3CO"), 19.2, 14.5 (C-$6_{A"}$, C-$6_{B"}$), 17.3, 14.4 (C-$6_{A'}$, C-$6_{B'}$) ppm. MS (MALDI-TOF): calcd. for $C_{32}H_{44}O_{16}$ $[M]^+$ 684.26; found 706.80 $[M + Na]^+$. $C_{32}H_{44}O_{16}$ (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-α-D-rhamnopyranosyl-(1→3)-5-*O*-acetyl-2-*O*-benzyl-D-rhamnofuranoside (36): ^{1}H NMR (400 MHz, $CDCl_3$) (α anomer): δ 7.34 (m, 10 H, Ar-H), 6.20 (d, $J_{1,2}$ = 2.7 Hz, 1 H, 1_A-H), 5.92 (m, 1 H, OCH_2CH=CH_2), 5.30 (dd, J_{vic} = 18.0, J_{gem} = 1.8 Hz, 1 H, *trans* OCH_2CH=C*H*H), 5.19–5.05 (m, 3 H, 2_B-H,

5_A-H, *cis* $OCH_2CH=CHH$), 4.90 (d, J_{gem} = 11.1 Hz, 1 H, $OCHHPh$), 4.78 (d, $J_{1,2}$ = 1.5 Hz, 1 H, 1_B-H), 4.66–4.56 (m, 3 H, 3 $OCHHPh$), 4.39 (t, $J_{3,4} = J_{3,2}$ = 4.8 Hz, 1 H, 3_A-H), 4.22–4.01 (m, 4 H, 4_A-H, 5_B-H, $OCH_2CH=CH_2$), 3.94 (dd, $J_{2,3}$ = 4.8, $J_{2,3}$ = 2.7Hz, 1 H, 2_A-H), 3.84 (dd, $J_{3,4}$ = 9.2, $J_{3,2}$ = 3.0 Hz, 1 H, 3_B-H), 3.38 (t, $J_{4,3} = J_{4,5}$ = 4.8Hz, 1 H, 4_B-H), 2.14, 2.06, 2.05 (3s, 9 H, 3 CH_3CO), 1.29 (d, $J_{6,5}$ = 6.3Hz, 3 H, 6_A-H), 1.18 (d, $J_{6,5}$ = 6.3Hz, 3 H, 6_B-H) ppm. ^{13}C NMR (CDC_{l3}, 75MHz) (α anomer): δ = 170.0, 169.8, 169.6 (3 CO), 138.7, 137.1 (2 C_{ipso}-Bn), 134.5 ($OCH_2CH=CH_2$), 128.5–127.4 (C-Ar), 117.3 ($OCH_2CH=CH_2$), 99.6, 98.4 (C-1_A, C-1_B), 81.4, 80.8, 79.8, 77.3, 76.7, 75.0, 72.6, 70.8, 69.4, 68.6, 68.5 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, $2OCH_2Ph$, $OCH_2CH=CH_2$), 21.3, 21.1, 21.0 (3 CH_3CO), 18.0, 16.4 (C-6_A, C-6_B) 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-α-D-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzyl-α-D-rhamnopyranoside (37): $[\alpha]_D$ = +69.6 (*c* = 1.0, CH_2Cl_2). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.26 (m, 15 H, Ar-H), 6.13 (d, $J_{1,2}$ = 2.0 Hz, 1 H, 1_A-H), 5.86 (m, 1 H, $OCH_2CH=CH_2$), 5.43 (dd, $J_{2,1}$ = 1.8, $J_{2,3}$ = 3.4 Hz, 1 H, 2_B-H), 5.25 (dd, J_{vic} = 17.0, J_{gem} = 1.6 Hz, 1 H, *trans* $OCH_2CH=CHH$), 5.12 (dd, J_{vic} = 10.0, J_{gem} = 1.6 Hz, 1 H, *cis* $OCH_2CH=CHH$), 5.08 (d, $J_{1,2}$ = 1.6 Hz, 1 H, 1_B-H), 4.93 (d, J_{gem} = 11.0 Hz, 1 H, $OCHHPh$), 4.84 (d, J_{gem} = 11.0 Hz, 1 H, $OCHHPh$), 4.76 (d, J_{gem} = 11.8 Hz, 1 H, $OCHHPh$), 4.67 (d, J_{gem} = 11.8 Hz, 1 H, $OCHHPh$), 4.63 (d, J_{gem} = 10.8 Hz, 1 H, $OCHHPh$), 4.61 (d, J_{gem} = 11.0 Hz, 1 H, $OCHHPh$), 4.09 (dd, J_{gem} = 12.8, J_{vic} = 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_A H), 4.03 (dd, J_{gem} = 12.8, J_{vic} = 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_B-H), 3.78

(m, 2 H, 5_A-H, 5_B-H), 3.72 (dd, $J_{2,1}$ = 1.6, $J_{2,3}$ = 2.2Hz, 1 H, 2_A-H), 3.70 (t, $J_{4,5}$ = $J_{4,3}$ = 9.4Hz, 1 H, 4_B-H), 3.39 (t, $J_{4,5}$ = $J_{4,3}$ = 9.4 Hz, 1 H, 4_AH), 2.11 (s, 3 H, CH_3CO), 2.07 (s, 3 H, CH_3CO), 1.32 (d, $J_{6,5}$ = 6.1Hz, 3 H, 6_A-H), 1.24 (d, $J_{6,5}$ = 6.2Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.0, 169.1 (2 CO), 138.6, 137.8, 137.5 (3 C_{ipso}-Bn), 134.6 ($OCH_2CH=CH_2$), 129.0–127.6 (C-Ar), 117.1 ($OCH_2CH=CH_2$), 99.4 (C-1_B), 91.2 (C-1_A), 80.1, 79.8, 77.4, 77.3, 76.7, 75.4, 75.1, 72.6, 70.7, 70.6, 69.2, 69.1 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, 3 OCH_2Ph, $OCH_2CH=CH_2$), 21.0, 20.9 (2 CH_3CO), 18.0, 17.9 (C-6_A, C-6_B) ppm. MS (MALDITOF): calcd. for $C_{40}H_{48}O_{11}$ $[M]^+$ 704.32; found 727.39 $[M + Na]^+$. $C_{40}H_{48}O_{11}$ (704.80): calcd. C 68.16, H 6.86; found C 68.23, H 6.97.

Acetyl 3-*O*-Allyl-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranosyl-(1→3)-5-*O*-acetyl-2-*O*-benzyl-α-D-rhamnofuranoside (38α): $[\alpha]_D$ = +5.9 (*c* = 0.8, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.07–7.26 (m, 15 H, Ar-H), 6.22 (d, $J_{1,2}$ = 3.0Hz, 1 H, 1_A-H), 5.87 (m, 1 H, $OCH_2CH=CH_2$), 5.38 (dd, $J_{2,3}$ = 3.4, $J_{2,1}$ = 2.0 Hz, 1 H, 2 $_B$-H), 5.28 (dd, J_{vic} = 18.0, J_{gem} = 1.5 Hz, 1 H, *trans* $OCH_2CH=CHH$), 5.18 (q, $J_{5,6}$ = $J_{5,4}$ = 6.0Hz, 1 H, 5_A-H), 5.12 (dd, J_{vic} = 10.0, J_{gem} = 1.5 Hz, 1 H, *cis* $OCH_2CH=CHH$), 4.94 (d, J_{gem} = 11.0 Hz, 1 H, $OCHHPh$), 4.91 (d, $J_{1,2}$ = 2.0Hz, 1 H, 1 $_B$-H), 4.64 (m, 3 H, 3 $OCHHPh$), 4.43 (t, $J_{3,4}$ = $J_{3,2}$ = 5.4 Hz, 1 H, 3_A-H), 4.20 (m, 2 H, 4_A-H, $OCHHCH=CH_2$), 4.09 (m, 2 H, 5_B-H, $OCHHCH=CH_2$), 3.96 (m, 2 H, 2_A-H, 3_B-H), 3.49 (t, $J_{4,5}$ = $J_{4,3}$ = 9.0 Hz, 1 H, 4_B-H), 2.08 (s, 3 H, CH_3CO), 2.06 (s, 3 H, CH_3CO), 1.31 (d, $J_{6,5}$ = 6.0 Hz, 3 H, 6_A-H), 1.21 (d, $J_{6,5}$ = 6.0 Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 169.9, 169.8, 165.5 (3 CO), 138.6, 137.1 (2 C_{ipso}-Bn), 134.6 ($OCH_2CH=CH_2$), 133.1 (C_{ipso}-Bz), 129.9–127.6 (C-Ar), 117.3 ($OCH_2CH=CH_2$), 99.5, 98.3 (C-1_A, C-1_B), 81.4, 80.8, 79.7, 77.4, 76.6, 75.0, 72.5, 70.6, 69.7, 68.6, 68.5 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, 2 OCH_2Ph,

O*C*H_2CH=CH_2), 21.4, 21.2 (2 *C*H_3CO), 18.2, 16.4 (C-6_A, C-6_B) ppm. MS (MALDI-TOF): calcd. for $C_{40}H_{46}O_{12}$ $[M]^+$ 718.30; found 741.03 $[M + Na]^+$. $C_{40}H_{46}O_{12}$ (718.79): calcd. C 66.84, H 6.45; found C 66.68, H 6.32.

Acetyl 3-*O*-Allyl-2-*O*-benzoyl-4-*O*-benzyl-α-D-rhamnopyranosyl-(1→3)-5-*O*-acetyl-2-*O*-benzyl-α-D-rhamnofuranoside (38β): $[\alpha]_D$ = –6 (*c* = 0.3, CH_2Cl_2). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.07–7.28 (m, 15 H, Ar-H), 6.25 (d, $J_{1,2}$ = 4.5 Hz, 1 H, 1_A-H), 5.88 (m, 1 H, OCH$_2$C*H*=CH$_2$), 5.36 (dd, J_{vic} = 18.0, J_{gem} = 1.6 Hz, 1 H, *trans* OCH$_2$CH=C*H*H), 5.30 (dd, $J_{2,3}$ = 3.4, $J_{2,1}$ = 2.0 Hz, 1 H, 2_B-H), 5.13 (m, 2 H, 5_A-H, *cis* OCH$_2$CH=C*H*H), 4.95 (d, J_{gem} = 11.0 Hz, 1 H, OC*H*HPh), 4.85 (d, $J_{1,2}$ = 1.8 Hz, 1 H, 1_B-H), 4.69 (d, J_{gem} = 13.0 Hz, 1 H, OC*H*HPh), 4.63 (d, J_{gem} = 11.0 Hz, 1 H, OC*H*HPh), 4.61 (d, J_{gem} = 13.0 Hz, 1 H, OC*H*HPh), 4.32 (dq, $J_{5,4}$ = 10.0, $J_{5,6}$ = 6.0 Hz, 1 H, 5_B-H), 4.23 (m, 2 H, 3_A-H, OC*H*HCH=CH$_2$), 4.10 (dd, J_{gem} = 13.0, J_{vic} = 6.0 Hz, 1 H, OC*H*HCH=CH$_2$), 4.00 (dd, *J*3,4 = 10.0, $J_{3,2}$ = 3.4Hz, 1 H, 3_B-H), 3.94 (dd, $J_{4,5}$ = 8.5, $J_{4,3}$ = 4.0 Hz, 1 H, 4_A-H), 3.85 (t, $J_{2,3}$ = $J_{2,1}$ = 4.5 Hz, 1 H, 2_A-H), 3.50 (t, $J_{4,5}$ = $J_{4,3}$ = 10.0 Hz, 1 H, 4_B-H), 2.11 (s, 3 H, CH_3CO), 2.09 (s, 3 H, CH_3CO), 1.36 (d, $J_{6,5}$ = 6.0 Hz, 3 H, 6_A-H), 1.15 (d, $J_{6,5}$ = 6.0Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 170.5, 170.3, 165.6 (3 CO), 138.9, 137.1 (2 C_{ipso}-Bn), 135.2, 134.8 (OCH$_2$*C*H=CH$_2$, C_{ipso}-Bz), 129.9–127.4 (C-Ar), 117.3 (OCH$_2$CH=*C*H$_2$), 99.8, 93.2 (C-1_A, C-1_B), 82.7, 80.1, 77.7, 77.2, 76.6, 75.0, 72.6, 70.1, 69.8, 68.6, 68.3 (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 O*C*H$_2$Ph, O*C*H$_2$CH=CH$_2$), 21.4, 21.3 (2 *C*H_3CO), 18.1, 16.9 (C-6_A, C-6_B) ppm. MS (MALDI-TOF): calcd. for $C_{40}H_{46}O_{12}$ $[M]^+$ 718.30; found 740.56 $[M + Na]^+$. $C_{40}H_{46}O_{12}$ (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_2Cl_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, 1_A-H), 5.84 (m, 1 H, OCH_2CH=CH_2), 5.68 (dd, $J_{2,1}$ = 1.6, $J_{2,3}$ = 3.4 Hz, 1 H, 2_B-H), 5.25–5.21 (m, 2 H, 1_B-H, *trans* OCH_2CH=C*H*H), 5.08 (dd, J_{vic} = 10.2, J_{gem} = 1.8 Hz, 1 H, *cis* OCH_2CH=C*H*H), 4.95 (d, J_{gem} = 11.0 Hz, 1 H, OC*H*HPh), 4.90 (d, J_{gem} = 11.0 Hz, 1 H, OC*H*HPh), 4.79 (d, J_{gem} = 12.0 Hz, 1 H, OC*H*HPh), 4.72–4.62 (m, 3 H, 3 OC*H*HPh), 4.19 (dd, J_{gem} = 12.0, J_{vic} = 5.0 Hz, 1 H, OC*H*HCH=CH_2), 4.12–4.05 (m, 2 H, 3_A-H, OC*H*HCH=CH_2), 3.98 (dd, $J_{3,2}$ = 3.2, $J_{3,4}$ = 9.2 Hz, 1 H, 3_B-H), 3.91–3.65 (m, 4 H, 2_A-H, 4_A-H, 5_A-H, 5_B-H), 3.51 (t, $J_{4,5}$ = $J_{4,3}$ = 9.6 Hz, 1 H, 4_B-H), 2.06 (s, 3 H, CH_3CO), 1.32 (d, $J_{6,5}$ = 6.2 Hz, 3 H, 6_B-H), 1.29 (d, $J_{6,5}$ = 6.2Hz, 3 H, 6_A-H) ppm. ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 169.2, 165.5 (2 CO), 138.6, 137.8, 137.5 (3 C_{ipso}-Bn), 134.6 (OCH2*C*H=CH2), 133.1 (C_{ipso}-Bz), 129.8–125.3 (C-Ar), 117.1 (OCH_2CH=*C*H_2), 99.5 (C-1_B), 91.2 (C-1_A), 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-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, 3 O*C*H_2Ph, O*C*H_2CH=CH_2), 21.0 (*C*H_3CO), 18.2, 18.1 (C-6_A, C-6_B) ppm. MS (MALDI-TOF): calcd. for $C_{45}H_{50}O_{11}$ $[M]^+$ 766.34; found 789.42 [M + Na]$^+$. $C_{45}H_{50}O_{11}$ (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, 1_A-H), 5.41 (dd, $J_{5,4}$ = 9.9, $J_{5,6}$ = 3.6 Hz, 1 H, 3_B-H), 5.20 (dd, $J_{2,3}$ = 3.6, $J_{2,1}$ = 1.8Hz, 1 H, 2_B-H), 5.06 (t, $J_{4,3}$ = $J_{4,5}$ = 9.9 Hz, 1 H, 4_B-H), 5.00 (s, 1 H, 1_B-H), 4.93 (d, J_{gem} = 11.1 Hz, 1 H, OC*H*HPh), 4.71 (d, J_{gem} = 11.4 Hz, 1 H, OC*H*HPh), 4.62 (d, J_{gem} = 11.4 Hz, 1 H, OC*H*HPh), 4.60 (d, J_{gem} = 11.1 Hz, 1 H, OC*H*HPh), 4.18

(dq, $J_{5,4}$ = 9.9, $J_{5,6}$ = 6.3 Hz, 1 H, 5_B-H), 4.12 (d, $J_{4,3}$ = 2.4 Hz, 1 H, 4_A-H), 3.99 (q, $J_{5,6}$ = 6.6 Hz, 1 H, 5_A-H), 3.69–3.61 (m, 2 H, 2_A-H, 3_A-H), 2.16, 2.11, 2.03, 2.00 (4 s, 12 H, 4 CH_3CO), 1.18 (d, $J_{6,5}$ = 6.6 Hz, 3 H, 6_A-H), 0.98 (d, $J_{6,5}$ = 6.3 Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) (α anomer): δ = 170.3, 169.9, 169.7, 169.3 (4 CO), 137.9, 137.7 (2 C_{ipso}-Bn), 128.9–127.7 (C-Ar), 94.1 (C-1_B), 90.6 (C-1_A), 76.1, 75.6, 74.3, 73.4, 73.0, 70.9, 70.3, 69.0, 68.9, 66.5 (C-2_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, 2 O*C*H_2Ph), 21.0–20.7 (4 *C*H_3CO), 17.0, 16.8 (C-6_A, C-6_B) ppm. MS (MALDI-TOF): calcd. for $C_{34}H_{42}O_{13}$ $[M]^+$ 658.26; found 680.88 $[M + Na]^+$. $C_{34}H_{42}O_{13}$ (658.69): calcd. C 62.00, H 6.43; found C 62.18, H 6.61.

4,5-di-*O*-acetyl-2,3-di-*O*-benzyl-D-quinovose (42): $[\alpha]_D$ +10 (*c* 0.4; CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.73 (s, 1H, CHO), 7.36-7.23 (m, 10H, H-Ar), 5.35 (dd, 1H, $J_{4,5}$ 6.4 Hz, $J_{4,3}$ 3.0 Hz, H-4), 5.10 (quintet, 1H, $J_{5,4}$=$J_{5,6}$ 6.4 Hz, H-5), 4.77 (d, 1H, J_{gem} 12.0 Hz, OC*H*HPh), 4.57 (s, 2H, OCH_2Ph), 4.52 (d, 1H, J_{gem} 12.0 Hz, OC*H*HPh), 3.92 (m, 2H, H-2, H-3), 2.02 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.19 (d, 3H, $J_{6,5}$ 6.4 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 201.1 (CHO), 169.9, 169.7 (2 CO), 136.9, 136.8 (2 C_{ipso}-Bn), 128.6-128.0 (C-Ar), 80.6, 77.5, 74.1, 73.2, 72.1, 68.6 (C-2, C-3, C-4, C-5, 2 O*C*H_2Ph), 21.2, 20.8 (2 *C*H_3CO), 15.8 (C-6). MALDI TOF-MS: calcd for $C_{24}H_{28}O_7$ (m/z), 428.18; found, 451.01 $[M+Na]^+$. Anal Calcd for $C_{24}H_{28}O_7$: C, 67.28; H, 6.59. Found: C, 67.09; H, 6.49.

1,1,4,5-tetra-*O*-acetyl-2,3-di-*O*-benzyl-D-quinovose acetal (43): $[\alpha]_D$ +19 (*c* 0.5; CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.28 (m, 10H, H-Ar), 6.97 (d, 1H, $J_{1,2}$ 3.2 Hz, H-1), 5.24 (d, 1H, $J_{4,5}$ 5.9 Hz, $J_{4,3}$ 3.2 Hz, H-4), 5.16 (quintet, 1H, $J_{5,4}$=$J_{5,6}$ 6.2 Hz, H-

5), 4.78 (d, 1H, J_{gem} 10.9 Hz, OC*H*HPh), 4.74 (d, 1H, J_{gem} 11.6 Hz, OC*H*HPh), 4.71 (d, 1H, J_{gem} 11.6 Hz, OC*H*HPh, 4.52 (d, 1H, J_{gem} 10.9 Hz, OC*H*HPh), 3.81 (m, 2H, H-2, H-3), 2.09 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.21 (d, 3H, $J_{6,5}$ 6.2 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 170.0, 168.7, 168.5 (4 CO), 137.7, 137.5 (2 C_{ipso}-Bn), 128.6-127.8 (C-Ar), 88.1 (C-1), 78.0, 76.1, 74.7, 74.1, 73.3, 69.2 (C-2, C-3, C-4, C-5, 2 O*C*H_2Ph), 21.1, 20.9, 20.8, 20.7 (4 *C*H_3CO), 15.8 (C-6). MALDI TOF-MS: calcd for $C_{28}H_{34}O_{10}$ (m/z), 530.22, found, 552.89 $[M+Na]^+$. Anal Calcd for $C_{28}H_{34}O_{10}$: C, 63.39; H, 6.46. Found: C, 63.11; H, 6.24.

1.11 1,5-di-*O*-acetyl-2,3-di-*O*-benzyl-D-quinovofuranose (44): ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36-7.24 (m, 10H_α + 10H_β, H-Ar), 6.35 (d, 1H_α, $J_{1,2}$ 4.2 Hz, H-1$_\alpha$), 6.16 (bs, 1H_β, H-1$_\beta$), 5.24 (dq, 1H_β, $J_{5,6}$ 7.4 Hz, $J_{5,6}$ 6.3 Hz, H-5$_\beta$), 5.18 (dq, 1H_α, $J_{5,6}$ 6.2 Hz, $J_{5,4}$ 4.5 Hz, H-5$_\alpha$), 4.64 (m, 2H_α+ 1H_β, 2 OC*H*HPh$_\alpha$ + OC*H*HPh$_\beta$), 4.58 (d, 1H_α, J_{gem} 12.0 Hz, OC*H*HPh$_\alpha$), 4.55 (d, 1H_β, J_{gem} 12.0 Hz, OC*H*HPh$_\beta$), 4.50 (d, 1H_α, J_{gem} 11.5 OC*H*HPh$_\alpha$), 4.48 (d, 1H_β, J_{gem} 12.0 Hz, OC*H*HPh□), 4.41 (d, 1H_β, J_{gem} 12.0 Hz, OC*H*HPh$_\beta$), 4.36 (dd, 1H_α, $J_{4,3}$ 5.8 Hz, $J_{4,5}$ 4.5 Hz, H-4$_\alpha$), 4.29 (dd, 1H_β, $J_{4,5}$ 7.4 Hz, $J_{4,3}$ 5.0 Hz, H-4$_\beta$), 4.12 (m, 2H_α, H-2$_\alpha$, H-3$_\alpha$), 4.04 (bs, 1H_β, H-2$_\beta$), 4.03 (dd, 1H_β, $J_{3,4}$ 5.0 Hz, $J_{3,2}$ 1.3 Hz, H-3$_\beta$), 2.09 (s, 3H_α, CH_3CO_α), 2.04 (s, 3H_β, CH_3CO_β), 1.97 (s, 3H_α, CH_3CO_α), 1.91 (s, 3H_β, CH_3CO_β), 1.34 (d, 3H_α, $J_{6,5}$ 6.2 Hz, H-6$_\alpha$), 1.30 (d, 3H_α, $J_{6,5}$ 6.2 Hz, H-6$_\alpha$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 169.9 (2 CO_α), 170.1, 169.8 (2 CO_β), 137.6, 137.2 (2 C_{ipso}-Bn$_\alpha$), 137.4, 137.1 (2 C_{ipso}-Bn$_\beta$), 128.5-127.5 (C-Ar), 100.2 (C-1$_\beta$), 94.4 (C-1$_\alpha$), 84.5, 84.2, 80.5, 72.1, 72.0, 68.6 (C-2$_\beta$, C-3$_\beta$, C-4$_\beta$, C-5$_\beta$, 2 O*C*H_2Ph$_\beta$), 83.2, 80.0, 77.0, 73.3, 72.6, 69.0 (C-2$_\alpha$, C-3$_\alpha$, C-4$_\alpha$, C-5$_\alpha$, 2 O*C*H_2Ph$_\alpha$), 21.2-21.1 (2 *C*H_3CO_α + 2 *C*H_3CO_β), 17.2 (C-6$_\beta$), 15.8

($C\text{-}6_\alpha$). MALDI TOF-MS: calcd for $C_{24}H_{28}O_7$ (m/z), 428.18; found, 451.39 $[M+Na]^+$. Anal Calcd for $C_{24}H_{28}O_7$: C, 67.28; H, 6.59. Found: C, 66.99; H, 6.38.

2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-1,1,4,5-tetra-*O*-acetyl-2-*O*-benzyl-D-quinovose Acetal (46): $[\alpha]_D = -15$ ($c = 0.1$, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.36 (m, 5 H, Ar-H), 6.95 (d, $J_{1,2}$ = 3.2 Hz, 1 H, 1_A-H), 5.33 (s, $J_{4,3}$ = 2.3 Hz, 1 H, 4_A-H), 5.28 (dd, $J_{3,4}$ = 10.0, $J_{3,2}$ = 4.8Hz, 1 H, 3_B-H), 5.19 (dd, $J_{2,3}$ = 4.8, $J_{2,1}$ = 3.2 Hz, 1 H, 2_B-H), 5.11 (q, $J_{5,6}$ = 6.3 Hz, 1 H, 5_A-H), 5.06 (t, $J_{4,3} = J_{4,5}$ = 10.0 Hz, 1 H, 4_B-H), 5.03 (br. s, 1 H, 1_B-H), 4.75 (d, J_{gem} = 11.6 Hz, 1 H, OC*H*HPh), 4.70 (d, J_{gem} = 11.5 Hz, 1 H, OC*H*HPh), 4.06 (dd, $J_{3,2}$ = 6.8, $J_{3,4}$ = 2.3Hz, 1 H, 3_A-H), 4.00 (dq, $J_{5,4}$ = 10.0, $J_{5,6}$ = 6.4 Hz, 1 H, 5_B-H), 3.80 (dd, $J_{2,3}$ = 6.8, $J_{2,1}$ = 3.2 Hz, 1 H, 2_A-H), 2.10, 2.07, 2.06, 2.05, 2.04, 2.03, 1.97 (7 s, 21 H, 7 CH_3CO), 1.24 (d, $J_{6,5}$ = 6.3 Hz, 3 H, 6_A-H), 1.21 (d, $J_{6,5}$ = 6.4 Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 170.1, 170.0, 169.9, 169.7, 168.6, 168.5, 168.4 (7 CO), 134.7 (C_{ipso}-Bn), 128.4–127.9 (C-Ar), 100.8, 98.9 ($C\text{-}1_A$, $C\text{-}1_B$), 87.7, 74.5, 74.1, 72.9, 70.9, 69.6, 69.0, 68.8, 67.5 ($C\text{-}2_A$, $C\text{-}2_B$, $C\text{-}3_A$, $C\text{-}3_B$, $C\text{-}4_A$, $C\text{-}4_B$, $C\text{-}5_A$, $C\text{-}5_B$, O*C*H_2Ph), 21.1–20.7 (7 *C*H_3CO), 17.3, 15.8 ($C\text{-}6_A$, $C\text{-}6_B$) ppm. MS (MALDI-TOF): calcd. for $C_{33}H_{44}O_{17}$ $[M]^+$ 712.29; found 734.61 $[M + Na]^+$. $C_{33}H_{44}O_{17}$ (712.69): calcd. C 55.61, H 6.22; found C 55.42, H 6.10.

Acetyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-5-*O*-acetyl-2-*O*-benzyl-D-quinovofuranoside (47): ^{1}H NMR (300 MHz, $CDCl_3$) (β-anomer): δ = 7.31 (m, 5 H, Ar-H), 6.21 (s, 1 H, 1_A-H), 5.25–5.00 (m, 4 H, 2_B-H, 3_B-H, 4_B-H, 5_A-H), 4.76 (s, 1 H, 1_B-H), 4.69 (d, J_{gem} = 11.7 Hz, 1 H, OC*H*HPh), 4.60 (d, J_{gem} = 11.7 Hz, 1 H, OC*H*HPh), 4.36 (dd, $J_{4,5}$ = 7.2, $J_{4,3}$ = 4.5 Hz, 1 H, 4_A-H), 4.29 (d, $J_{3,4}$ = 4.5 Hz, 1 H, 3_A-H), 4.08 (s, 1

H, 2_A-H), 3.79 (dq, $J_{5,4}$ = 9.3, $J_{5,6}$ = 6.3 Hz, 1 H, 5_B-H), 2.15, 2.09, 2.06, 2.04, 1.98 (5 s, 15 H, 5 CH_3CO), 1.38 (d, $J_{6,5}$ = 6.3 Hz, 3 H, 6_A-H), 1.24 (d, $J_{6,5}$ = 6.3 Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) (β-anomer): δ = 170.1–169.6 (5 CO), 136.8 (C_{ipso}-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, O*C*H_2Ph), 21.3–20.7 (5 *C*H_3CO), 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 $[M + Na]^+$. $C_{29}H_{38}O_{14}$ (610.60): calcd. C 57.04, H 6.27; found C 57.00, H 6.24.

Acetyl 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-*O*-benzyl-D-quinovopyranoside (48): ^1H NMR (400 MHz, $CDCl_3$) (α-anomer): δ = 7.34–7.24 (m, 10 H, Ar-H), 6.25 (d, $J_{1,2}$ = 3.6 Hz, 1 H, 1_A-H), 5.39–5.28 (m, 3 H, 1_B-H, 2_B-H, 3_B-H), 4.99 (t, $J_{4,3}$ = $J_{4,5}$ = 9.9 Hz, 1 H, 4_B-H), 4.86 (d, J_{gem} = 11.1 Hz, 1 H, OC*H*HPh), 4.72 (d, J_{gem} = 11.1 Hz, 1 H, OC*H*HPh), 4.63 (d, J_{gem} = 11.1 Hz, 1 H, OC*H*HPh), 4.50 (d, J_{gem} = 11.1 Hz, 1 H, OC*H*HPh), 4.10 (t, $J_{3,4}$ = $J_{3,2}$ = 9.6Hz, 1 H, 3_A-H), 4.05 (dq, $J_{5,4}$ = 9.9, $J_{5,6}$ = 6.3 Hz, 1 H, 5_B-H), 3.91 (dq, $J_{5,4}$ = 9.6, $J_{5,6}$ = 6.3 Hz, 1 H, 5_A-H), 3.66 (dd, $J_{2,3}$ = 9.6, $J_{2,1}$ = 3.3 Hz, 1 H, 2_A-H), 3.17 (t, $J_{4,3}$ = $J_{4,5}$ = 9.6 Hz, 1 H, 4_A-H), 2.13, 2.07, 2.01, 1.94 (4 s, 12 H, 4 CH_3CO), 1.32 (d, *J*6,5 = 6.3 Hz, 3 H, 6_A-H), 0.91 (d, $J_{6,5}$ = 6.3 Hz, 3 H, 6_B-H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz) (α-anomer): δ = 170.0, 169.8, 169.7, 169.4 (4 CO), 137.6, 136.9 (2 C_{ipso}-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 O*C*H_2Ph), 21.0–20.8 (4 *C*H_3CO), 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-

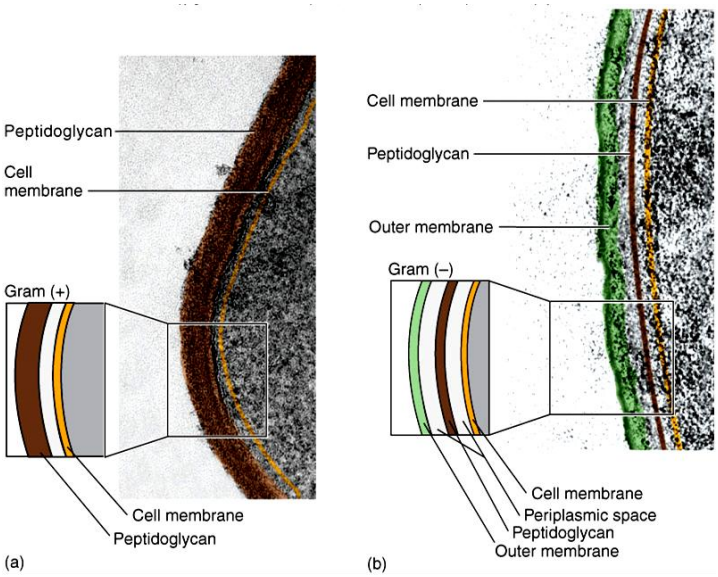


FIG. 3.1: Gram-Positive And Gram-Negative Cell Walls. The Gram-positive envelope is from *Bacillus lichteniformis* (left), and the Gram-negative micrograph is of *Aquaspirillum serpens* (right).

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

[173] 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.

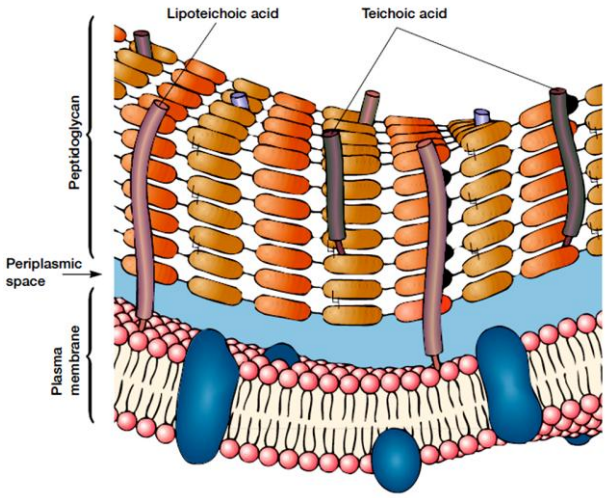


FIG.3.2: The Gram-positive envelope.

[173] (a) Matias, V.R.; Al-Amoudi, A.; Dubochet, J.; Beveridge, T.J. *J Bacteriol.* **2003,** *185,* 6112; (b) Vollmer, W.; Höltje, J.V. *J. Bacteriol.* **1990**, *186*, 5978.

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 β-(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**

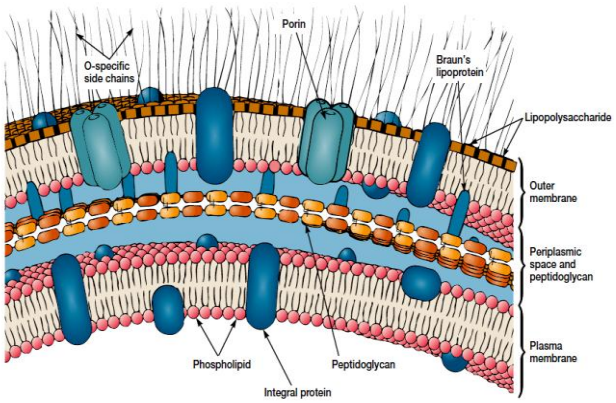


FIG. 3.3: The Gram-negative envelope.

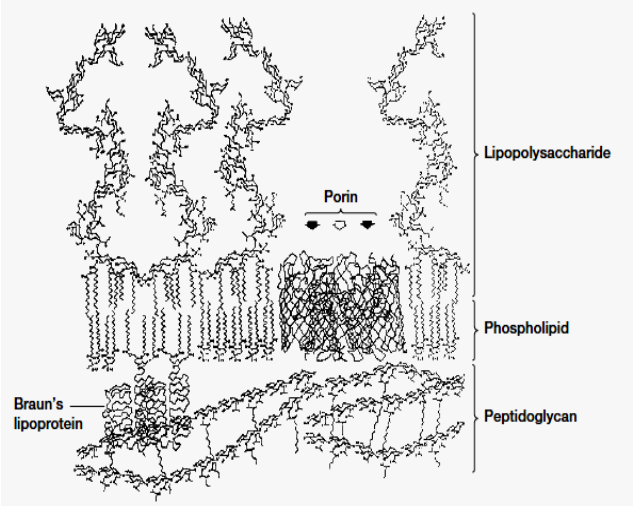


FIG. 3.4: A chemical model of the Gram-negative outer membrane

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-octulu-sonic acid). The core oligosaccharides possess a more conservative structure when compared with those of the O-specific polysaccharides. [174] 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 [175] and teichoic acids [176] and is involved in the division processes. [177]

---

[174] Holst, O. In *Endotoxin in Health and Disease*, Brade, H.; Opal, S.M.; Vogel, S.N.; Morrison, C.D. Ed., Marcel Dekker Inc, New York, **1999**.
[175] Dramsi, S.; Davison, S.; Magnet, S.; Arthur, M. *FEMS Microbiol Rev.* **2008**, *32*, 307.
[176] Neuhaus, F.C.; Baddiley, J. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 686.
[177] Nanninga, N. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 110.

The main structural features of peptidoglycan are linear glycan strands cross-linked by short peptides. The glycan strands are made up of alternating β-1→4 linked *N*-acetyl-D-

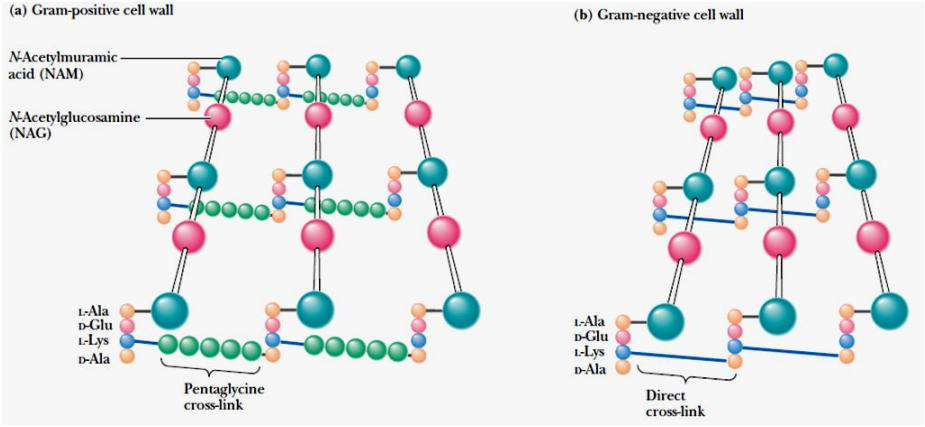


FIG. 3.5: Schematic representation of Gram-positive and Gram-negative peptidoglycan.

glucosamine (Glc*N*Ac) and *N*-acetylmuramic acid (Mur*N*Ac) 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 γ-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. [178] The peptide structure of a Gram-negative bacterium is commonly L-Ala-γ-D-Glu-*meso*-DAP-D-Ala-D-Ala, where the dibasic *meso*-

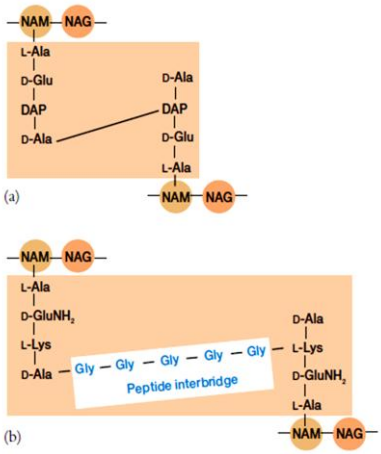


FIG. 3.6 Peptidoglycan Cross-Links: (a) *E. coli* peptidoglycan with direct cross-linking, typical of many Gram-negative bacteria. (b) *Staphylococcus aureus* peptidoglycan. *S. aureus* is a Gram-positive bacterium.

[178] Schleifer, K.H.; Kandler, O. *Bacteriol. Rev.* **1972**, *36*, 407.

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). [179]

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, [180] insects [181] and plants, by PGRP proteins ("***PG Recognition Protein***") and can stimulate innate immunity that is the first line of defense against infections by microbes: [182] 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

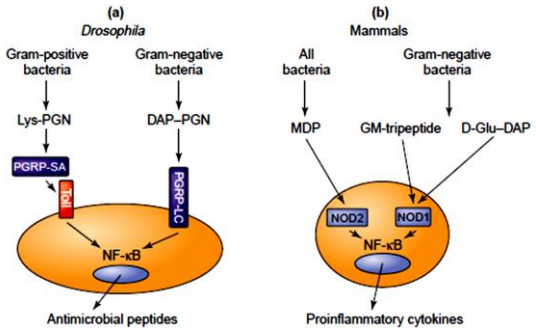


FIG. 3.7: Recognition of peptidoglycans (PGNs) by immune-competent cells in *Drosophila* and mammals. A major distinction exists between PGN accessibility of Gram-positive and Gram-negative bacteria. While Gram-positive bacteria have their PGN exposed for direct recognition by small PGRPs, Gram-negative bacteria have their PGN between the cytoplasmatic membrane and outer membrane . Hence, their detection by PGRPs occurs most likely via recognition of PGN fragments release during PG turmover.

[179] Navarre, W.W.; Schneewind, O. *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 174.
[180] Le Bourhis, L.; Wertz, C. *Microbes and Infection* **2007**, *9*, 629.
[181] (a) Gobert, V.; Gottar, M.; Matskevich, A.A.; Rutschmann, S.; Royet, J.; Belvin, M.; Hoffmann, J.A.; Ferrandon, D. *Science* **2003**, *302*, 2126; (b) Chang, C.I.; Pili-Floury, S.; Herve, M.; Parquet, C.; Chelliah, Y.; Lemaitre, B.; Mengin-Lecreulx, D.; Deisenhofer, J. *PLoS Biol.* **2004**, *2*, E277.
[182] Hoffmann, J.A., Kafatos, F.C., Janeway, C.A., Ezekowitz, R.A.B. *Science* **1999**, *284***,** 1313.

and to 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. [183] 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, [184] PGN structures can be modified in both the peptide stem FIG. 3.8) and bridge regions and in the glycan strands. [185]

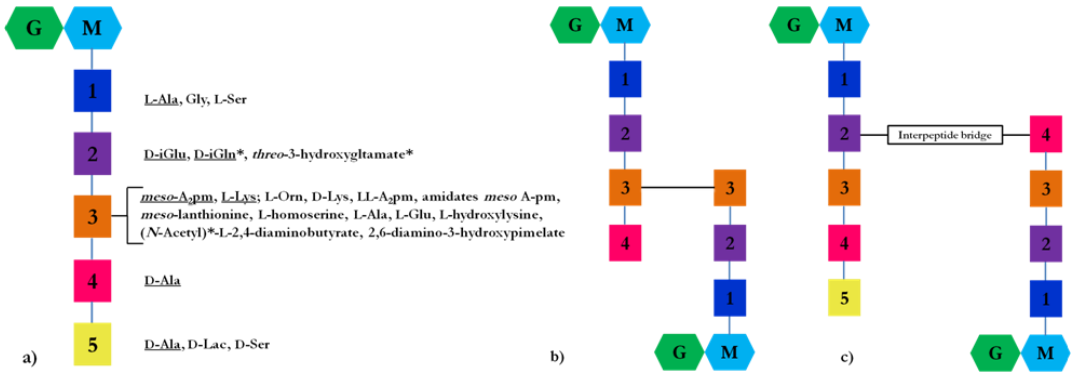


FIG. 3.8: Most common variation in peptide stem (a) and in the cross-linking (b and c). Amino acids with a star (*) are formed by a secondary modification during peptidoglycan biosynthesis.

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

[183] (a) Gust, A.A.; Biswas, R.; Lenz, H.D.; Rauhut, T.; Ranf, S.; Kemmerling, B.; Götz, F.; Glawischnig, E.; Lee, G.; Felix, G.; Nürnberger, T. *J. Biol. Chem.* **2007**, *282*, 32338; (b) Silipo, A.; Erbs, G.; Shinya, T.; Dow, J.M.; Parrilli, M.; Lanzetta, R.; Shibuya, N.; Newman, M.A.; Molinaro, A. *Glycobiology* **2010**, *20*, 406.
[184] Humann, J.; Lenz, L.L. *J. Innate Immun.* **2009**, *1*, 88.
[185] For a detailed review on structural variation of peptidoglycan structures see: Vollmer, W.; Blanot, D.; de Pedro, M.A. *FEMS Microbiol Rev.* **2008**, *32*, 149.

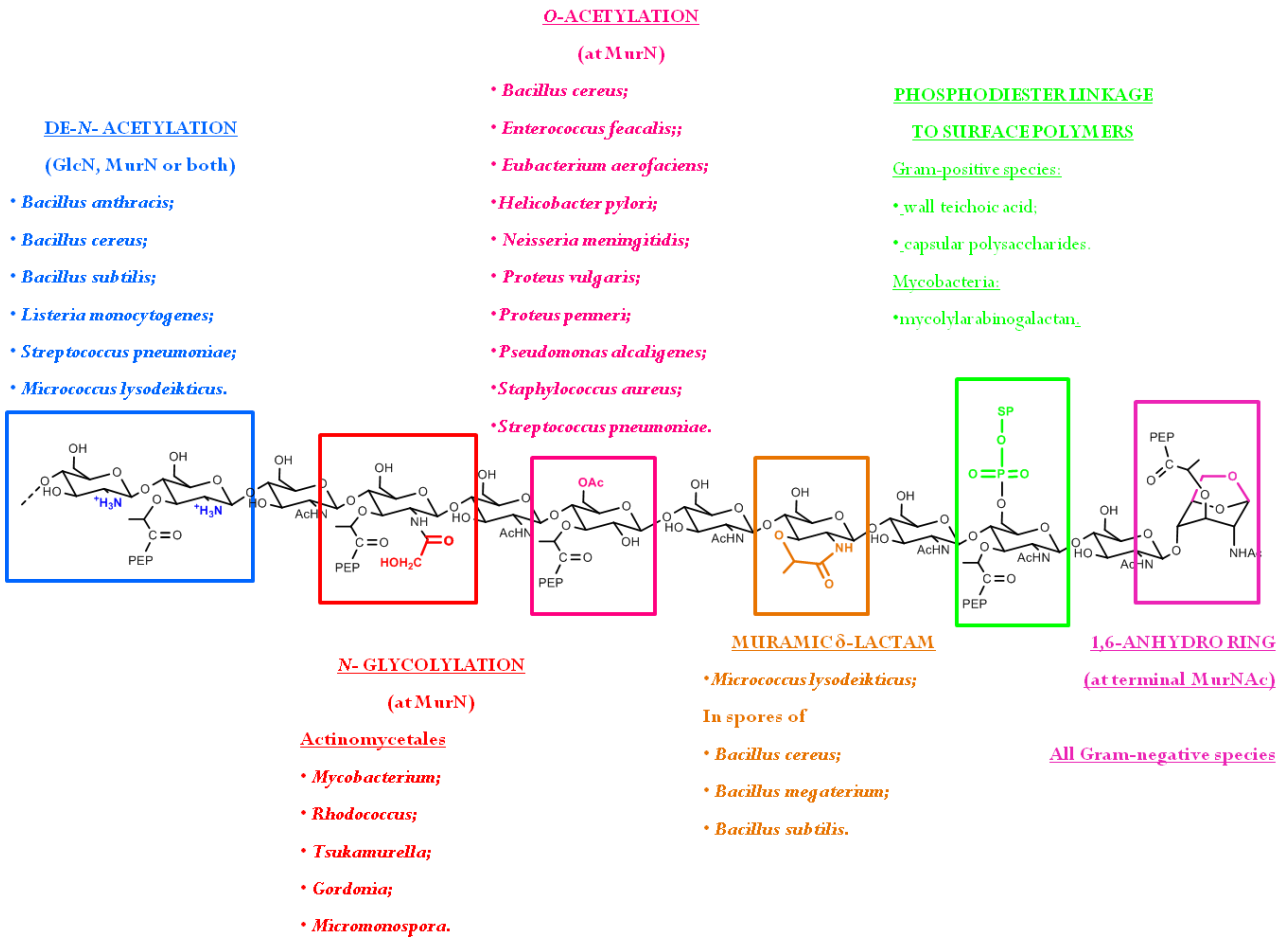


FIG. 3.9: Modification in the glycan strands. The name of some species containing the modifications in their peptidoglycan are indicated.

bacteria), the attachment of surface polymers (*e.g.* teichoic acids or capsular polysaccharides via phosphodiester bonds to their Glc*N*Ac or Mur*N*Ac residues). Among these modifications *N*-deacetylation has acquired great relevance because of its influence on recognition processes. The presence of deaceylated 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. [186] It is worth noting that the activity of the lysozyme can be restored by chemical acetylation of the substrate. [187] 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. [188] The role of peptidoglycan in virulence has been largely investigated for the human-pathogen Gram-positive *Lysteria monocytogenes* [189] 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 phythopathogenic Gram-

[186] (a) Blake, C.C.; Koenig, D.F.; Mair, G.A.; North, AC.; Phillips, D.C.; Sarma, V.R. *Nature* **1965**, *206*, 757; (b) Vocadlo, D.J.; Davies, G.J.; Laine, R.; Withers, S.G. *Nature* **2001**, *412*, 835.
[187] (a) Westmacott, D.; Perkins, H.R. *J. Gen. Microbiol.* **1979**, *115*, 1; (b) Vollmer, W.; Tomasz, A. *J. Biol. Chem.* **2000**, *275*, 20496.
[188] Peschel, A. *Trends Microbiol.* **2002**, *10*, 179.
[189] Boneca, I.G.; Dussurget, O.; Cabanes, D.; Nahori, M.A.; Sousa, S.; Lecuit, M.; Psylinakis, E.; Bouriotis, V.; Hugot, J.P.; Giovannini, M.; Coyle, A.; Bertin, J.; Namane, A.; Rousselle, J.C.; Cayet, N.; Prévost, M.C.; Balloy, V.; Chignard, M.; Philpott, D.J.; Cossart, P.; Girardin, S.E. *Proc. Nat. Acad. Sci. U.S.A.* **2007**, *104*, 997.

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 tiphymurium*, *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

[190] Erbs, G.; Silipo, A.; Aslam, S.; De Castro, C.; Liparoti, V.; Flagiello, V.; Pucci, P.; Lanzetta, R.; Parrilli, M.; Molinaro, A.; Newmann, M.A.; Cooper, R.M. *Chem.Biol.* **2008**, *15*, 438.
[191] (a) Dworkin, J.; Shah, I.M. *Nature Microb.* **2010**, *8*, 890; (b) Keep, N.H.; Ward, J.M.; Cohen-Gonsaud, M.; Henderson, B. T. *TRENDS in Microbiology* **2006**, *14*, 271.
[192] Oliver, J.D. *J. Microbiol.* **2005**, *43*, 93.
[193] Hu, Y.; Coates, A.R.M. In *Dormancy and Low-Growth States in Microbial Deseases*, Coates A.R.M. Ed., Cambridge University Press, **2003**, 1.
[194] Atrih, A.; Foster, S.J. *Antonie Van Leeuwenhoek* **1999**, *75*, 299.

shortening of the chain length of glycan strands. [195] Also *Enterococcus feacalis* exhibits qualitatively similar modifications in peptidoglycan [196] and, recently, it has been shown that also *O*-acetylation is increased. [197]

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 [198] Actinobacter *Micrococcus luteus* (*lysodeiktikus*), whose cells could be resuscitated by exposure to non-dormant *Micrococcus luteus*, this suggesting that cell-cell interaction are required to exit from dormancy. [199] 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. [200] Inactivation of the *Micrococcus luteus rpf* gene was not possible, which indicated the essential nature of Rpf for cell survival. [201] 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. [202] A key enzyme for *M. tuberculosis* cell division is resuscitation promoting factor interacting protein (RipA), which is believed to cleave peptidoglycan cross-links [203] but its mechanism of action is still unknown and

---

[195] Signoretto, C.; Lleò; M.M.; Canepari, P. *Curr Microbiol.* **2002**, 44, 125.
[196] Signoretto, C.; Lleò, M.M.; Tafi, M.C.; Canepari, P. *Appl. Environ. Microbiol.* **2000**, *66*, 1953.
[197] Pfeffer, J.M.; Strating, H.; Weadge, J.T.; Clarke, A.J. *J. Bacteriol.* **2006**, *188*, 902.
[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 16SrRNAs isolated from 30S subunits of prokaryotic ribosomes. The vantages 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.
[199] Votyakova, T.V.; Kaprelyants, A.S.; Kell, D.B. *Appl. Env. Microbiol.* **1994**, *60*, 3284.
[200] Mukamolova, G.V.; Kaprelyants, A.S.; Young, D.I.; Young, M.; Bell, D.B. *Proc. Nat. Acad. Sci. U.S.A.* **1998**, *95*, 8916.
[201] Mukamolova, G.V.; Turapov, O.A.; Kazarian, K.; Telkov, M.; Kaprelyants, A.S.; Kell, D.B.; Young, M. *Mol. Microbiol.* **2002**, *46*, 611.
[202] (a) Downing, K.J.; Mischenko, V.V.; Shleeva, M.O.; Young, D.I.; Kaprelyants, A.S.; Apt, A.S.; Mizrahi, V. *Infect. Immunol.* **2005**, *73*, 3038; (b) Kana, B.D.; Gordhan, B.G.; Downing, K.J.; Sung, N.; Vostroktunova, G.; Machowski, E.E.; Tsenova, L.; Young, M.; Kaprelyants, A.; Kaplan, G.; Mizrahi, V. *Mol. Microbiol.* **2008**, *67*, 672.
[203] Hett, E,C.; Rubin, E.J. *Microbiol. Mol. Biol. Rev.* **2008**, *72*, 126.

is object of several studies. [204] 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. [205] This activity could be provided by Rpfs 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. [28] 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. [30a]

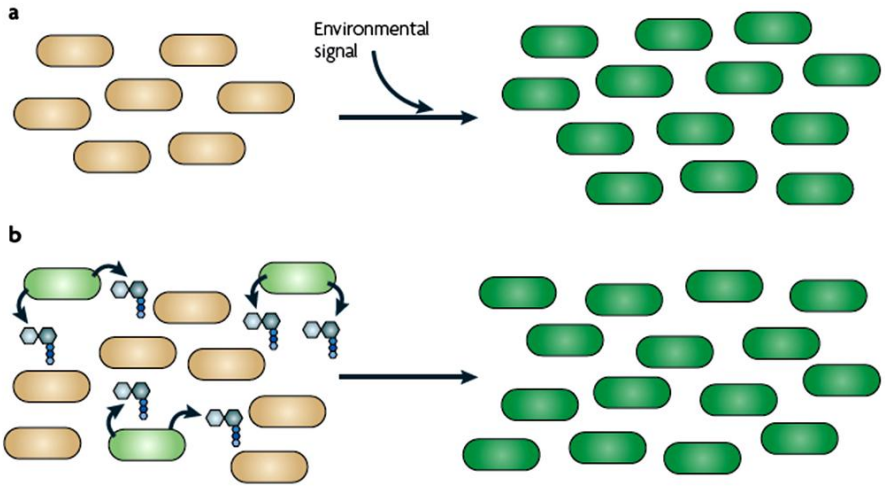


FIG. 3.10: Exit from dormancy triggered by growing cells. a) Dormant cells (beige) may sense some aspect of the environment before exiting dormancy and initiating growth (green cells). **b**) the presence of secreted signaling molecules such as cell wall muropeptides from growing cells (pale green) could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

[204] Ruggiero, A.; Marasco, D.; Squeglia, F.; Soldini, S.; Pedone, E.; Pedone, C.; Berisio, R. *Structure* **2010**, *18*, 1184.
[205] (a) Ravagnani, A.; Finan, C.L.; Young, M. *BMC Genomics* **2005**, *6*, 39; (b) Mukamolova, G.V.; Murzin, A.G.; Salina, E.G.; Demina, G.R.; Kell, D.B.; Kapprelyants, A.; Young, M. *Mol. Microbiol.* **2006**, *58*, 84; (c) Cohen-Gonsaud, M.; Barthe, P.; Bagnèris, C.; Henderson, B.; Ward, J.; Roumestand, C.; Keep, N.H. *Nat. Struct. Mol. Biol.* **2005**, *12*, 270.

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 Glc*N* 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 lysodeiktikus*. Although in literature are present several chemical syntheses of peptidoglycan fragments, [206] 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

[206] (a) Inamura, S.; Fukase, K.; Kusumoto, S. *Tetrahedron Lett.* **2001**, *42*, 7613;(b) Kubasch, N.; Schmidt, R. R. *Eur. J. Org. Chem.* **2002**, 2710; (c) Roy Chowdhury, A.; Siriwardena, A.; Boons, G.-J. *Tetrahedron Lett.* **2002**, *43*, 7805; (d) Hesek, D.; Suvorov, M.; Morio, K.; Lee, M.; Brown, S.; Vakulenko, S. B.; Mobashery, S. *J. Org. Chem.* **2004**, *69*, 778; (e) Hesek, D.; Lee, M.; Morio, K.;Mobashery, S. *J. Org. Chem.* **2004**, *69*, 2137; (f) Roy Chowdury, A.; Boons,G.-J. *Tetrahedron Lett.* **2005**, *46*, 1675; (g) Roy Chowdhury, A.; Wolfert, M.A.; Boons, G.-J. *ChemBioChem* **2005**, *6*, 2088; (h) Inamura, S.; Fujimoto, Y.;Kawasaki, A.; Shiokawa, Z.; Woelk, E.; Heine, H.; Lindner, B.; Inohara, N.; Kusumoto, S.; Fukase, K. *Org. Biomol. Chem.* **2006**, *4*, 232; (i) Narayan, R. S.;Van Nieuwenhze, M. S. *Eur. J. Org. Chem.* **2007**, 1399; (j) Kawasaki, A.; Karasudani, Y.; Otsuka, Y.; Hasegawa, M.; Inohara, N.; Fujimoto, Y.; Fukase, K. *Chem. Eur. J.* **2008**, *14*, 10318; (k) Hesek, D.; Lee, M.; Zhang, M.; Noll, B.C.; Mobashery, S. *J. Am. Chem. Soc.* **2009**, *131*, 5187.

(*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 β-2-amino-2-deoxyglucosides. [211] They generally present a *N*-2 protecting group capable of efficient participation via acyloxonium ion that guarantees 1,2-*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

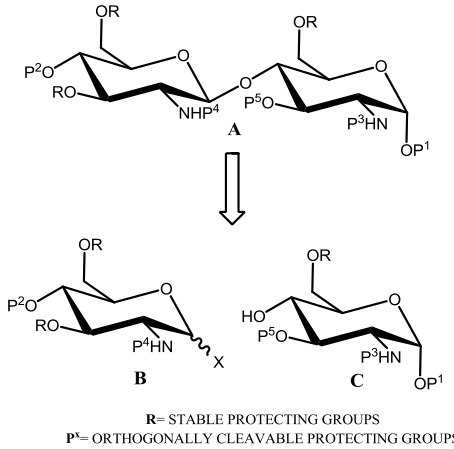


FIG. 3.11: Protecting group pattern on glycosyl donor, acceptor and disaccaride.

[207] Saha, S. L.; Van Nieuwenhze, M. S.; Hornback, W. J.; Aikins, J. A.; Blaszczak, L. C. *Org. Lett.* **2001**, *3*, 3575.
[208] (a) Paulsen, H. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 155; (b) Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2001**, *123*, 6819; (c) Liao, L.; Auzanneau, F.-I. *Org. Lett.* **2003**, *5*, 2607; (d) Lucas, R.; Hamza, D.; Lubineau, A.; Bonnaffé, D. *Eur. J. Org. Chem.* **2004**, 2107.
[209] Crich, D.; Vinod, A. U. *J. Org. Chem.* **2005**, *70*, 1291 and references cited therein.
[210] Crich, D.; Vinod, A. U. *Org. Lett.* **2003**, *5*, 1297.
[211] A comprehensive review: Bongat, A. F. G.; Demchenko, A. V. *Carbohydr. Res.* **2007**, *342*, 374.

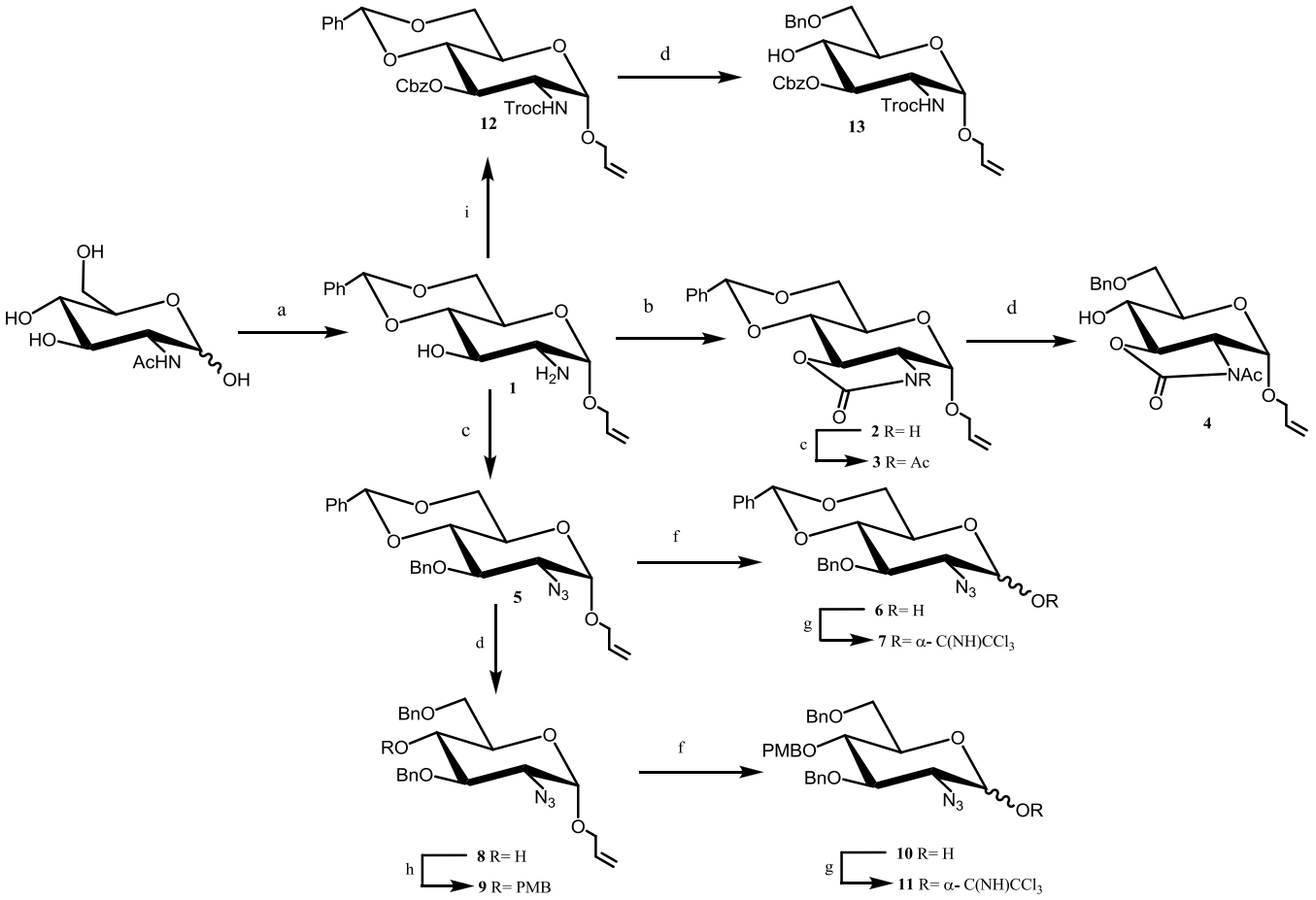


FIG.3.12: Reagents and conditions: a) ref. 13; b) triphosgene, 2:1 v/v CH_3CN/sat. aq. $NaHCO_3$, rt, 60%; c) AcCl, DIPEA, CH_2Cl_2, rt, 92%; d) Et_3SiH, TFA, AW-300 4Å MS, CH_2Cl_2, 5°C, 57% for **4**, 74% for **8**, 77% for **13**; e) i. TfN_3, $CuSO_4$, Et_3N, py, 0°C; ii. BnBr, NaH, DMF, rt, 71% over two steps; f) $PdCl_2$, 1:1 v/v CH_2Cl_2/MeOH, rt, 58% for **6**, 80% for **10**; g) Cl_3CCN, DBU, CH_2Cl_2, rt, 78% for **7**, 70% for **11**; h) PMBCl, NaH, DMF, 0°C, 92%; i) i. TrocCl, $NaHCO_3$, 2:1 H_2O/CH_3CN, rt; ii. CbzCl, DMAP, CH_2Cl_2, 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_3SiH/TFA in CH_2Cl_2; 57%) [214] afforded acceptor **4**. Conversely, the treatment of **1** with triflyl azide in pyridine [215] and subsequent benzylation 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 α-trichloroacetimidate donor **7**. Unfortunately the coupling between **4** and **7** in a β-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_3CCN and DBU afforded the desired α-trichloroacetimidate **11** in 70% yield.

---

[212] Gavard, O.; Hersant, Y.; Alais, J.; Duverger, V.; Dilhas, A.; Bascou, A.; Bonnaffé, D. *Eur. J. Org. Chem.* **2003**, 3603.
[213] Boysen, M.; Gemma, E.; Lahmann, M.; Oscarson, S. *Chem. Commun.* **2005**, 3044.
[214] de Ninno, M.P.; Etienne, J.B.; Duplantier, K.C. *Tetrahedron Lett.* **1995**, *36*, 669.
[215] Yan, R. B.; Yang, F.; Wu, Y.; Zhang, L.-H.; Ye, X.-S. *Tetrahedron Lett.* **2005**, *46*, 8993.
[216] (a) Jensen, H. H.; Nordstrøm, L. U.; Bols, M. *J. Am. Chem. Soc.* **2004**, *126*, 9205.

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-

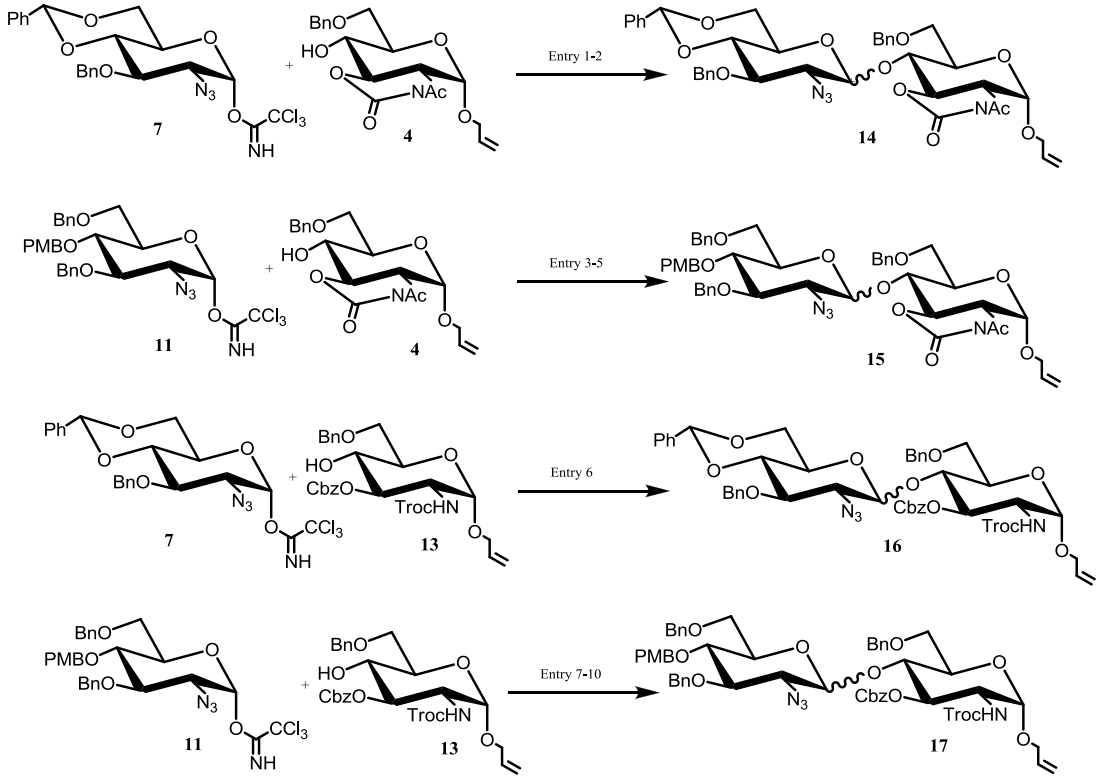


Table 3.1: Glycosylation Reactions ([A] Donor/acceptor molar ratio=1.6 unless otherwise stated; [B] Promoter equivalents calculated with respect to the donor; [C] Isolated yield unless otherwise stated; [D] detected by TLC and MALDI analysis; [E] Anomeric ratio measured by the isolation of two anomers;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

Entry	*Acceptor*	*Donor*[A]	*Promoter*[B]	*Solvent*	*T*	*Disaccharide product*[C]
1	**4**	**7**	TMSOTf (0.02 eq.)	CH_3CN	-20°C	No reaction
2	**4**	**7**	TMSOTf (0.2 eq.)	CH_3CN	rt	**14** (traces)[D]
3	**4**	**11**	TMSOTf (0.02 eq.)	Pivalonitrile	Rt	**15** (traces)[D]
4	**4**	**11**	$BF_3{\cdot}OEt_2$ (0.6 eq.)	3:2 v/v CH_2Cl_2/hexane	-30°C	**15** (68%; β/α 1:1)[E]
5[F]	**4**	**11**	$BF_3{\cdot}OEt_2$ (0.15 eq.)	3:2 v/v CH_2Cl_2/hexane	-60°C	**15** (68%; β/α 1.3:1)[E]
6	**14**	**7**	$BF_3{\cdot}OEt_2$ (0.2 eq.)	3:2 v/v CH_2Cl_2/hexane	-80°C	**16** (traces)[D]
7	**14**	**11**	$BF_3{\cdot}OEt_2$ (0.2 eq.)	3:2 v/v CH_2Cl_2/hexane	-80°C	**17** (81%; only β)[E]
8	**14**	**11**	$BF_3{\cdot}OEt_2$ (0.2 eq.)	3:2 v/v CH_2Cl_2/hexane	-30°C	**17** (40%; only β)[E]
9	**14**	**11**	$BF_3{\cdot}OEt_2$ (0.2 eq.)	3:2 v/v CH_2Cl_2/ CH_3CN	-30°C	**17** (43%; only β)[E]
10	**14**	**11**	TMSOTf (0.02 eq.)	3:2 v/v CH_2Cl_2/hexane	-80°C	**17** (71%; β/α 1:4)[E]

deoxyglycosyl-α-trichloroacetimidates under S_N2 conditions. [217] Therefore, the coupling between **4** and **11** was attempted at low temperature in a CH_2Cl_2–hexane solvent mixture with $BF_3{\cdot}OEt_2$ 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 β-anomer (entry 5). The low β/α 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 imido-protecting groups. [218] Compound **13** was synthesized from key building block **1** by *N*-trichloroethoxycarbonylation and protection of the 3-hydroxy as benzyloxycarbonyl group (**12**, 70% over two steps), which were then subjected to Et_3SiH/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 $BF_3{\cdot}OEt_2$ catalysis in a CH_2Cl_2-hexane solvent system at -78°C (typical S_N2 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).

[217] Schmidt, R.R.; Behrendt, M.; Toepfer, A. *Synlett* **1990**, 694.
[218] Xue, J.; Khaja, S. D.; Locke, R. D.; Matta, K. L. *Synlett* **2004**, 861.

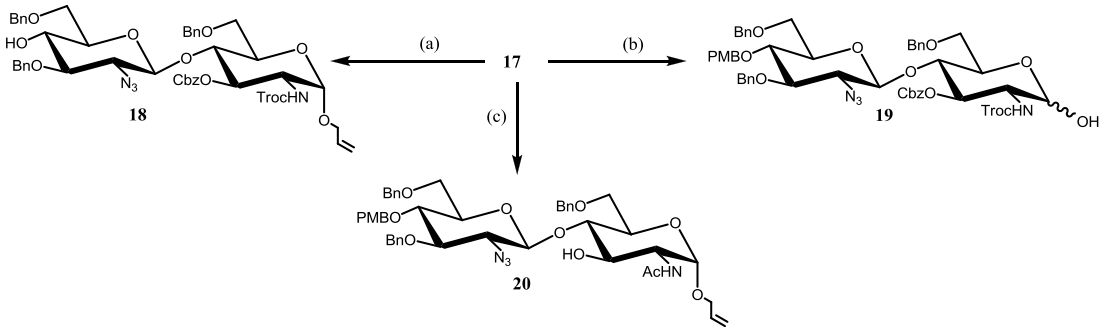
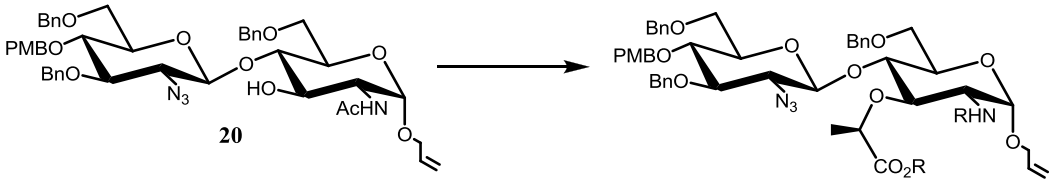


FIG. 3.13: Reagents and conditions: (a) DDQ, 18:1 v/v CH_2Cl_2/H_2O, T=-20°C, 58%; (b) $PdCl_2$, 1:1 v/v CH_2Cl_2/MeOH, rt, 43%(c) 5:1 v/v THF/2M aq. KOH, 50°C; ii. 10:10:1 v/v/v CH_2Cl_2/MeOH/Ac_2O, rt, 67% over two steps;

Disaccharide **17** was readily transformed also into alcohol **20** by cleavage of Troc- and Cbz-protecting 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). [34j] 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.



Entry	Reaction conditions	Products (isolated yield %)
1	(*S*)-2-chloropropionic acid (5eq.), NaH (11.2 eq), THF, 40°C, on.	No product
2	(*S*)-2-chloropropionic acid (5eq.), NaH (15.6 eq), 1:1 v/v DMF/dioxane, 95°C, on. [207k]	**21** R=OH, R^1=Ac (14%)
3	Triflyl-(*S*)-2-propionic acid ethyl ester (3 eq.), NaH (9 eq.), CH_2Cl_2, rt, on.	**22** R=Et, R^1=H (50%)
4	Triflyl-(*S*)-2-propionic acid ethyl ester (2 eq.), NaH (3 eq.), CH_2Cl_2, rt, 90 min.	**23** R=Et, R^1=H (63%)

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.

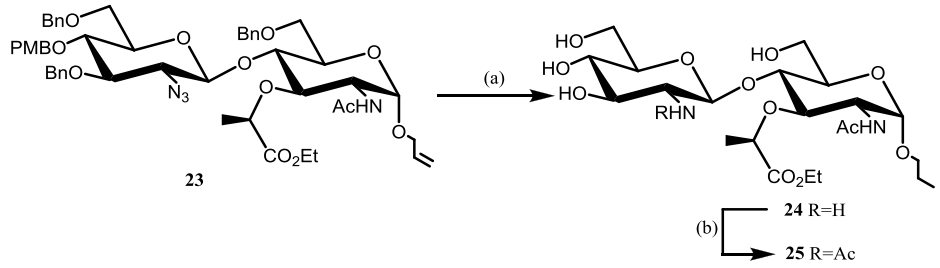


FIG.3.14: Reagents and conditions: (a) $Pd(OH)_2$, 9:1 v/v MeOH/AcOH, H_2, rt, 65%; (b) 9:1 v/v MeOH/Ac_2O, 96%.

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, [205] 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.

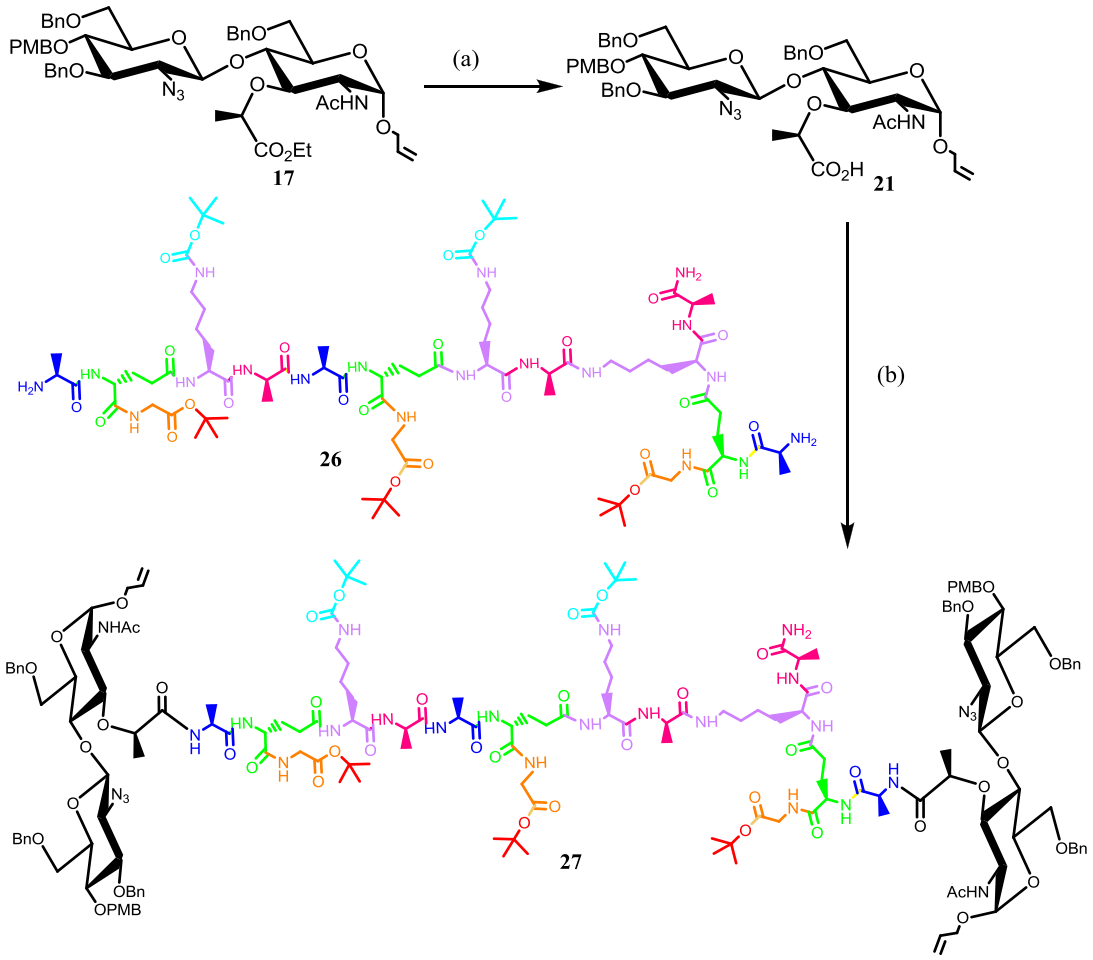


FIG. 3.15: Reagents and conditions: (a) LiOH, 2:1:0.5 v/v/v THF/dioxane/H20, rt, 82%; (b) PyBOP, DIPEA, CH_2Cl_2, rt, on.

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 carbamato 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 (lysodeiktikus)* has been also synthesized for the inecstigation 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: C*H*Cl_3 at δ=7.26 ppm; ^{13}C: *C*DCl_3 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 CH_3CN 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_3CN/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_2SO_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,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_3CN/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 ethylendiamine in ethyl acetate (195 μL of ethylendiamine 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_2SO_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_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51-7.36 (m, 5H, H-Ar), 5.91 (m, 1H, OCH_2CH=CH_2), 5.62 (s, 1H, C*H*Ph), 5.35 (ddd, 1H, J_{vic} 17.2 Hz, $^4J_{H,H}$ 3.0 Hz, J_{gem} 1.5 Hz, *trans* OCH_2CH=C*H*H), 5.28 (dd, 1H, J_{vic} 10.4 Hz, J_{gem} 1.5 Hz, *cis* OCH_2CH=C*H*H), 5.15 (d, 1H, $J_{1,2}$ 2.9 Hz, H-1), 5.07 (bs, 1H, NH), 4.84 (dd, 1H, $J_{3,2}$=11.3 Hz, $J_{3,4}$=10.2 Hz, H-3), 4.29 (m, 2H, H-6a, OC*H*HCH=CH_2), 4.09 (m, 2H, H-4, OC*H*HCH=CH_2), 3.90 (m, 2H, H-5, H-6b), 3.74 (dd, 1H, $J_{2,3}$ 11.3 Hz, $J_{2,1}$ 2.9 Hz, H-2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.1 (N*C*OO), 136.5 (C_{ipso}), 132.8 (OCH_2CH=CH_2), 129.2, 128.3, 126.2 (C-Ar), 118.5 (OCH_2CH=CH_2), 101.4 (*C*HPh),

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, O*C*H_2CH=CH_2). MALDI TOF-MS: calcd for $C_{17}H_{19}NO_6$ (m/z), 333.12; found, 334.16 $[M+H]^+$. Anal Calcd for $C_{17}H_{19}NO_6$: 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 CH_2Cl_2 (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 CH_2Cl_2 and washed with 1 M $NaHCO_3$. The organic layer was collected, dried with Na_2SO_4, filtered and purified by chromatography (16% ethyl acetate in petroleum ether) to give **3** as a amorphous solid (206.5 mg, 83.1%):^{1}H NMR (600 MHz, $CDCl_3$): δ 7.50-7.34 (m, 5H, H-Ar), 5.85 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.80 (d, 1H, $J_{1,2}$=2.7 Hz, H-1), 5.58 (s, 1H, C*H*Φ), 5.31 (d, 1H, J_{vic}=17.6 Hz, *trans* OCH$_2$CH=C*H*H), 5.27 (d, 1H, J_{vic} 10.3 Hz, *cis* OCH$_2$CH=C*H*H), 4.75 (t, 1H, $J_{3,2}$ = $J_{3,4}$= 11.7 Hz, H-3), 4.32 (dd, 1H, J_{vic}=16.2 Hz, $J_{5,6}$=10.2 Hz, H-6a,), 4.27 (dd, 1H, J_{vic}= 12.6 Hz, J_{gem}=5.2 Hz, OC*H*HCH=CH$_2$), 4.23 (dd, 1H, J_{vic}= 12.6 Hz, J_{gem}=6.24 Hz, OC*H*HCH=CH$_2$), 4.7 (t, 1H, $J_{4,5}$ = $J_{3,4}$= 11.7 Hz, H-4); 3.95-3.91 (m, 2H, H-5, H6b), 3.88 (dd, 1H, $J_{2,1}$ =2.7 Hz, $J_{2,3}$=11.7 Hz, H-2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.11 (*C*OCH$_3$), 152.8 (N*C*OO), 136.3 (C$_{ipso}$), 132.9 (OCH$_2$*C*H=CH$_2$), 129.1, 128.1, 125.2 (C-Ar), 118.2 (OCH$_2$CH=*C*H$_2$), 101.0 (*C*HPh), 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, O*C*H$_2$CH=CH$_2$), 23.6 (CO*C*H$_3$). MALDI TOF-MS: calcd for $C_{17}H_{19}NO_6$ (m/z), 357.13; found, 358.16 $[M+H]^+$. Anal Calcd for $C_{19}H_{21}NO_7$: 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 CH_2Cl_2(6.0 mL). Et_3SiH (393 μL, 2.46 mmol) and a 2.4 M solution of TFA in CH_2Cl_2 were then added and the mixture was stirred at 5°C over night. The mixture was then filtered, diluted in CH_2Cl_2 (10 mL) and washed with a 1 M $NaHCO_3$. The organic layer was collected, dried with Na_2SO_4, filtered and purified by chromatography (20% ethyl acetate in petroleum ether) to give **4** as a yellow oil (98.6 mg, 53.1%):^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35-7.38 (m, 5H, H-Ar), 5.85 (m, 1H, OCH_2CH=CH_2), 5.68 (d, 1H, $J_{1,2}$=2.7 Hz, H-1), 5.24 (dd, 1H, J_{vic}=17.2 Hz, *trans* OCH_2CH=C*H*H), 5.19 (dd, 1H, J_{vic}=10.3 Hz, *cis* OCH_2CH=C*H*H), 4.64-4.59 (m, 3H, H-3, OC*H*HPh), 4.54 (d, 1H, J_{gem}=12.1 Hz, OCH*H*Ph), 4.18 (dd, 1H, J_{vic}= 12.7 Hz, J_{gem}=5.5 Hz, OC*H*HCH=CH_2), 4.10-4.03 (m, 2H, H-4, OC*H*HCH=CH_2), 3.80 (dd, 1H, J_{gem}=9.5 Hz, $J_{5\text{-}6}$=3.7 Hz, H-6a), 3.75-3.66 (m, 3H, H-2, H-5, H-6b), 2.48 (s, 3H, NCOC*H*$_3$). ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.3 (*C*OCH_3), 152.3 (N*C*OO), 136.3 (C_{ipso}), 133.2 (OCH_2CH=CH_2), 128.5, 127.9, 127.7 (C-Ar), 118.1 (OCH_2CH=CH_2), 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, *C*H_2Ph O*C*H_2CH=CH_2), 23.7 (CO*C*H_3). MALDI TOF-MS: calcd for $C_{19}H_{23}NO_7$ (m/z), 377.15; found, 378.23 $[M+H]^+$. Anal Calcd for $C_{19}H_{23}NO_7$: 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 disolved in pyridine (5.0 mL) and treated with Et_3N (930 μL, 6.67 mmol) and $CuSO_4$ (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-evapored 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_2Cl_2 (20 mL) and washed with water. The organic layer was collected, dried with Na_2SO_4, 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%):^{1}H NMR (500 MHz, $CDCl_3$): δ 7.56-7.36 (m, 10H, H-Ar), 6.0 (m, 1H, OCH_2CH=CH_2), 5.63 (s, 1H, C*H*Φ), 5.41 (d, 1H, J_{vic}=17.0 Hz, *trans* OCH_2CH=C*H*H), 5.3 (d, 1H, J_{vic}=8.4 Hz, *trans* OCH_2CH=C*H*H), 5.01 (d, 1H, J_{gem}= 11 Hz, OC*H*HPh), 4.98 (d, 1H, $J_{1,2}$=3.0 Hz, H-1), 4.86 (d, 1H, J_{gem}= 11 Hz, OC*H*HPh), 4.33 (dd, J_{gem}= 10 Hz, $J_{5,6}$=5.5 Hz, H-6a), 4.26 (dd, 1H, J_{vic}= 12.7 Hz, J_{gem}=4.5 Hz, OC*H*HCH=CH_2), 4.17 (t, 1H, H-3), 4.10 (dd, 1H, J_{vic}= 12.7 Hz, J_{gem}=6 Hz, OCH*H*CH=CH_2), 3.97 (m, 1H, H-5), 3.82-3.74 (m, 2H, H-4, H-6b), 3.46 (dd, 1H, J_{1-2}= 3 Hz, J_{2-3}= 10 Hz, H-2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 137.8, 137.1 (C_{ipso}), 133.0 (OCH_2CH=CH_2), 128.9, 128.4, 128.3, 128.2, 128.1, 128.0, 128.7, 126.0, 125.9 (C-Ar), 118.1 (OCH_2CH=CH_2), 101.3 (*C*HPh), 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, *C*H_2Ph O*C*H_2CH=CH_2). MALDI TOF-MS: calcd for $C_{23}H_{25}N_3O_5$ (m/z), 423.18; found, 423.8 $[M+H]^+$. Anal Calcd for $C_{23}H_{25}N_3O_5$: C, 65.24; H, 5.95; N, 9.92. Found: C, 65.5; H, 5.89; N 9.95.

2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranose (6): A solution of **5** (99.1 mg, 0.234 mmol) in 9:1 v/v MeOH/CH_2Cl_2 (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_2Cl_2 (8 mL) and washed with water (8 mL). The organic layer was collected, dried over anhydrous Na_2SO_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, C*H*Φ),, 5.58 (s, 1H, C*H*Φ), 5.25 (d, 1H, $J_{1,2}$=3.5 Hz, H-1α), 4.98 (d, 1H, , J_{gem}= 11.1 Hz, OC*H*HPh), 4.94 (d, 1H, , J_{gem}= 11.2 Hz, OC*H*HPh), 4.82 (d, 1H, , J_{gem}= 11.2 Hz, OC*H*HPh), 4.81 (d, 1H, , J_{gem}= 11.1 Hz, OC*H*HPh), 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_{1-2}= 3.6 Hz, J_{2-3}= 9.8 Hz, H-2). MALDI TOF-MS: calcd for $C_{20}H_{21}N_3O_5$ (m/z), 384.15; found, 384.8 $[M+H]^+$. Anal Calcd for $C_{20}H_{21}N_3O_5$: C, 62.65; H, 5.52; N, 10.96. Found: C, 62.5; H, 5.89; N 9.95.

2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranosyl trichloroacetimidate (7): to a solution of compound **6** (51.5 mg, 0.134 mmol) in CH_2Cl_2 (900 μL) Cl_3CCN (200 μL, 2.01 mmol) and a 0.40 M solution of DBU in CH_2Cl_2 (100 μL, 40.2 μmol) wew added. After 2 h the mixture is concentred and subjected to column cromatography (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, N*H*), 7.51-7.32 (m, 5H, H-Ar), 6.39 (d, 1H, $J_{1,2}$=3.7 Hz, H-1), 5.63 (s, 1H, C*H*Φ), 5.03 (d, 1H, J_{gem}= 10.8Hz, OC*H*HPh), 4.85 (d, 1H, J_{gem}= 10.8Hz, OC*H*HPh), 4.34 (dd, 1H, $J_{6,5}$=4.9 Hz, J_{gem}=7.4 Hz, H-6a), 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}= $J_{6,}$

$_6$=10.8Hz, H-6b), 3.80 (t, 1H, H-4), 3.73 (dd, 1H, 1H, $J_{1\text{-}2}$= 3.7 Hz, $J_{2\text{-}3}$= 9.9 Hz, H-2). MALDI TOF-MS: unstable. Anal Calcd for $C_{22}H_{21}Cl_3N_4O_5$: 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.

Allyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-α-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_2Cl_2(6.5 mL). Et_3SiH (425 μL, 2.66 mmol) and a 2.4 M solution of TFA in CH_2Cl_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_2Cl_2 (5 mL) and washed with a 1 M $NaHCO_3$. The organic layer was collected, dried with Na_2SO_4, filtered and purified by chromatography (11% ethyl acetate in petroleum ether) to give **8** as a yellow oil (155.3 mg, 69%):^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43-7.32 (m, 10H, H-Ar), 5.93 (m, 1H, OCH_2CH=CH_2), 5.36 (dd, 1H, J_{vic}=17.5 Hz, J_{gem}=1.5 Hz, *trans* OCH_2CH=C*H*H), 5.27 (dd, 1H, J_{vic}=12.6 Hz, J_{gem}=1 Hz, *cis* OCH_2CH=C*H*H), 4.97 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 4.94 (d, 1H, J_{vic}=11.5 Hz, OC*H*HPh), 4.82 (d, 1H, J_{vic}=11 Hz, OC*H*HPh), 4.62 (d, 1H, J_{vic}=12 Hz, OC*H*HPh), 4.55 (d, 1H, J_{vic}=12 Hz, OC*H*HPh), 4.22 (dd, 1H, J_{vic}= 12.6 Hz, J_{gem}=5.0 Hz, OC*H*HCH=CH_2), 4.06 (dd, 1H, J_{vic}= 12.6 Hz, J_{gem}=6.5 Hz, OC*H*HCH=CH_2), 3.87 (t, 1H, H-6a), 3.80 (m, 1H, H-5), 3.73 (m, 3H, H-3, H-4, H-6b), 3.35 (dd, 1H, $J_{1\text{-}2}$= 3.5 Hz, $J_{2\text{-}3}$= 10 Hz, H-2). MALDI TOF-MS: calcd for $C_{23}H_{27}N_3O_5$ (m/z), 425.20; found, 448.2 $[M+Na]^+$. Anal Calcd for $C_{23}H_{27}N_3O_5$: 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, 0.366mmol) was dissolved in DMF (1 mL) and to the solution *p*-methoxybenzy 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 CH_2Cl_2 (6 mL) and washed with water. The organic layer was collected, dried with Na_2SO_4, filtered and purified by chromatography (16 to 7% ethyl acetate in petroleum ether) to give **5** as a yellow oil (181.1 mg, 92%): ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60-7.50 (m, 10H, H-Ar), 7.25 (d, 2H, J_{vic}= 14 Hz, H-Ar), 7.02 (d, 2H, J_{vic}= 13.5 Hz, H-Ar), 6.12 (m, 1H, OCH_2CH=CH_2), 5.54 (dd, 1H, J_{vic}=17.1 Hz, J_{gem}=0.9 Hz, *trans* OCH_2CH=C*H*H), (dd, 1H, J_{vic}=10.5Hz, *cis* OCH_2CH=C*H*H), 5.19 (d, 1H, , $J_{1,2}$=3.3 Hz, H-1), 5.10 (s, 2H, CH_2PhOMe), 4.95 (d, 1H, J_{vic}=10.5 Hz, OC*H*HPh), 4.86 (d, 1H, J_{gem}=12.3 Hz, OC*H*HPh), 4.71 (d, 1H, J_{gem}=12.3 Hz, OC*H*HPh), 4.66 (d, 1H, J_{gem}=10.2 Hz, OC*H*HPh), 4.41 (dd, 1H, J_{vic}= 12.9 Hz, J_{gem}=4.8 Hz, OC*H*HCH=CH_2), 4.28-4.19 (m, 2H, H-3, OC*H*HCH=CH_2), 4.06-3.84 (m, 7H, H-6a, H-6b, H-4, H-5, OCH_3), 3.62 (dd, J_{1-2}= 3.6 Hz, J_{2-3}= 10.2 Hz, H-2). MALDI TOF-MS: calcd for $C_{31}H_{35}N_3O_6$ (m/z), 524.25; found, 547.77 $[M+Na]^+$. Anal Calcd for $C_{31}H_{35}N_3O_6$: 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 trichloro-acetimidate (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/CH_2Cl_2 (1.8 mL) was treated with $PdCl_2$ (16.6 mg, 93 µmol). The mixture was vigorously stirred at room temperature for 2 h, diluted with CH_2Cl_2 (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:^{1}H NMR (400 MHz, $CDCl_3$): 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, OC*H*HPh, OC*H*$_2$*p*OMePh), 4.82 (d, 1H, J_{gem}=10.8 Hz, OC*H*HPh), 4.73 (t, 2H, OC*H*$_2$*p*OMePh), 4.61-4.41 (m, 7H, 6 OC*H*HPh, H-1β), 4.05-3.97 (m, 2H), 3.81 (s, 6H, 2 OCH$_2$*p*O*Me*Ph), 3.65-3.37 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$): 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, (C-2α, C-3α, C-4α, C-5α, C-6α, C-2β, C-3β, C-4β, C-5β, C-6β, 4 *C*H$_2$Bn, 2*C*H$_2$PhOCH$_3$, 2CH$_2$PhO*C*H$_3$). MALDI TOF-MS: calcd for $C_{28}H_{31}N_3O_6$ (m/z), 505.56; found, 548.77 $[M+Na]^+$. Anal Calcd for $C_{28}H_{31}N_3O_6$: 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*-*p*methoxy benzyl-2-deoxy-α -D-glucopyranose trichloroacetimidate (11): to a solution of compound **10α** and **10β** (74.9 mg, 0.15 mmol) in CH_2Cl_2 (1 mL) Cl_3CCN (220 μL, 2.19 mmol) and a 0.40 M solution of DBU in CH_2Cl_2 (110.4 μL, 44 μmol) were added. After 2 h the mixture is concentred and subjected to column cromatography (12.5 to 20% ethyl acetate in *n*-hexane) to give **11** (67.7 mg, 70.2%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.70 (N*H*), 7.40-7.36 (10H, H-Ar), 7.07 (d, 2H, $J_{o\text{-}m}$=8.6 Hz, H*m*PMB), 6.82 (d, 2H, $J_{o\text{-}m}$=8.6 Hz, H*m*PMB), 6.44 (1H, $J_{1,2}$=3.5 Hz, H-1), 4.96 (d, 1H, J_{gem}=10.8 Hz, OC*H*HPh), 4.90 (d, 1H, J_{gem}=10.7 Hz, OC*H*HPh), 4.75 (d, 1H, J_{gem}=10.3 Hz, OC*H*HPhOMe), 4.64 (d, 1H, J_{gem}=12Hz, OC*H*HPh), 4.50 (d, 1H, J_{gem}=10.3 Hz, OC*H*HPhOMe), 4.49 (1H, J_{gem}=12

Hz, OC*H*HPh), 4.05-3.97 (m, 2H, H-6a, H-3), 3.89-3.79 (m, 5H, H-6b, H-5, OCH_3), 3.71-3.65 (m, 2H, H-2, H-4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6 (*C*=NH), 159.4 (*C*-OMe), 137.7 (C_{ipso}), 129.7-127.7 (C-Ar), 113.8 (*C*mPMB), 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 *C*H_2Bn, *C*H_2PhOCH_3, CH_2PhO*C*H_3). MALDI TOF-MS: unstable. Anal Calcd for $C_{30}H_{31}Cl_3N_4O_6$ (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*-benzyloxycarbonyl-2-*N*-trichloroethoxycarbonyl-amino-2-deoxy-α-D-glucopyranoside (12): Compound **2** (265.1 mg , 0.865 mmol) was dissolved in 2:1 v/v H_20/CH_3CN (5.7 mL) and treated with $NaHCO_3$ (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 (10 mL) and washed with water (8 mL). The organic layer was collected, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was dissolved in CH_2Cl_2 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: $[\alpha]_D$ +30.2 (*c* 1.05 in CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.46-7.31 (10 H, m, H-Ar), 5.90 (, m, 1 H, OCH_2CH=CH_2), 5.52 (s, 1H, C*H*Φ), 5.44-5.06 (m, 6H, H-3, N*H*, *trans* OCH_2CH=C*H*H, *cis* OCH_2CH=C*H*H, CO*C*H_2CCl_3), 4.94 (d, 1-H, H-1, $J_{1,2}$=3.6 Hz), 4.66 (s, 2H, CO*C*H_2Φ), 4.34-3.91 (m, 6-H, H-2, H-6a, H-5, OC*HH*CH=CH_2), 3.83-3.71 (m, 2H, H-4, H-6b). ^{13}C NMR (100 MHz, $CDCl_3$): 155.13 (*C*=O), 154.23 (*C*=O), 136.84 (C_{ipso}), 132.94 (C_{ipso}, OCH_2*C*H=CH_2), 129.07-126.16 (C-Ar), 118.65 (OCH_2CH=*C*H_2), 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,

CH_2-Φ, *C*H_2CCl_3, O*C*H_2CH=CH_2). MALDI TOF-MS: calcd for $C_{27}H_{28}Cl_3NO_9$ (m/z), 615.08; found, 538.69 $[M+Na]^+$. Anal Calcd for $C_{27}H_{28}Cl_3NO_9$: 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*-benzyloxycarbonyl-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 CH_2Cl_2(7.5 mL). Et_3SiH (465 μL, 3.01 mmol) and a 5.1 M solution of TFA in CH_2Cl_2 (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 CH_2Cl_2 (10 mL) and washed with a 1 M $NaHCO_3$. The organic layer was collected, dried with Na_2SO_4, filtered and purified by column chromatography (17% ethyl acetate in petroleum ether) to give **12** as a yellow oil (266.0 mg, 77%): $[\alpha]_D$ +56.3 (*c* 0.88 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.26 (m, 10H, H-Ar), 5.87 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.35 (d, 1H, J_{NH-H2}=9.8 Hz), 5.29 (dd, 1H, J_{vic}=17.2 Hz, J_{gem} 1.4 Hz, *trans* OCH$_2$CH=C*H*H), 5.22 (dd, 1H, J_{vic}=10.3 Hz, J_{gem} 1.2 Hz, *cis* OCH$_2$CH=C*H*H), 5.15 (s, 2H, COC*H*$_2$Φ), 4.99 (m, 1H, H-3), 4.91 (d, 1H, $J_{1,2}$=3.6 Hz, H-1), 4.69-4.61 (m, 3H, C*H*HΦ, C*H*$_2$Cl$_3$), 4.55 (d, 1H, C*H*HΦ, J_{gem}=12.0 Hz), 4.19 (dd, 1H, J_{vic}= 5.27 Hz, J_{gem}= 12.8 Hz, OC*H*$_2$CH=CH$_2$), 4.04-3.97 (m, 2H, H-2, OC*H*$_2$CH=CH$_2$), 3.84-3.83 (bs, 2H, H-4, H-5), 3.78 (dd, 1H, J_{gem}= 10.7 Hz, J_{6a-5}= 2.8 Hz, H-6a), 3.71 (dd, 1H, J_{gem}= 10.4 Hz, J_{6b-5}= 2.6 Hz, H-6b). ^{13}C NMR (100 MHz, $CDCl_3$): 155.74 (*C*=O), 154.15 (*C*=O), 137.60 (C_{ipso}), 134.77 (C_{ipso}), 133.12 (OCH$_2$*C*H=CH$_2$), 128.55-127.61 (C-Ar), 118.24 (OCH$_2$CH=*C*H$_2$), 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, *C*H$_2$-Φ,

CH_2CCl_3, O*C*$H_2CH{=}CH_2$). MALDI TOF-MS: calcd for $C_{27}H_{30}Cl_3NO_9$ (m/z), 617.10; found, 540.53 $[M+Na]^+$. Anal Calcd for $C_{27}H_{30}Cl_3NO_9$: 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 $BF_3{\cdot}OEt_2$ 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 cromatography (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** $[\alpha]_D$ +29.1 (*c* 0.54 in CH_2Cl_2); ^{1}H 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, $OCH_2C\mathit{H}{=}CH_2$), 5.72 (d, 1H, $J_{1,2}$=2.7 Hz, H-1 $_A$), 5.26 (dd, 1H, J_{vic}=18.7 Hz, J_{gem} 1.4 Hz, *trans* $OCH_2CH{=}C\mathit{H}H$), 5.21 (dd, 1H, J_{vic}=10.4 Hz, J_{gem} 1.3 Hz, *cis* $OCH_2CH{=}C\mathit{H}H$), 4.83 (s, 2H, OCH_2PhOMe), 4.26-4.65 (m, 5H, H-3_A, CH_2Ph), 4.57 (d, 1H, J_{gem}=13.4 Hz, C*H*HΦ), 4.51 (d, 1H, C*H*HΦ, J_{vic}=10.8 Hz), 4.20-4.14 (m, 3H, H-1_B, H-3_B, O$CH_2CH{=}CH_2$); 4.07 (dd, 1H, J_{vic}= 5.27 Hz, J_{gem}= 12.8 Hz, O$CH_2CH{=}CH_2$), 3.90 (dd, 1H, J_{gem}= 10.4 Hz, $J_{6Ba\text{-}5B}$=2.6 Hz, H-6_Ba), 3.81-3.59 (m, 10H, H-2_A, H-4_A, H-

5_A, H-6_Aa, H-6_Bb, H-4_B, H-6_Bb, OCH_3), 2.45 (s, 3H, $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2 (C=O), 159.4 (C_{ipso} PMB), 152.9 (C=O), 138.6, 137.8, 135.1 (3 C_{ipso} Bn), 133.2 ($OCH_2CH=CH_2$), 130.8 (C_{ipso} PMB), 130.1-125.1 (C-Ar), 118.4 ($OCH_2CH=CH_2$), 113.8 (C-Ar), 101.2 (C-1_B), 95.0 (C-1_A), 83.2 (C-3_B), 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-4_B, C-3_A, C-5_B, OCH_2*p*OMePh, 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, 898.60 $[M+Na]^+$. Anal Calcd for $C_{47}H_{52}N_4O_{12}$: C, 65.27; H, 6.06; N, 6.48. Found: C, 65.79; H, 5.99; N, 6.99. **Compound 15α**: $[\alpha]_D$ +29.6 (*c* 0.47 in CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): 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, $OCH_2CH=CH_2$), 5.73 (d, 1H, $J_{1,2}$=2.8 Hz, H-1_A), 5.41 (d, 1H, $J_{1,2}$=2.8 Hz, H-1_B), 5.25 (dd, 1H, J_{vic}=17.2 Hz, J_{gem} 1.5 Hz, *trans* $OCH_2CH=CHH$), 5.20 (dd, 1H, J_{vic}=10.4 Hz, J_{gem}=1.2 Hz, *cis* $OCH_2CH=CHH$), 4.85 (s, 2H, OCH_2PhOMe), 4.76 (t, 1H, $J_{3,4}$=$J_{3,2}$= 9.9 Hz, H-3_A), 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-4_A), 4.30 (d, 1H, J_{gem}=12.0 Hz, CHHΦ), 4.20 (dd, 1H, J_{vic}= 5.27 Hz, J_{gem}= 12.8 Hz, $OCH_2CH=CH_2$), 4.07 (dd, 1H, J_{vic}= 5.33 Hz, J_{gem}= 12.6 Hz, $OCH_2CH=CH_2$), 3.82-376 (m, 6H, H-3_B, H-5_A, H-2_A, OCH_3). 3.71-3.55 (m, 4H, H-4_B, H-5_B, H-6_Aa, H-6_Ab), 3.45 (dd, 1H, J_{gem}= 10.6 Hz, J_{6Ba-5B}=2.8 Hz, H-6_Ba), 3.45 (dd, 1H, , $J_{1,2}$=2.8 Hz, $J_{3,2}$= 9.5 Hz, H-2_B), 3.35 (d, 1H, J_{gem}= 10.6 Hz, H-6_Bb), 2.5 (s, 3H, $COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2 (C=O), 160.0 (C_{ipso} PMB), 152.9 (C=O), 137.9, 135.2, 133.2 (3 C_{ipso} Bn), 130.9 ($OCH_2CH=CH_2$), 130.2, 129.6, 128.8, 128.5, 128.3, 127.9, 127.9, 127.7, 127.4 (C-Ar), 118.1 ($OCH_2CH=CH_2$), 113.8 (C-Ar), 96.3 (C-1_B), 95.0 (C-1_A), 80.2 (C-3_B), 77.6,

75.4, 74.7, 73.6, 73.5, 71.9, 71.8, 71.7, 70.7, 69.5, 68.2, 67.8, 63.3, 59.9 (C-4_B, C-3_A, C-5_B, O*C*H_2*p*OMePh, C-4_A, 3 O*C*H_2Ph, C-5_A, C-6_B, O*C*H_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-*O*-benzyl-4-*O*-*p*-methoxybenzyl-2-deoxy-β -D-gluco-pyranosyl-(1→4)-6-*O*-benzyl-3-*O*-benzyloxycarbonyl-2-*N*-trichloroethoxy-carbonylamino-2-deoxy-α-D-glucopyranoside (17): a mixture of acceptor **13** (13.5 mg, 21.8 μmol) and of donor **11** (22.8 mg, 35.0 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 (370 μL). A 31.1 mM solution of BF_3·OEt_2 in CH_2Cl_2 (230 μL, 7.45 μmol) was the added and the mixture was stirred at -80°C for 30 min. Et_3H (5 μL) was then added and the mixture was filtered on a Celite pad. The mixture was purified by column cromatography (10 to 15% ethyl acetate in *n*-hexane) to give **17** (7.3 mg, 35%) as a white crystalline compound: $[\alpha]_D$ +7 (*c* 0.6 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 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$_2$C*H*=CH$_2$), 5.28 (2 H, m, NH, *trans* OCH$_2$CH=C*H*H), 5.22 (1 H, br d, J_{vic} 10.4 Hz, *cis* OCH$_2$CH=C*H*H), 5.08 (1 H, t, $J_{3,2}$=$J_{3,4}$ 10.1 Hz, H-3_A), 5.06 (1 H, d, J_{gem} 12.5 Hz, OC*H*HCCl$_3$), 4.97 (1 H, d, J_{gem} 12.5 Hz, OC*H*HCCl$_3$), 4.93 (1 H, dd, $J_{1,2}$ 3.5 Hz, H-1_A), 4.81 (2 H, s, O*CH*$_2$*p*OMePh), 4.69-4.60 (4 H, m, 4 OC*H*HPh), 4.51 (1 H, d, J_{gem} 12.1 Hz, OC*H*HPh), 4.47 (2 H, s, OC*H*$_2$Ph), 4.45 (1 H, d, J_{gem} 10.5 Hz, OC*H*HPh), 4.21 (1 H, dd, J_{gem} 12.5 Hz, J_{vic} 5.2 Hz, OC*H*HCH=CH$_2$), 4.13 (1 H, d, $J_{1,2}$

7.2 Hz, H-1_B), 4.07-3.91 (5 H, m, H-2_A, H-4_A, H-5_A, H-$6a_A$, OC*H*HCH=CH_2), 3.79 (3 H, s, OMe), 3.72 (1 H, dd, J_{gem} =9.8 Hz, $J_{6b,5}$ 1.7 Hz, H-$6b_A$), 3.61 (1 H, d, J_{gem} 10.8 Hz, $J_{6a,5}$ 1.7 Hz, H-$6a_B$), 3.47 (2 H, m, H-4_B, H-6_Bb), 3.23 (3 H, m, H-2_B, H-3_B, H-5_B); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.3 (C_{ipso} PMB), 155.3, 154.1 (O*C*OOCH$_2$Ph, N*C*OOCH$_2$CCl$_3$), 138.4, 138.0, 137.7, 135.2 (4 C_{ipso} Bn), 133.1 (OCH$_2$*C*H=CH$_2$), 130.1 (C_{ipso} PMB), 129.4-125.0 (C-Ar), 118.4 (OCH$_2$CH=*C*H$_2$), 113.8 (C-Ar), 100.8 (C-1_B), 96.3 (C-1_A), 83.3 (C-3_B), 77.6 (C-4_B), 75.7 (C-3_A), 75.4 (C-5_B, O*C*H$_2$*p*OMePh), 74.7 (OCH$_2$Ph), 74.5 (C-4_A, O*C*H$_2$Ph), 73.5 (2 O*C*H$_2$Ph), 70.3 (C-5_A), 69.5 (O*C*H$_2$CCl$_3$), 68.7 (C-6_B), 68.6 (O*C*H$_2$CH=CH$_2$), 67.7 (C-6_A), 66.4 (C-2_B), 55.3 (OMe), 54.1 (C-2_A). MALDI TOF-MS: calcd for $C_{55}H_{59}Cl_3N_4O_{14}$ (m/z), 1104.31; found, 1127.07 $[M+Na]^+$. Anal Calcd for $C_{55}H_{59}Cl_3N_4O_{14}$: 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-gluco-pyranosyl-(1→4)-6-*O*-benzyl-3-*O*-benzyloxycarbonyl-2-*N*-trichloroethoxy-carbonylamino-2-deoxy-α-D-gluco-pyranoside (18): Compound **17** (46 mg, 0.041 mmol) was dissolved in 18:1 v/v CH_2Cl_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_2Cl_2 and washed with water. The mixture was diluted with CH_2Cl_2 and then washed with water. The organic layer was collected and treated with Na_2SO_4, filtered and the solvent was evaporated. The residue was purified by column cromatography (30% ethyl acetate in *n*-hexane) to give **18** as a white oil (23.5 mg, 58%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40-7.23 (m, 20H, H-Ar), 5.86 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.31-5.21 (m, 3H, H-3A, OCH$_2$C*H*=C*H*$_2$), 5.05 (d, 1H, J_{gem}= 8.5 Hz, COOC*H*HPh), 5.00 (d, 1H, J_{gem}= 8.6 Hz,

COOC*H*HPh), 4.92 (d, 1H, $J_{1,2}$=3.6 Hz, H-1_A), 4.87 (d, 1H, (d, 1 H, J_{gem}=11.1 Hz, OC*H*HPh), 4.79 (d, 1 H, J_{gem}=11.1 Hz, OC*H*HPh), 4.70 (d, 1 H, J_{gem}=11.9 Hz, OC*H*HPh), 4.69 (d, 1 H, J_{gem}=11.9 Hz, OC*H*HPh), 4.61 (d, 1 H, J_{gem}=11.9 Hz, OC*H*HPh), 4.48 (d, 1 H, J_{gem}=11.9 Hz, OC*H*HPh), 4.29 (s, 2H, OCH_2Cl_3), 4.17 (dd, 1H, , J_{vic}= 5.2 Hz, J_{gem}= 12.6 Hz, OC*H*HCH=CH_2), 4.11-4.39 (m, 5H, OC*H*HCH=CH_2, H-2_A, H-1_B, H-4_A, H-6_Aa), 3.86 (dd, 1H, J_{4-5}= 10 Hz, H-5_A), 3.72 (d, 1H, J_{gem}= 9.6 Hz, H-6_Ab), 3.62 (dd, 1H, J_{gem}= 9.7 Hz, J_{6Ba-5}= 4.5 Hz, H-6_Ba), 3.48 (t, 1H, J_{4-3}= J_{4-5}= 8.8 Hz, H-4_B), 3.34 (dd, 1H, J_{6gem}= 9.7 Hz, J_{6Bb-5}= 7.2 Hz, H-6_Bb), 3.17-3.11 (m, 3H, H-3_B, H-2_B, H-5_B); ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.2 (O*C*OOCH$_2$Ph), 154.1 (N*C*OOCH$_2$CCl_3), 138.1, 137.7, 137.3 (3 C_{ipso}), 137.3-127.6 (C-Ar), 118.5 (OCH$_2$CH=*C*H$_2$), 100.8 (C-1_B), 96.3 (C-1_A), 95.4, 82.5, 75.7, 74.9, 74.4, 74, 2, 73.7, 73.5, 72.4, 71.5, 70.2, 69.5, 68.6, 67.5, 65.3 (C-3_B, C-4_B C-3_A, C-5_B, O*C*H$_2$Ph, C-4_A, O*C*H$_2$Ph, 2 O*C*H$_2$Ph, C-5_A, O*C*H$_2$CCl_3, O*C*H$_2$CH=CH$_2$ C-6_A, C-2_B), 53.9 (C-2_A). MALDI TOF-MS: calcd for $C_{47}H_{51}Cl_3N_4O_{13}$ (m/z), 984.25; found, 1008.33 $[M+Na]^+$. Anal Calcd for $C_{47}H_{51}Cl_3N_4O_{13}$: C, 57.24; H, 5.21; N, 10.75; Cl, 5.68. Found: C, 57.65; H, 5.44; N, 10.12; Cl, 5.76.

2-Azido-3,6-di-*O*-benzyl-4-*O*-*p*-methoxybenzyl-2-deoxy-β -D-gluco-pyranosyl-(1→4)-2-acetamido-6-*O*-benzyl-3-*O*-benzyloxycarbonyl-2-deoxy-α-D-gluco-pyranose (19): Compound **17** (46.9 mg, 42.3 μmol) was dissolved in 1:1 v/v CH_2Cl_2/MeOH(500 μL) and the solution was treated with $PdCl_2$ (3.5 mg, 19 μmol) and stirred for 2 h. The mixture was then diluted with CH_2Cl_2 (4,5 mL) and filtered on a Celite pad. The solvent was evaporated and the produc was isolated by column cromatography (10 to 12.5/ toluene in ethyl acetate) to give **19** as a yellow oil (19.4 mg, 43%, α/β 9:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42-7.18 (m, 15H, H-Ar), 7.06 (2 H, d,

J_{ortho} 8.5 Hz, H-Ar PMB), 6.81 (2 H, d, J_{ortho} 8.5 Hz, H-Ar PMB), 5.47 (d, 1H, J_{NH-H2a}=7.6 Hz, NH), 5.29 (d, 1H, J_{1-2}= 3.0 Hz, H-1A), 5.17 (t, 1H, J_{2-3}= J_{3-4}= 10 Hz, H-3A), 5.11 (d, 1H, J_{gem}=13 Hz, OC*H*HΦ), 5.03-5.00 (m, 4H, COOCH_2Φ, 2 OC*H*HΦ), 4.84 (s, 2H, CH_2PhOMe), 4.70-4.65 (m, 3H, 1 OC*H*HΦ, COOCH_2Cl_3), 4.54 (d, 1H, J_{gem}=12 Hz, OC*H*HΦ), 4.50-4.47 (m, 2H, 2 OC*H*HΦ), 4.18-4.16 (bm, 1H, H-5_A), 4.06 (dd, 1H, J_{2-3}= 10 Hz, J_{1-2}= 3.0 Hz, H-2_A), 4.00 (t, 1H, J_{4-5}= J_{3-4}= 9.6 Hz, H-4_A), 3.90 (dd, 1H, J_{5-6}= 3.5 Hz, J_{gem}=10.5 Hz, H-6_A), 3.81 (s, 3H, OCH_3), 3.76 (d, 1H, , J_{gem}=10.5 Hz, H-6_B), 3.64 (d, 1H, , J_{gem}=11Hz, H-6_B), 3.51-3.46 (m, 2H, H-4_B, H-6_B), 3.28-3.24 (m, H-2_B, H-3_B, H-5_B). ^{13}C NMR (125 MHz, $CDCl_3$): ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.3 (C_{ipso} PMB), 155.3, 154.1 (O*C*OOCH$_2$Ph, N*C*OOCH$_2$CCl$_3$), 138.3, 137.9, 137.8, 135.7 (4 C_{ipso} Bn), 130.1 (C_{ipso} PMB), 129.4-125.0 (C-Ar), 113.8 (C-Ar), 100.8 (C-1_B), 91.7 (C-1_A), 83.3 (C-3_B), 79.2 77.5, 77.3, 75.3, 75.0, 74.4, 73.5, 70.0, 69.6, 68.6, 68.0, 66.4 (C-4_A, C-5_A, C-4_B, C-5_B, C-6_B, C-6_A, C-3_A, C-2_B, O*C*H$_2$*p*OMePh, O*C*H$_2$CCl$_3$, O*C*H$_2$Ph, 3 O*C*H$_2$Ph), 55.2 (OMe), 54.3 (C-2_A). MALDI TOF-MS: calcd for $C_{52}H_{55}Cl_3N_4O_{14}$ (m/z), 1064.28; found, 1628.28 $[M+Na]^+$. Anal Calcd for $C_{52}H_{55}Cl_3N_4O_{14}$: C, 58.57; H, 5.20; Cl 9.97; N, 5.25. Found: C, 58.42; H, 5.33; Cl 10.0; N, 5.43.

Allyl 2-azido-3,6-di-*O*-benzyl-4-*O*-*p*-methoxybenzyl-2-deoxy-β -D-gluco-pyranosyl-(1→4)- 2- acetamido-6-*O*-benzyl-2-deoxy-α-D-glucopyranoside (20): Compoun **17** (33.5 mg, 30 μmol) is dissolved in 10:1 v/v THF/KOH 2M (3.3 mL) and the mixture was stirred for 6 h at 50°C. The solvent was then evaporated, the residue was dissolved in 1:1 v/v CH_2Cl_2/MeOH and Ac_2O (300 μL, 3.17 mmol) was added. After 4 h the mixture was concentrated and the produc was purified by column cromatography (20% of *n*-hexane in ethyl acetate) to give **21** as a white powder (17 mg,

67%). $[\alpha]_D$=+34.0 (*c* 1.2 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37-7.31 (m, 15H, H-Ar), 7.06 (2 H, d, J_{ortho}= 8.6 Hz, H-Ar PMB), 6.81 (2 H, d, J_{ortho}= 8.6 Hz, H-Ar PMB), 5.83 (m, 1H, OCH_2CH=CH_2), 5.62 (d, 1H, $J_{NH\text{-}H2A}$=8.6 Hz, N*H*), 5.28 (dd, 1H, J_{vic}=17.2 Hz, J_{gem} 1.5 Hz, *trans* OCH_2CH=C*H*H), 5.20 (dd, 1H, J_{vic}=10.4 Hz, J_{gem}=1.4 Hz, *cis* OCH_2CH=C*H*H), 4.96 (1 H, d, $J_{1,2}$=3.6 Hz, H-1_A), 4.84 (s, 2H, O*C*H_2PhOMe), 4.69 (d, 1 H, J_{gem}=10.1 Hz, O*CH*HPh), 4.67 (d, 1 H, J_{gem}=12.0 Hz, O*CH*HPh), 4.55 (d, 1 H, J_{gem}=11.9 Hz, O*CH*HPh), 4.53 (d, 1 H, J_{gem}=11.9 Hz, O*CH*HPh), 4.45 (d, 1 H, J_{gem}=11.9 Hz, O*CH*HPh), 4.44 (d, 1 H, J_{gem}=11.9 Hz, O*CH*HPh), 4.12 (m, 4H, O*CH*HCH=CH_2, H-3_A, H-1_B, H-2_A), 4.95 (dd, 1H, , J_{vic}= 5.33 Hz, J_{gem}= 12.6 Hz, O*CH*HCH=CH_2), 3.86-3.75 (m, 6H, OCH_3, H-6_Aa, H-6_Ab, H-5_A), 3.70-3.59 (m, 3H, H-4_B, H-6_Ba, H-6_Bb), 3.35-3.33 (m, 3H, H-3b, H-5_B, H-2_B), 2.00 ($COCH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9 (C=O), 159.5 (C_{ipso} PMB), 138.2, 137.7, 133.7 (3 C_{ipso} Bn), 129.8 (OCH_2CH=CH_2), 129.6, 128.5, 128.4, 127.9, 127.8 (C-Ar), 117.5 (OCH_2CH=CH_2), 113.9 (C-Ar), 101.7 (C-1_B), 96.5 (C-1_A), 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-3_B, C-4_B, C-3_A, C-5_B, O*C*H_2*p*OMePh, C-4_A, 3 O*C*H_2Ph, C-5_A, C-6_B, O*C*H_2CH=CH_2, C-6_A, C-2_B, C-2_A), 53.3 (OMe), 23.5 (*C*H_3CO). MALDI TOF-MS: calcd for $C_{46}H_{54}N_4O_{11}$ (m/z), 838.38; found, 862.38 $[M+Na]^+$. Anal Calcd for $C_{46}H_{54}N_4O_{11}$: 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-gluco-pyranosyl-(1→4)- 2-acetamido-6-*O*-benzyl-3-*O*-((*R*)-1'-carboxyethyl)-2-deoxy-α-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

min at 95°C. The mixture was treated with (*S*)-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 CH_2Cl_2 and then washed with water. The organic layer was collected and treated with Na_2SO_4, filtered and the solvent was evaporated. The residue was purified by column cromatography (2% methanol in dichloroethane) to give **21** as a white oil (42.4 mg, 14%). $[\alpha]_D$ +13 (*c* 0.3 in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36-7.26 (15H, m, H-Ar), 7.06 (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, $OCH_2CH=CH_2$), 5.26 (1 H, br d, J_{vic} 17.0 Hz, *trans* $OCH_2CH=CHH$), 5.21 (1 H, d, $J_{1,2}$ 3.0 Hz, H-1_A), 5.18 (1 H, br d, J_{vic} 11.0 Hz, *cis* $OCH_2CH=CHH$), 4.81 (2 H, s, OCH_2*p*OMePh), 4.71 (1 H, d, J_{gem} 12.1 Hz, OC*H*HPh), 4.68 (1 H, d, J_{gem} 10.5 Hz, OC*H*HPh), 4.63 (1 H, q, J_{vic} 6.5 Hz, CH_3CHO), 4.54-4.45 (4 H, m, 4 OC*H*HPh), 4.25 (1 H, d, $J_{1,2}$ 7.5 Hz, H-1_B), 4.12 (1 H, dd, J_{gem} 13.5 Hz, J_{vic} 5.5 Hz, OC*H*HCH=CH_2), 4.06 (1 H, t, $J_{4,3}$= $J_{4,5}$ 10.0 Hz, H-4_A), 4.00-3.92 (3 H, m, H-2_A, H-$6a_A$, OC*H*HCH=CH_2), 3.79 (3 H, s, OMe), 3.76-3.64 (6 H, m, H-3_A, H-4_B, H-5_A, H-$6b_A$, H-$6a_B$, H-$6b_B$), 3.28 (1 H, dd, $J_{2,3}$ 9.5 Hz, $J_{2,1}$ 7.5 Hz, H-2_B), 3.23 (1 H, t, $J_{3,4}$=$J_{3,2}$ 9.5 Hz, H-3_B), 3.16 (1H, br d, $J_{5,4}$ 9.6 Hz, H-5_B), 2.02 (3H, s, CH_3CO), 1.41 (3H, d, J_{vic} 6.5 Hz, CH_3CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.0 (COOH), 171.1 (NH*C*OCH_3), 159.5 (C_{ipso} PMB), 137.9, 137.8, 137.6 (3 C_{ipso} Bn), 133.8 ($OCH_2CH=CH_2$), 130.2 (C_{ipso} PMB), 129.4-127.7 (C-Ar), 117.5 ($OCH_2CH=CH_2$), 113.8 (C-Ar), 100.7 (C-1_B), 96.0 (C-1_A), 83.3 (C-3_B), 77.5 (C-3_A), 76.9 (C-4_A), 76.1 (C-4_B), 75.4 (OCH_2*p*OMePh), 75.2 (CH_3CH), 74.7 (C-5_B), 74.5 (OCH_2Ph), 73.5 (OCH_2Ph), 73.3 (OCH_2Ph), 70.6 (C-5_A), 70.5 (C-6_B), 68.6 ($OCH_2CH=CH_2$), 67.8 (C-6_A), 66.6 (C-2_B), 55.3 (OMe), 54.0 (C-2_A), 23.1 (CH_3CO), 18.3 (CH_3CH). MALDI TOF-MS: calcd for

$C_{49}H_{58}Cl_3N_4O_{13}$ (m/z), 910.40; found, 933.29 [M+Na]. Anal Calcd for $C_{49}H_{58}N_4O_{13}$: 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^+ resin was added until a pH=7. The mixture was filtered, the solvent was evaporated and the product was purified by column cromatography (98% to 96% dicloromethane in methanol) to give **21** as a white oil (32.8 mg, 82%).

Allyl 2-azido-3,6-di-*O*-benzyl-4-*O*-*p*-methoxybenzyl-2-deoxy-β -D-gluco-pyranosyl-(1→4)-2-amino-6-*O*-benzyl-3-O-((*R*)-1'-(ethoxycarbonyl)ethyl)-2-deoxy -α-D-glucopyranoside (22): Compound **20** (342 mg, 0.407 mmol) was co-evaporated three times with toluene and then under Ar dissolved in CH_2Cl_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_2Cl_2 and then washed with water. The organic layer was collected and treated with Na_2SO_4, filtered and the solvent was evaporated. The residue was purified by column cromatography (70% ethyl acetate in *n*-hexane) to give **22** as a white oil (209.4 mg, 50%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37-7.32 (15H, m, H-Ar), 7.09 (2 H, d, J_{ortho}= 8.5 Hz, H-Ar PMB), 6.84 (2 H, d, J_{ortho}= 8.5 Hz, H-Ar PMB), 5.88 (1 H, m, OCH_2CH=CH_2), 5.29 (1 H, d, J_{vic}=17.0 Hz, *trans* OCH_2CH=C*H*H), 5.22 (d, 1 H, J_{vic}= 11.0 Hz, *cis* OCH_2CH=C*H*H), 5.18 (d, 1H, $J_{1,2}$=3.5 Hz, H-1_A), 4.84-4.82 (s, 2H,

OC*H*$_2$*p*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, OCH$_2$C*H*$_3$). ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.3 (*C*OOEt), 159.3 (C_{ipso} PMB), 137.9-137.5 (3 C_{ipso} Bn), 133.4 (OCH$_2$*C*H=CH$_2$), 130.0 (C_{ipso} PMB), 129.3-127.6 (C-Ar), 118.2 (OCH$_2$CH=*C*H$_2$), 113.8 (C-Ar), 100.4 (C-1$_B$), 95.1 (C-1$_A$), 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-2$_A$, C-3$_A$, C-3$_B$, C-4$_A$, C-4$_B$, C-5$_A$, C-5$_B$, C-6$_A$, C-6$_B$, O*C*HCH$_3$, 3 O*C*H$_2$Ph, O*C*H$_2$PhOMe, O*C*H$_2$CH=CH$_2$), 61.2 (O*C*H$_2$CH$_3$), 55.2, 54.6 (C-2$_A$, OCH$_3$), 18.4 (*C*H$_3$CH), 14.0 (OCH$_2$*C*H$_3$). MALDI TOF-MS: calcd for $C_{49}H_{60}N_4O_{13}$ (m/z), 868.39; found, 891.21 [M+Na]. Anal Calcd for $C_{49}H_{60}N_4O_{13}$: 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*-*p*methoxy benzyl-2-deoxy-β -D-gluco-pyranosyl-(1→4)- 2-acetamido-6-*O*-benzyl-3-*O*-((*R*)-1'-(ethoxtcarbonyl)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 CH_2Cl_2 (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 CH_2Cl_2 and then washed with water. The organic layer was collected and treated with Na_2SO_4, filtered and the solvent was evaporated. The residue was purified by column cromatography (50% ethyl acetate in *n*-hexane) to give **23** as a white oil (53.9 mg, 60%). $[\alpha]_D$ +25.0 (*c* 2.0 in CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): 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, OCH_2CH=CH_2), 5.37 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1_A), 5.25 (1 H, br d, J_{vic} 17.0 Hz, *trans* OCH_2CH=C*H*H), 5.15 (1 H, br d, J_{vic} 11.0 Hz, *cis* OCH_2CH=C*H*H), 4.83 (2 H, s, OCH_2*p*OMePh), 4.73 (1 H, d, J_{gem} 12.0 Hz, OC*H*HPh), 4.69 (1 H, d, J_{gem} 10.5 Hz, OC*H*HPh), 4.66 (1 H, q, J_{vic} 7.5 Hz, CH_3CHO), 4.54 (1 H, d, J_{gem} 11.5 Hz, OC*H*HPh), 4.50 (1 H, d, J_{gem} 10.5 Hz, OC*H*HPh), 4.46 (1 H, d, J_{gem} 12.0 Hz, OC*H*HPh), 4.43 (1 H, d, J_{gem} 11.5 Hz, OC*H*HPh), 4.25 (1 H, d, $J_{1,2}$ 8.0 Hz, H-1_B), 4.22 (2 H, m, OCH_2CH_3), 4.11 (1 H, dd, J_{gem} 13.5 Hz, J_{vic} 5.5 Hz, OC*H*HCH=CH_2), 4.06 (1 H, t, $J_{4,3}$= $J_{4,5}$ 10.0 Hz, H-4_A), 3.99 (1 H, dd, J_{gem} 13.5 Hz, J_{vic} 5.5 Hz, OC*H*HCH=CH_2), 3.95 (dd, 1H, $J_{2,3}$ 9.0 Hz, $J_{2,1}$ 3.5 Hz, H-2_A), 3.80-3.64 (m, 10H, H-3_A, H-4_B, H-5_A, H-$6a_A$, H-$6b_A$, H-$6a_B$, H-$6b_B$, OCH_3), 3.30 (1 H, t, $J_{2,3}$=$J_{2,1}$ 8.0 Hz, H-2_B), 3.25 (1 H, t, $J_{3,4}$=$J_{3,2}$ 9.5 Hz, H-3_B), 3.16 (1 H, br d, $J_{5,4}$ 9.6 Hz, H-5_B), 2.04 (3H, s, CH_3CO), 1.36 (3 H, d, J_{vic} 7.5 Hz, C*H*$_3$CHO), 1.30 (3 H, t, J_{vic} 7.5 Hz, OCH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.3 (*C*OOEt), 170.1 (NH*C*OCH_3), 159.3 (C_{ipso} PMB), 137.9-137.7 (3 C_{ipso} Bn), 134.1 (OCH_2CH=CH_2), 130.2 (C_{ipso} PMB), 129.3-127.6 (C-Ar), 116.9 (OCH_2CH=CH_2), 113.8 (C-Ar), 100.6 (C-1_B), 95.9 (C-1_A), 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-2_A, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, C-6_A, C-6_B, O*C*HCH_3, 3 O*C*H_2Ph, O*C*H_2PhOMe, O*C*H_2CH=CH_2), 61.2 (O*C*H_2CH_3), 55.2, 54.4 (C-2_A, OCH_3), 23.1 (*C*H_3CO), 18.5 (*C*H_3CH), 14.1 (OCH_2CH_3). MALDI TOF-MS: calcd for $C_{51}H_{62}N_4O_{13}$ (m/z), 938.43 $[M+H]^+$; found, 961.21 [M+Na]. Anal Calcd for $C_{51}H_{62}N_4O_{13}$: 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-*O*-((*R*)-1'-(ethoxtcarbonyl)ethyl)-2-deoxy-α-D-glucopyranoside (24): Compound **23** (24.8 mg, 0.026 mmol) was dissolved in 2.2:1 v/v MeOH/CH_2Cl_2 (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 fpr 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_2O): δ 4.86 (d, 1H, $J_{1\text{-}2}$=3 Hz, H-1_A), 4.44 (q, 1H, J_{gem}=7 Hz, C*H*(CH_3)COOEt), 4.16-4.05 (m, 2H), 3.96-3.13 (m, 11H), 3.00 (t, $J_{2\text{-}3}$= $J_{1\text{-}2}$=8.5 Hz, H-2_B), 1.92 (s, 3H, COC*H*$_3$), 1.46 (m, 2H, $OCH_2CH_2CH_3$), 1.31 (d, 3H, J_{gem}=7 Hz, CH(C*H*$_3$)COOEt), 1.16 (t, 3H, $COOCH_2CH_3$), 0.77 (t, 3H, J_{gem}=7 Hz, $OCH_2CH_2CH_3$). MALDI TOF-MS: calcd for $C_{22}H_{40}N_2O_{12}$ (m/z), 524.56; found, 546.28 $[M+Na]^+$. Anal Calcd for $C_{22}H_{40}N_2O_{12}$: C, 50.37; H, 7.59; N, 5.34. Found: C, 50.46; H, 7.73; N, 5.22.

Propyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)- 2-acetamido-3-*O*-((*R*)-1'-(ethoxtcarbonyl)ethyl)-2-deoxy-α-D-glucopyranoside (25): Compound 24 (3.9 mg, 7.43 μmol) was dissolved in 9:1 v/v MeOH/Ac_2O and the mixture was stirred at room temperature for 2 h. The solvent was evaporated and **25** coul be obtained as a white solid (2.8 mg, 95%). ^{1}H NMR (500 MHz, D_2O): δ 4.85 (d, 1H, $J_{1\text{-}2}$=3 Hz, H-1_A), 4.55 (q, 1H, J_{gem}=7 Hz, C*H*(CH_3)COOEt), 4.39 (d, 1H, $J_{1\text{-}2}$=8.5 Hz, H-1_B), 4.11 (q, 2H, J_{gem}=7 Hz, $COOCH_2CH_3$), 3.82 (dd, 1H, $J_{6Aa\text{-}5a}$= 2 Hz, J_{gem}= 12.5 Hz), 3.75-3.41 (m, 10H), 3.32-3.29 (m, 3H), 1.92 (s, 3H, COC*H*$_3$), 1.91 (s, 3H, COC*H*$_3$), 1.44 (m, 2H, $OCH_2CH_2CH_3$), 1.33 (d, 3H, J_{gem}=7 Hz, CH(C*H*$_3$)COOEt), 1.16 (t, 3H, $COOCH_2CH_3$), 0.76 (t, 3H, J_{gem}=7 Hz, $OCH_2CH_2CH_3$). MALDI TOF-MS: calcd for $C_{24}H_{42}N_2O_{13}$ (m/z), 567.27 $[M+H]^+$; found, 590.32 $[M+Na]^+$. Anal Calcd for $C_{24}H_{42}N_2O_{13}$: C, 50.88; H, 7.47; N, 4.94. Found: C, 50.92 H, 7.53; N, 4.88.

Chapter 4: Synthesis of Urea Neosaccharide

4.1 Introduction

Although the preparation of urea and its derivatives dates back to the early history of organic chemistry, the enourmous 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. [219] 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 accomodated. Urea moieties also act as strong hydrogen-bond donors and acceptors, [220] and have been frequently used as functional groups for the formation of supramolecular architectures. [221] Substituted ureas and bis-ureas have also been shown to give gels with organic liquids and water, [222] 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

[219] (a) Brown, M.E.; Hollingsworth, M.D. *Nature* **1995**, *376*, 323; (b) Harris, K.D.M. *Chem. Soc. Rev.* **1997**, *26*, 279.
[220] Choi, K.H.; Hamilton, A.D. *Coord. Chem. Rev.* **2003**, *240*, 101
[221] (a) Castellano, R.K.; Nuckolls, C.; Rebek Jr., J. *J. Am. Chem. Soc.* **1999**, *121*, 11156; (b) Cho, Y.L.; Rudkevich, D.M.; Shivanyuk, A.; Rissanen, K.; Rebek Jr., J. *Chem. Eur. J.* **2000**, *6*, 3788.
[222] (a)van Esch, J.; De Feyter; S.; Kellogg, R.M.; De Schryver, S.; .Feringa, B.L. *Chem. Eur. J.* **1997**, *3*, 1238; (b) Estroff, L.A.; Hamilton, A.D. *Angew. Chem.* **2000**, *112*, 3589; (d) de Loos, M.; van Esch, J.; Kellogg, R.M.; Feringa, B.L. *Angew. Chem.* **2001**, *113*, 633; e) Kölbel, M.; Menger, F.M. *Langmuir* **2001**, *17*, 4490; f) Beginn, U.; Tartsch, B. *Chem. Commun.* **2001**, 1924–1925; g) Tamaru, S.-I.; Uchino, S.-Y.; Takeuchi, M.; Ikeda, M.; Hatano, T.; Shinkai, S. *Tetrahedron Lett.* **2002**, *43*, 3751; h) Wang, G.; Hamilton, A. D. *Chem. Commun.* **2003**, 310; i) Babu, P.; Sangeetha, N. M.; Vijayakumar, P.; Mitra, U.; Rissanen, K.; Raju, A. R. *Chem. Eur. J.* **2003**, *9*, 1922; j) George, S. J.; Ajayaghosh, A. *Chem. Eur. J.* **2005**, *11*, 3217.

oligoglycosylphosphates and nucleotides). [223] Some carbohydrate-based ureas exhibit in fact relevant biological properties such as the antibiotics SF-1993, [224] CV-1, [225] and the family of glycocinnamoiylspermidines (FIG. 4.1, **1-4**). [226]

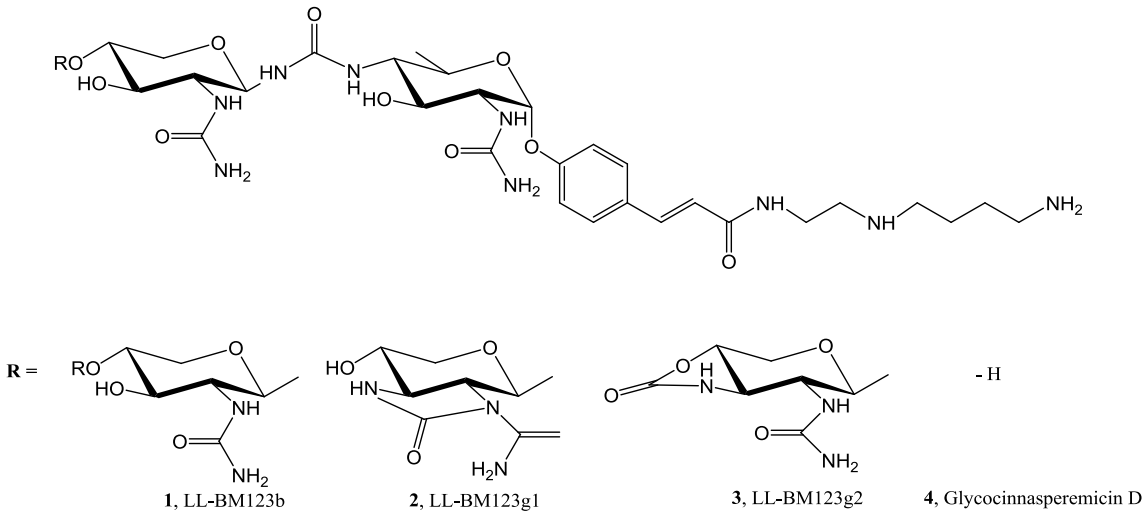


Fig. 4.141: Aminoglycosidic antibiotics of the glycocinnamoylspermidine family.

In 2005, Ichikawa and co-workers described the total synthesis of the glycocinnasperemicin D **4**, a broad spectrum antibiotic against Gram-negative organisms, which contains two highly functionalised aminosugars connected by an urea linkage. [227] 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. [228] Another example of

[223] Jiménez Banco, J.L.; Ortega-Caballero, F.; Ortiz Mellet, C.O.; Garcìa Fernàndez, J.M. *Beilstein Journal of Organic Chemistry* **2010**, *6*, 1.
[224] Omoto, S.; Shomura, T.; Suzuki, H.; Inouye, S. *J. Antibiot.* **1979**, *32*, 436.
[225] (a) Ichimura, M.; Koguchi, T.; Yasuzawa, T.; Tomita, F. *J. Antibiot.* **1987**, *40*, 723; (b) Yasuzawa, T.; Yoshida, M.; Ichimura, M.; Shirahata, K.; Sano, H. *J. Antibiot.* **1987**, *40*, 727.
[226] Ellestad, G.A.; Cosulich, D.B.; Broschard, R.W.; Martin, J.H.; Kunstmann, M.P.; Morton, G.O.; Lancaster, J.E.; Fulmor, W.; Lovell, F.M. *J. Am. Chem. Soc.* **1978**, *100*, 2515.
[227] Nishiyama, T.; Isobe, M.; Ichikawa, Y. *Angew. Chem. Int. Ed.* **2005**, *44*, 4372.
[228] (a) Ichikawa, Y.; Matsukawa, Y.; Tamura, M.; Ohara, F.; Isobe, M.; Kotsuki, H. *Chem. Asian J.* **2006**, *1*, 717; (b) Ichikawa, Y.; Matsukawa, Y.; Isobe, M. *J. Am. Chem. Soc.* **2006**, *128*, 3934.

natural disaccharide analogue containing a pseudoamide linkage is trehazolin **7**, [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-trehalose. Although several synthetic methodologies had been successfully applied to prepare this inhibitor, Chiara and co-workers have described a novel complementary appreoach in which the oxazoline ring is generated by S_N2 nucleophilic displacement reaction from the β-hydroxyurea **5** via the triflate intermediate **6** (FIG. 4.2). [230]

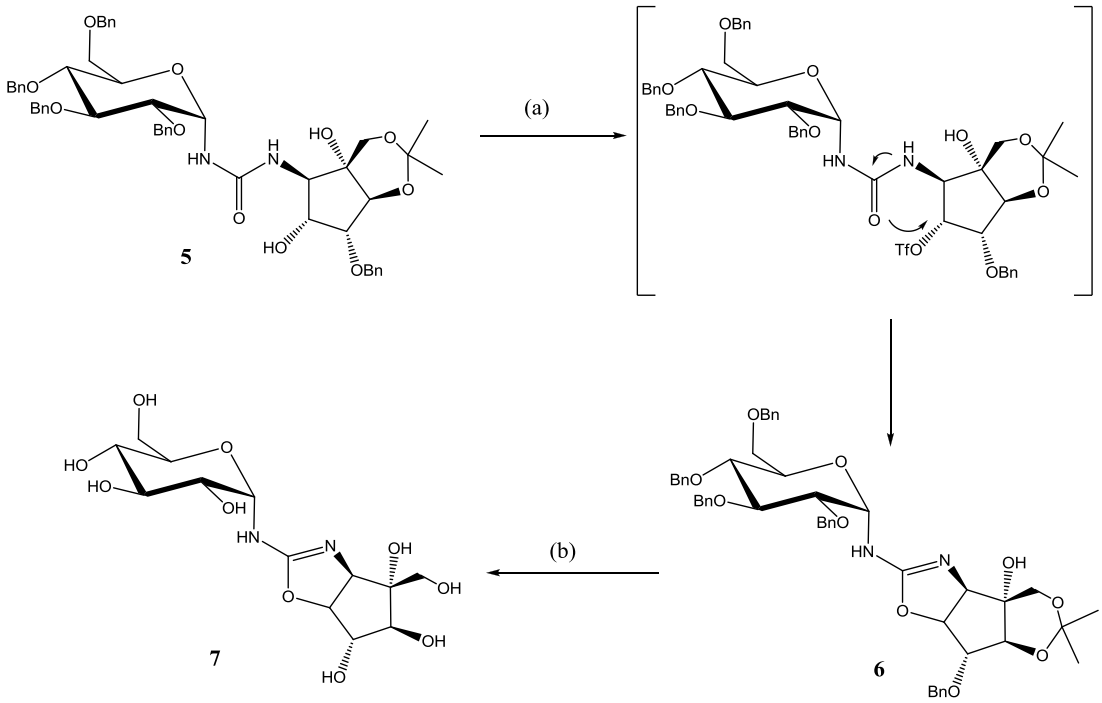


FIG. 4.242: Synthesis of trehazoline , via triflate, from β-hydroxyurea: *Reagents and conditions*: (a) Tf_2O, py, CH_2Cl_2, from -20°C to rt; (b) i. H_2, $Pd(OH)_2/C$, EtOH, TFA; ii. 2N HCl, rt.

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

[229] Ando, O.; Satake, H.; Itoi, K.; Sato, A.; Nakajima, M.; Takahashi, S.; Haruyama, H.; Ohkuma, Y.; Kinishita, T.; Enokita, R. *J. Antibiot.* **1991**, *44*, 1165.
[230] Storch de Gracia, I.; Bobo, S.; Martin-Ortega, M.D.; Chiara, J.L. *Org. Lett.* **1999**, *1*, 1705.

mimics: i) nucleophilic addition of sugar derivatives to carbohydrate isocyanate [231] 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). [232]

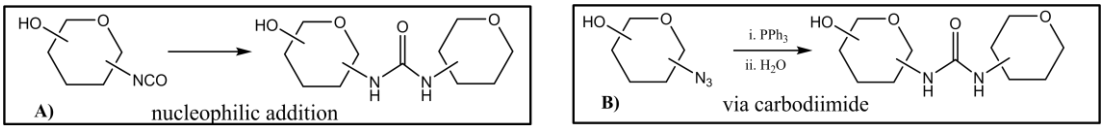


FIG. 4.3: Approaches to access pseudoamyde-type oligosaccaride mimics.

A particular area of interest in glycomimetics is that of neosaccharides, that are oligosaccharides linked together without using the anomeric centre.[233] To the best of our knowledge, urea-linked neosaccharides were reported only twice. [234] Herein, it is reported an access [235] to a novel class of neosaccharides starting from an unprecedented urea-linked glucosamine dimer obtained from amino-alcohol **8** through a modification of the known [236] oxazolidinone closure reaction.

---

[231] (a) Ichikawa, Y.; Nishiyama, T.; Isobe, M. *J. Org. Chem.* **2001**, *66*, 4200; (b) Prosperi, D.; Ronchi, S.; Lay, L.; Rencurosi, A.; Russo, G. *Eur. J. Org. Chem.* **2004**, 395; (c) Ichikawa, Y.; Matsukawa, Y.; Nishiyama, T.; Isobe, M. *Eur. J. Org. Chem.* **2004**, 586; (d) Ichikawa, Y.; Ohara, F.; Kotsuki, H.; Nakano, K. *Org. Lett.* **2006**, *8*, 5009; (e) Park, N.H.; Nguyen, H.M. *Org. Lett.* **2009**, *11*, 2433.

[232] (a) Jiménez Banco, J.L.; Bootello, P.; Benito, J.M.; Ortiz Mellet, C.; Garcìa Fernàndez, J.M. *J. Org. Chem.* **2006**, *71*, 5136; (b) Benito, J.M.; Rodriguez-Lucena, D.; Jiménez Banco, J.L.; Ortiz Mellet, C.; Garcìa Fernàndez, J.M. *J. Iclusion Phenom. Macrocyclic Chem.* **2007**, *57*, 147; (c) Rodriguez-Lucena, D.; Benito, J.M.; Álvarez, E.; Jaime, C.; Perez-Miron, J.; Garcìa Fernàndez, J.M. *J. Org. Chem.* **2008** *73*, 2967; (d) Rodriguez-Lucena, D.; Ortiz Mellet, C.; Jaime, C.; Burusco, K.K.; Garcìa Fernàndez, J.M.; Benito, J.M. *J. Org. Chem.* **2009**, *74*, 2997.

[233] Akhtar, T.; Cumpstey, I. *Tetrahedron Lett.* **2007**, *48*, 8673 and references cited therein.

[234] (a) Ávalos, M.; Babiano, R.; Cintas, P.; Hursthouse, M.B.; Jiménez, J.L.; Light, M.E.; Palacios, J.C.; Pérez, E.M.S. *Eur. J. Org. Chem.* **2006**, 657; (b) García-Moreno, M.I.; Benito, J.M.; Ortiz Mellet, C.; García Fernandez, J.M. *J. Org. Chem.* **2001**, *66*, 7604.

[235] Cirillo, L.; Silipo, A.; Bedini, E.; Parrilli, M. *Eur. J. Org. Chem.* **2010**, 4062.

[236] (a) Benakli, K.; Zha, C.; Kerns, R.J. *J. Am. Chem. Soc.* **2001**, *123*, 9461; (b) Crich, D.; Vinod, A.U. *Org. Lett.* **2003**, *5*, 1297; (c) Manabe, S.; Ishii, K.; Ito, Y. *J. Am. Chem. Soc.* **2006**, *128*, 10666; (d) Geng, Y.; Zhang, L.-H.; Ye, X.-S. *Tetrahedron* **2008**, *64*, 4949; (e) Olsson, J.D.M.; Eriksson, L.; Lahmann, M.;

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. [18b] 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

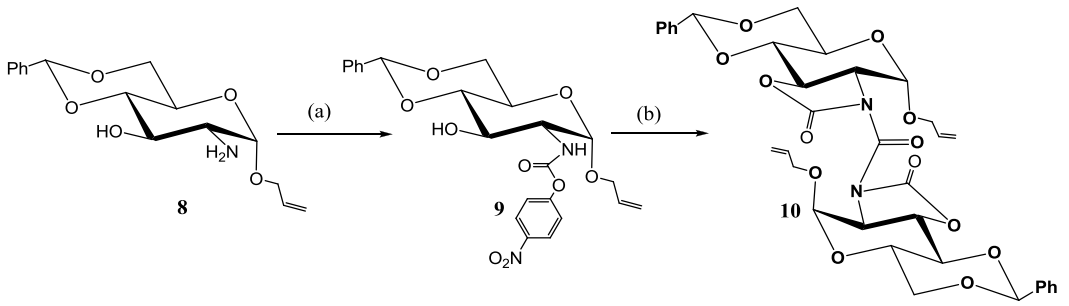


FIG. 4.4: Reagents and Conditions: (a) *p*NO_2PhOCOCl, $NaHCO_3$, 3:2 v/v CH_3CN/H_2O, 0°C; (b) NaH, DMF.

constituted by two oxazolidinone protected glucosamine derivatives held together by a ureido-linkage (**10**).This unprecedented system has been then largely studied and the

Oscarson, S. *J. Org. Chem.* **2008**, *73*, 7181; (f) Nagai, Y.; Ito, N.; Sultana, I.; Sagai, T. *Tetrahedron* **2008**, *64*, 9599.

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 - [237] 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

[237] For the synthesis of the glucose derivative see: Gavard, O.; Hersant, Y.; Alais, J.; Duverger, V.; Dilhas, A.; Bascou, A.; Bonnaffé, D. *Eur. J. Org. Chem.* **2003**, 3603.

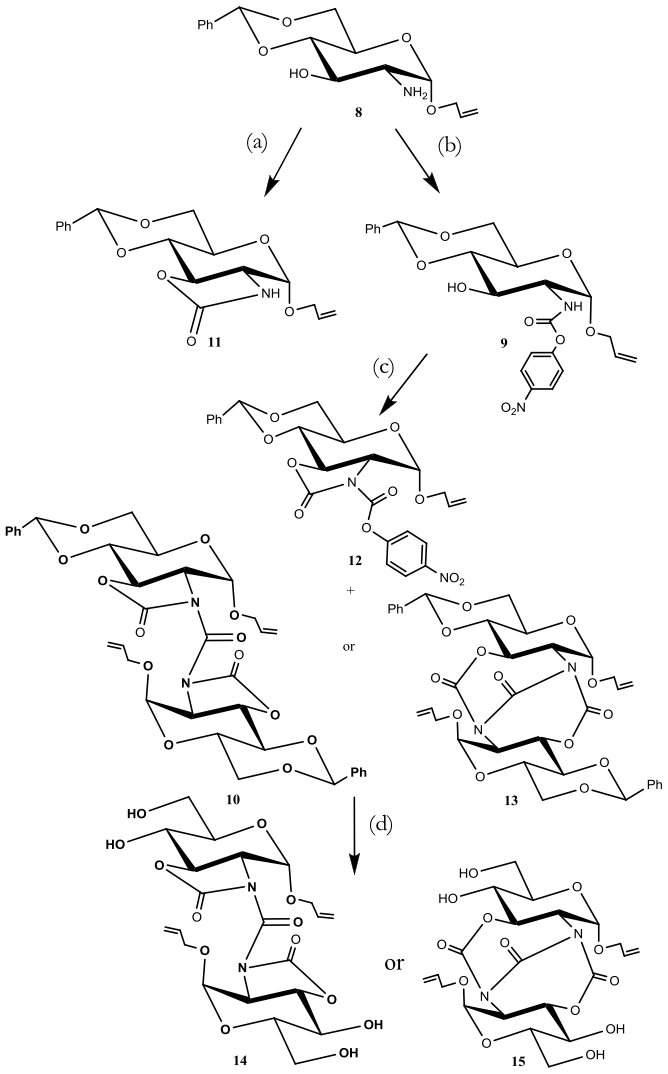


FIG. 4.5: Synthesis of urea-linked neodisaccharide from amino alcohol 8. Reagents and conditions: (a) *p*-$NO_2PhOCOCl$, 3:2 CH_3CN/H_2O, 40°C, 80%; (b) *p*-$NO_2PhOCOCl$, 3:2 CH_3CN/H_2O, 0°C; (c) NaH, DMF, 30°C, 72% from **8**, (**10**/**13**:**12**=6.2:1); (d) CSA, 4:1 dioxane/ H_2O, 60°C, 73%.

Table 4.1: Oxazolidinone 11 *versus* uncyclized carbamate 9 formation from 8 with NPCC (5 equiv.) and $NaHCO_3$ (5 euiv.) in 2:3 (v/v) H_2O/CH_3CN.

entry	T	time	**11**/**9**[a]
1	40°C	30 min	100:0
2	20°C	3 hours	19:81
3	0°C	3 hours	17:83
4	0°C	2 hours	6:94
5	0°C	50 min	0:100
6	0°C	30 min	0:100

[a] Percent molar ratio determined by ^1H 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 neodisaccharide 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 $^3J_{C\text{-}H}$ measured in NMR experiments were in good agreement with the calculated $^3J_{C\text{-}H}$ 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). [238]

Table 4.2: Experimental and theoretical values for selected $^3J_{C,H}$ coupling constants [Hz] of structures 14 and 15.

atom pairs	compound **14**/**15** (NMR data)	compound **14** (MD data)[a]	compound **15** (MD data)[a]
$^3J_{\text{CO ureido, H-2}}$	1.6/1.7[b]	1.6	5.1
$^3J_{\text{CO carbamate, H-2}}$	absent	0.9	6.5
$^3J_{\text{CO carbamate, H-3}}$	absent	0.9	7.7

[a] The values correspond to the weighted average $^3J_{C,H}$ coupling constants calculated from the molecular dynamics simulation
[b] The two values correspond to the $^3J_{C,H}$ coupling constants obtained from HSQMBC and J-HMBC 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. [239] 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*-

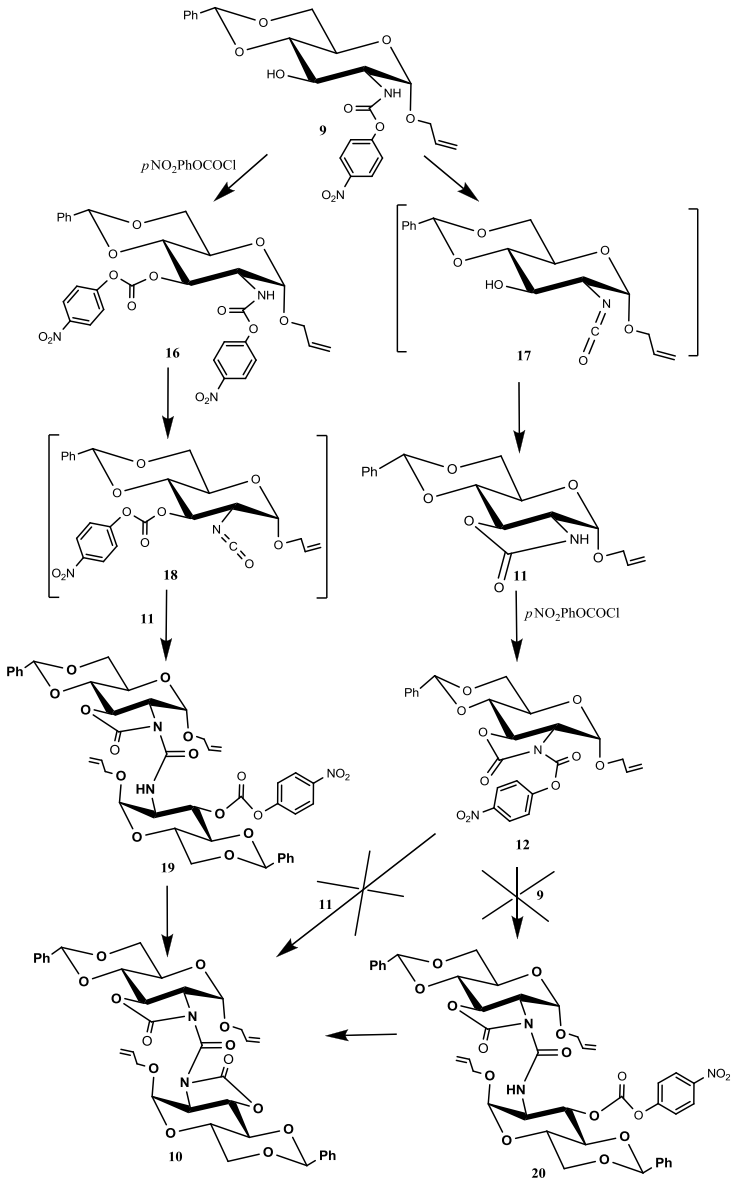


FIG. 4.6: Proposed mechanism for the formation of 10.

[239] (a) Gavard, O.; Hersant, Y.; Alais, J.; Duverger, V.; Dilhas, A.; Bascou, A.; Bonnaffé, D. *Eur. J. Org. Chem.* **2003**, 3603; (b) Sugawara, T.; Narisada, M. *Carbohydr. Res.* **1989**, *194*, 125; (c) Manabe, S.; Ishii, K.; Ito, Y. *J. Am. Chem. Soc.* **2006**, *128*, 10666; (c) Bodlenner, A.; Alix, A.; Weibel, J.-M.; Pale, P.; Ennifar, E.; Paillart, J.-C.; Walter, P.; Marquet, R.; Dumas, P. *Org. Lett.* **2007**, *9*, 4415; (d) Chen, G.; Pan, P.; Yao, Y.; Chen, Y.; Meng, X.; Li, Z. *Tetrahedron* **2008**, *64*, 9078-9087.

aryloxycarbonyl-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** [240] 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 dimerzation

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 [241] 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 ^{1}H-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

[240] Compound **16** was obtained by reacting **9** with DMAP and excess NPCC in DMF at 5°C.
[241] Cai, Y.; Ling, C.C.; Bundle, D.R. *J. Org. Chem.* **2009**, *74*, 580.

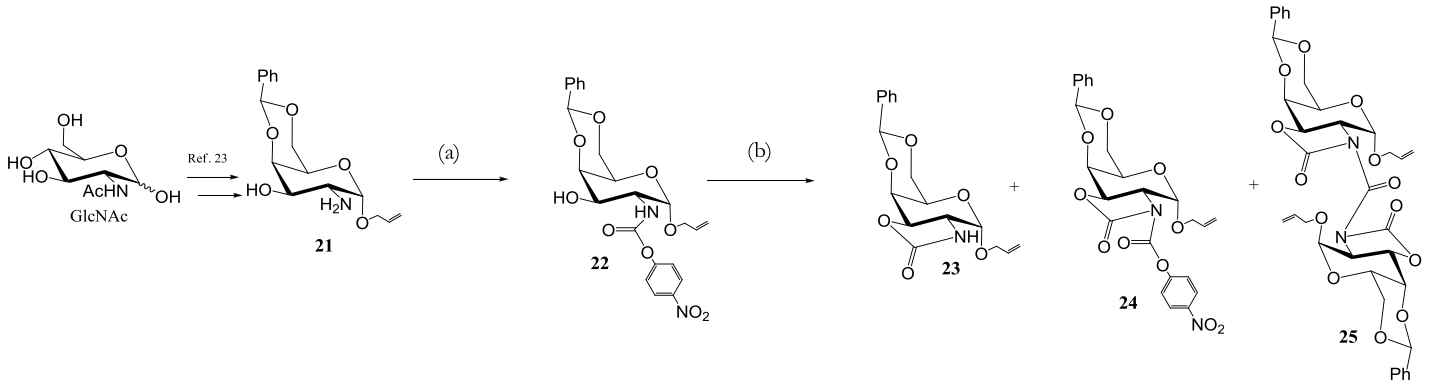


FIG. 4.7: Synthesis of galctosamine derived neosaccharide. Reagents and conditions: (a) *p*-NO_2PhOCOCl, 3:2 CH_3CN/H_2O;(b) NaH, DMF.

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

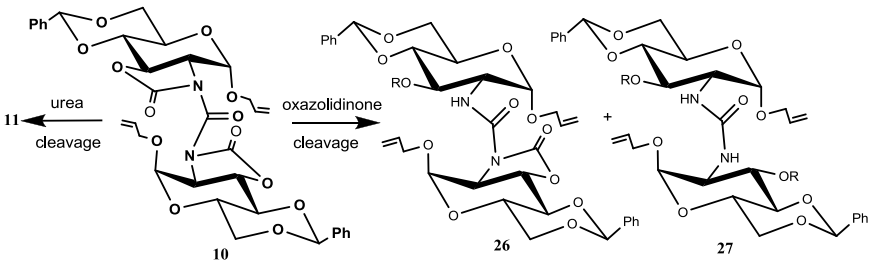
Entry	Concentration of **22**	*p*NO_2PhOCOCl eq. [a]	Base (eq.)	T (°C)	**23**	**24**	**25**
1	0.16 M	4	NaH (5)	0	33%	46%	8%
2	0.16 M	4	NaH (5)	30		43%	30%
3	0.16 M	4	NaH (5)	50		27%	43%
4	0.16 M	4	NaH (5)	70		27%	42%
5	0.05 M	4	NaH (5)	30		64%	32%
6	0.46 M	4	NaH (5)	30		13%	78%
7	0.46 M	4	NaH (5)	50		10%	32%
8	0.46 M	0	NaH (5)	30	90%		
9	0.46	1.01	NaH (5)	0	85%		
10	0.46 M	4	DBU (5)	30	20%		20%

[[a] the equivalents of *p*NO_2PhOCOCl 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



entry	reaction conditions	products (isolated yields)
1	LiOH, H_2O_2, 7:2 v/v THF/H_2O, -40 to 0°C, 15 min	**11** (42%)
		26a R=H (20%)
2	LiOH, LiCl, 3:1 v/v THF/H_2O, 0°C, 75 min	**11** (41%)
		26a R=H (33%)
		27a R=H (21%)
3	NaOMe, 9:1 v/v CH_2Cl_2/MeOH, -60°C, 40 min	**26b** R=COOMe (34%)
		27b R=COOMe (34%)
4	14:6:1 v/v/v $CHCl_3$/MeOH/Et_3N, rt, 2 days	**27b** R=COOMe (54%)
5	14:6:1:1 v/v/v/v $CHCl_3$/dioxane/H_2O/Et_3N, rt, 2 days	**26a** R=H (81%)
6	6:2:1 v/v/v dioxane/H_2O/Et_3N, 80°C, 14 hours	**27a** R=H (76%)

FIG. 4.8: Chemoselective Cleavage Of The Oxazolidinone-Urea-Oxazolidinone System 10.

was accomplished by employing mild Et_3N-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

[242] Wei, P.; Kerns, R.J. *Tetrahedron Lett.* **2005**, *46*, 6901.

chemoselective cleavage of the residual oxazolidinone ring was possible under the

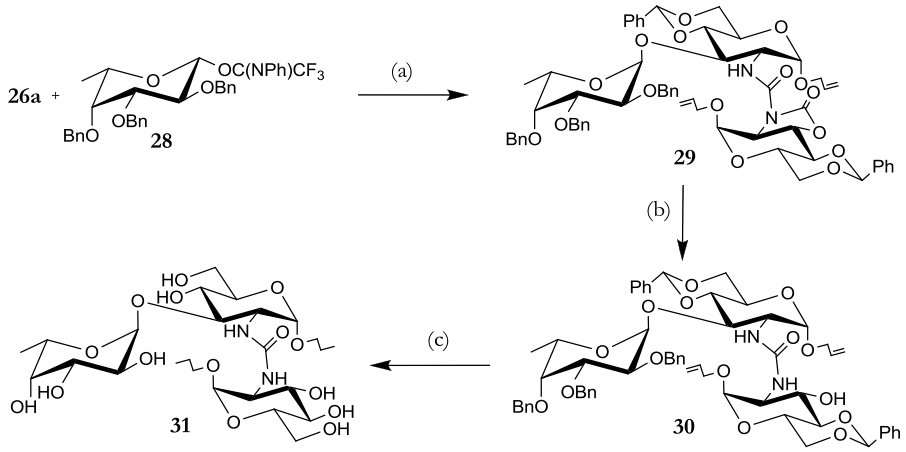


FIG. 4.9: Synthesis of neotrisaccharide 26. Reaction and conditions: (a) TMSOTf, AW-300 4Å MS, 1:1 v/v CH2Cl2/THF, T=-30°C, 76%; (b) 6:2:1 v/v/v dioxane/H20/Et3N, T=80°C, 58%; (c) Pd/C, 9:1 v/v MeOH/HCOOH, ultrasound bath, 40°C, 72%.

conditions developed above. Indeed, hydrolysis in 6:2:1 v/v/v dioxane/water/Et_3N gave alcohol **30** (58%). Then, a transfer hydrogenation under Perlin conditions [243] 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. [244] 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

[243] Rao, V.S.; Perlin, A.S. *Carbohydr. Res.* **1980**, *83*, 175.

[244] (a) Gattuso, G.; Nepogodiev, S.A.; Stoddart, J.F. *Chem. Rev.* **1998**, *98*, 1919; (b) Chong, P.Y.; Petillo, P.A. *Org. Lett.* **2000**, *2*, 1093; (c) Stichler-Bonaparte, J.; Vasella, A. *Helv. Chim. Acta* **2001**, *84*, 2355; (d) Locardi, E.; Stöckle, M.; Gruner, S.; Kessler, H. *J. Am. Chem. Soc.* **2001**, *123*, 8189; (e) Fan, L.; Hindsgaul, O. *Org. Lett.* **2002**, *4*, 4503; (f) van Well, R.M.; Marinelli, L.; Erkelens, K.; van der Marel, G.A.; Lavecchia, A.; Overkleeft, H.S.; van Boom, J.H.; Kessler, H.; Overhand, M. *Eur. J. Org. Chem.* **2003**, 2303; (g) Mayes, B.A.; Stetz, R.J.E.; Ansell, C.W.G.; Fleet, G.W.J. *Tetrahedron Lett.* **2004**, *45*, 153; (h) Velasco-Torrijos, T.; Murphy, P.V. *Tetrahedron Asimm.* **2005**, *16*, 261; (i) Bodine, K.D.; Gin, D.Y.; Gin, M.S. *Org. Lett.* **2005**, *7*, 4479; (j) Ménand, M.; Blais, J.-C.; Valéry, J.-M.; Xie, J. *J. Org. Chem.* **2006**, *71*, 3295; (k) Di Fabio, G.; Randazzo, A.; D'Onofrio, J.; Ausín, C.; Pedroso, E.; Grandas, A.; De Napoli, L.; Montesarchio, D. *J. Org. Chem.* **2006**, *71*, 3395; (l) Kim, K.S.; Lee, B.-Y.; Yoon, S.H.; Jeon, H.J.; Baek, J.Y.; Jeong, K.-S. *Org. Lett.* **2008**, *10*, 2373; (m) Muthana, S.; Yu, H.; Chao, H.; Cheng, J.; Chen, X. *J. Org. Chem.* **2009**, *74*, 2928; (n) Paul, S.; Raghothama, S.; Jayaraman, N. *Carbohydr. Res.* **2009**, *344*, 177; (o) Hsieh, Y.-C.; Chir, J.-L.; Zou, W.; Wu, H.-H.; Wu, A.-T. *Carbohydr. Res.* **2009**, *344*, 1020; (m) Porwanski, S.; Marsura, A. *Eur. J. Org. Chem.* **2009**, 2047; (p) Romero Zaliz, C.L.; Varela, O. *Tetrahedron Lett.* **2009**, *50*, 5677.

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%).

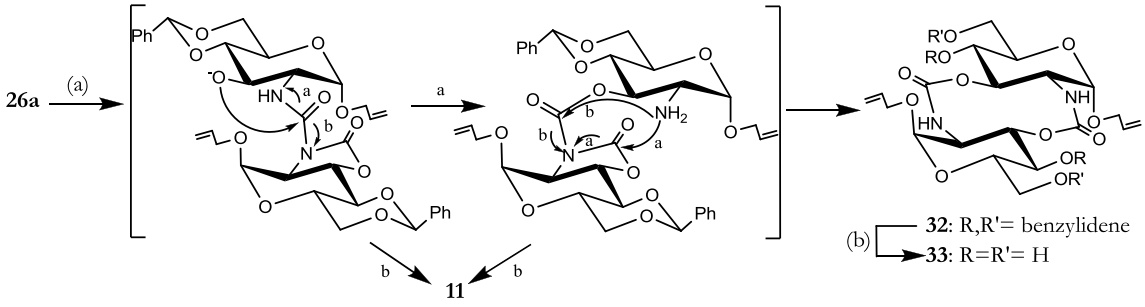


Fig. 4.10: Synthesis of carbamate-linked cyclic neosaccharide 33. *Reaction and conditions:* (a), DBU, DMF, 50°C; (b) CSA, T=60°C, 98%.

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).

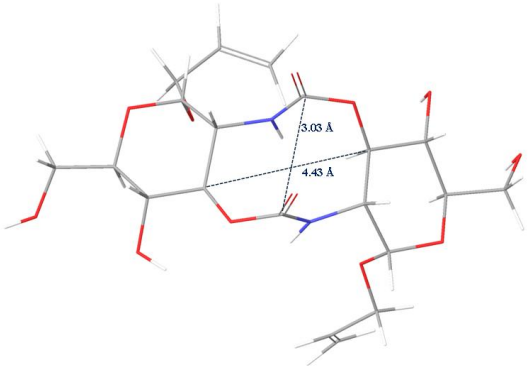


FIG. 4.11: View of the cyclic neodisaccharide 33.

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. [245] This versatile urea-urea motif has been employed to direct the formation of sheets, [246] fibers, [247] polymers, [248] gels [249] and two-dimensional molecular solids. [250] 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),

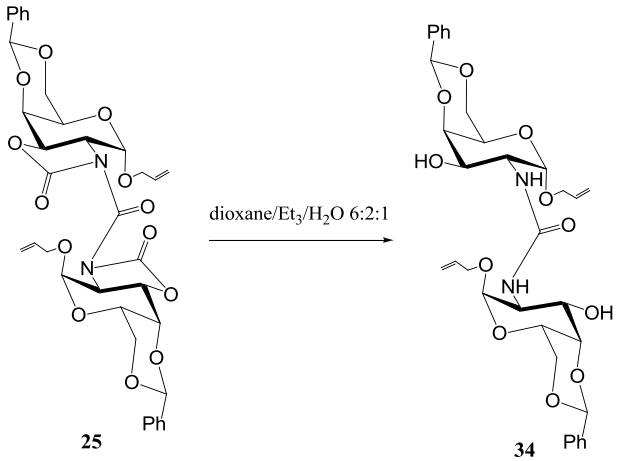


FIG. 4.12: Synthesis of galactosamine urea-linked disaccharide

^{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

---

[245] Yang, J.; Dewal, M.B.; Sobransingh, D.; Smith, M.D.; Xu, Y.; Shimizu, L.S. *J. Org. Chem.* **2009**, *74*, 102.
[246] (a) Custelcean, R. *Chem. Commun.* **2008**, 295; (b) Davis, R.; Berger, R.; Zentel, R. *Adv. Mat.* **2007**, *19*, 3878.
[247] Castellano, R.K.; Nuckolls, C.; Eichhorn, S.H.; Wood, M.R.; Lovinger, A.J.; Rebek, J. *Angew. Chem. Int. Ed.* **1999**, *38*, 2603.
[248] Castellano, R.K.; Clark, R.; Craig, S.L.; Nuckolls, C.; Rebek, J. *PNAS* **2000**, *97*, 12418.
[249] (a) van Esch, J.; De Feyter, S.; Kellog, R. M.; De Schryver, F.; Feringa, B. L. *Chem. Eur. J.* **1997**, *3*, 1238; (b) van Esch, J.; Kellogg, R. M.; Feringa, B. L. *Tetrahedron Lett.* **1997**, *38*, 281; (c) Carr, A. J.; Melendez, R.; Geib, S. J.; Hamilton, A. D. *Tetrahedron Lett.* **1998**, *39*, 7447; (d) Jeong, Y.; Hanabusa, K.; Masunaga, H.; Akiba, I.; Miyoshi, K.; Sakurai, S.; Sakurai, K. *Langmuir* **2005**, *21*, 586; (e) Suzuki, M.; Nakajima, Y.; Yumoto, M.; Kimura, M.; Shirai, H.; Hanabusa, K. *Langmuir* **2003**, *19*, 8622; (f) Estroff, L. A.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2000**, *39*, 3447; (g) Wang, G.; Hamilton, A. D. *Chem. Eur. J.* **2002**, *8*, 1954; (h) Jung, J. H.; Ono, Y.; Shinkai, S. *Chem. Eur. J.* **2000**, *6*, 4552; (i) Moreau, J. J. E.; Vellutini, L.; Wong Chi Man, M.; Bied, C. *J. Am. Chem. Soc.* **2001**, *123*, 1509.
[250] (a) Shao, X.; Chang, Y.-L.; Fowler, F. W.; Lauher, J. W. *J. Am. Chem.Soc.* **1990**, *112*, 6627; (b) Schauer, C. L.; Matwey, E.; Fowler, F. W. *J. Am.Chem. Soc.* **1997**, *119*, 10245; (c) Hollingsworth, M. D.; Brown, M. E.; Satarsiero, B. D.; Huffman, J. C.; Goss, C. R. *Chem. Mater.* **1994**, *6*, 1227.

of the chemical shift on the N*H* of the urea moieties in $CDCl_3$. In the graphs below is reported the variation of the chemical shift on the N*H* in function of the logarithm of the concentration (the experiments have been made by successive dilutions of a saturated solution of **27a** and **34**). A 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 unassociated monomers. Increasing the concentration

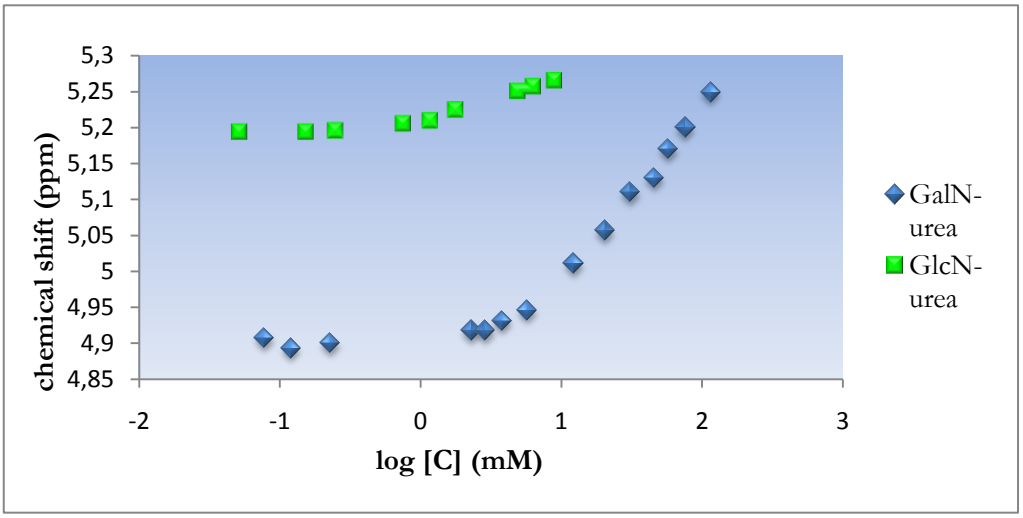


FIG. 4.13: Variation of the chemical shift of the NH proton of the urea linked neodisaccharides 27a and 34 in function of the concentration of the solution.

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 N*H*-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. [28] Although the different behaviuor 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 N*H*s in function of the concentration is due to the possibility of **27a** to form intramolecular hydrogen bonding in the unassociated

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.

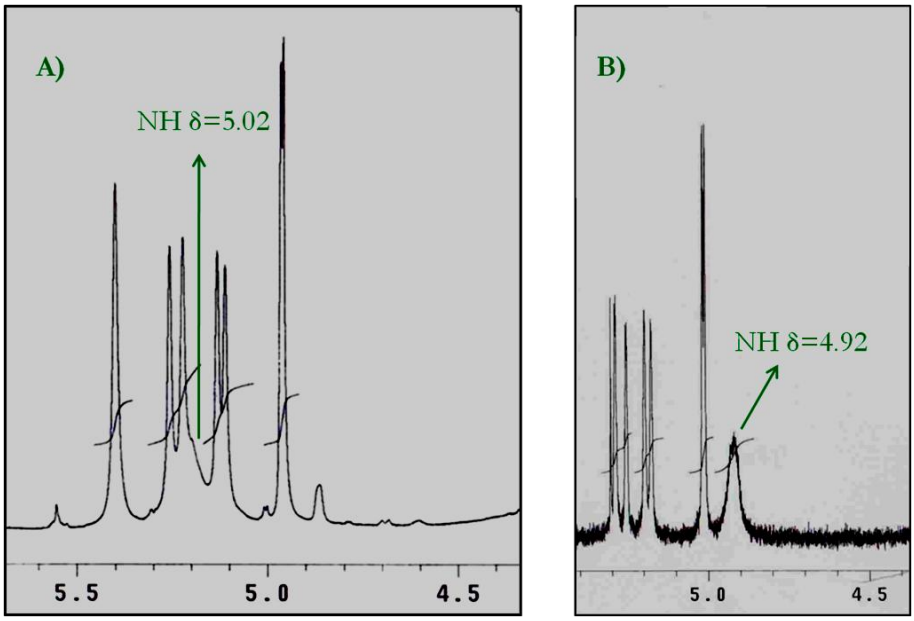


FIG. 4.14: Chemical shift of the N*H* of the urea derivative 34. Both spectra were recorded on a 500MHz instrument in $CDCl_3$ at 297K. A) [34]=76.7 mM; B) [34]=2.29 mM.

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 valuated by measuring the NMR chemical shift of the N*H* 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: C*H*Cl_3 at δ =7.26; ^{13}C: $CDCl_3$ at δ =77.0) Compounds **7**, **19** and **21** were analyzed in D_2O (acetone as internal standard, ^{1}H: (CH_3)$_2$CO at δ 2.22; ^{13}C: (CH_3)$_2$CO at δ 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_3CN 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_3CN/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_2SO_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*,*O*-carbonyl-2-deoxy-α-D-glucopyranoside (11). Compound **8** (95.4 mg, 0.310 mmol) was suspended in 2:1 v/v water/CH_3CN (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_3CN (750 μL) were added. After

30 min stirring at 40°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_2SO_4 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. $[\alpha]_D$ +33 (*c* 0.3; CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51-7.36 (m, 5H, H-Ar), 5.91 (m, 1H, OCH_2CH=CH_2), 5.62 (s, 1H, C*H*Ph), 5.35 (ddd, 1H, J_{vic} 17.2 Hz, $^4J_{H,H}$ 3.0 Hz, J_{gem} 1.5 Hz, *trans* OCH_2CH=C*H*H), 5.28 (dd, 1H, J_{vic} 10.4 Hz, J_{gem} 1.5 Hz, *cis* OCH_2CH=C*H*H), 5.15 (d, 1H, $J_{1,2}$ 2.9 Hz, H-1), 5.07 (bs, 1H, NH), 4.84 (dd, 1H, $J_{3,2}$=11.3 Hz, $J_{3,4}$=10.2 Hz, H-3), 4.29 (m, 2H, H-6a, OC*H*HCH=CH_2), 4.09 (m, 2H, H-4, OC*H*HCH=CH_2), 3.90 (m, 2H, H-5, H-6b), 3.74 (dd, 1H, $J_{2,3}$ 11.3 Hz, $J_{2,1}$ 2.9 Hz, H-2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 159.1 (N*C*OO), 136.5 (C_{ipso}), 132.8 (OCH_2CH=CH_2), 129.2, 128.3, 126.2 (C-Ar), 118.5 (OCH_2CH=CH_2), 101.4 (*C*HPh), 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, OCH_2CH=CH_2). MALDI TOF-MS: calcd for $C_{17}H_{19}NO_6$ (m/z), 333.12; found, 334.16 $[M+H]^+$. Anal Calcd for $C_{17}H_{19}NO_6$: 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)-α-D-glucopyranoside (12) and *N*,*N*'-Bis(1-*O*-allyl-4,6-*O*-benzylidene-2,3-*N*,*O*-carbonyl-2-deoxy-α-D-glucopyranos-2-yl)urea (10). Compound **8** (430 mg, 1.40 mmol) was suspended in 2:1 v/v water/CH_3CN (6.0 mL) and then cooled to 0°C. $NaHCO_3$ (585 mg, 6.96 mmol) and then a solution of 4-nitrophenyl chloroformate (1.83 g, 6.80 mmol) in CH_3CN (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_2SO_4 and concentrated to give a white gummy solid.

To obtain pure allyl 4,6-*O*-benzylidene-2-deoxy-2-(*p*-nitro-phenoxycarbonylamino)-α-D-glucopyranoside **9** for analytical purposes, a flash-chromatography (silica gel;15:1 to 2:1 petroleum ether-ethyl acetate) was performed: $[\alpha]_D$ +22.4 (*c* 1.4; CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (d, 2H, $J_{3',2'}$ 8.8 Hz, 2 H-3' *p*NO_2-Ar), 7.51-7.35 (m, 7H, H-Ar benzylidene, 2 H-2' *p*NO_2-Ar), 5.94 (m, 1H, OCH_2CH=CH_2), 5.58 (s, 1H, C*H*Ph), 5.47 (d, 1H, $J_{H,NH}$ 7.8 Hz, NH), 5.35 (bd, 1H, J_{vic} 17.2 Hz, *trans* OCH_2CH=C*H*H), 5.29 (bd, 1H, J_{vic} 10.4 Hz, *cis* OCH_2CH=C*H*H), 5.00 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1), 4.30 (dd, 1H, J_{gem} 10.2 Hz, $J_{6a,5}$ 4.7 Hz, H-6a), 4.25 (dd, 1H, J_{gem} 12.3 Hz, J_{vic} 4.7 Hz, OC*H*HCH=CH_2), 4.06 (dd, 1H, J_{gem} 12.3 Hz, J_{vic} 4.7 Hz, OC*H*HCH=CH_2), 4.02 (m, 2H, H-2, H-3), 3.89 (dt, 1H, $J_{5,4}$=$J_{5,6b}$ 10.2 Hz, $J_{5,6a}$ 4.7 Hz, H-5), 3.78 (t, 1H, J_{gem}=$J_{6b,5}$ 10.2 Hz, H-6b), 3.61 (t, 1H, $J_{4,3}$=$J_{4,5}$ 10.2 Hz, H-4), 2.58 (bs, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.7 (N*C*OO), 153.3, 145.1, 136.9 (3 C_{ipso}), 133.1 (OCH_2CH=CH_2), 129.4, 128.4, 126.2, 125.1, 121.9 (C-Ar), 118.4 (OCH_2CH=CH_2), 102.0 (*C*HPh), 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_2CH=CH_2). MALDI TOF-MS: calcd for $C_{23}H_{24}N_2O_9$ (m/z), 472.15; found, 495.28 $[M+Na]^+$. Anal Calcd for $C_{23}H_{24}N_2O_9$: 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_2Cl_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_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 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_2CH=CH_2), 5.64 (s, 1H, CHPh), 5.63 (d, 1H, $J_{1,2}$ 2.7 Hz, H-1), 5.32 (bd, 1H, J_{vic} 17.2 Hz, *trans* OCH_2CH=CHH), 5.22 (bd, 1H, J_{vic} 10.3 Hz, *cis* OCH_2CH=CHH), 4.90 (t, 1H, $J_{3,2}$= $J_{3,4}$ 11.0 Hz, H-3), 4.30 (m, 2H, H-6a, OCHHCH=CH_2), 4.11 (m, 2H, H-4, OCHHCH=CH_2), 4.05 (dd, 1H, $J_{2,3}$ 11.0 Hz, $J_{2,1}$ 2.7 Hz, H-2), 3.92 (m, 2H, H-5, H-6b); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.3, 145.8 (2 C_{ipso}), 150.3, 148.9 (2 NCOO), 136.2 (C_{ipso} benzylidene), 132.7 (OCH_2CH=CH_2), 129.3, 128.4, 126.1, 125.4, 122.2 (C-Ar), 118.8 (OCH_2CH=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_2CH=CH_2). MALDI TOF-MS: unstable. Anal Calcd for $C_{24}H_{22}N_2O_{10}$: 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_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50-7.36 (m, 5H, H-Ar), 5.85 (m, 1H, OCH_2CH=CH_2), 5.61 (s, 1H, CHPh), 5.53 (d, 1H, $J_{1,2}$ 2.8 Hz, H-1), 5.31 (ddd, 1H, J_{vic} 17.3 Hz, $^4J_{H,H}$ 3.1 Hz, J_{gem} 1.3 Hz, *trans* OCH_2CH=CHH), 5.22 (ddd, 1H, J_{vic} 10.4 Hz, $^4J_{H,H}$ 2.4 Hz, J_{gem} 1.3 Hz, *cis* OCH_2CH=CHH), 4.94 (dd, 1H, $J_{3,2}$ 11.4 Hz, $J_{3,4}$ 10.0 Hz, H-3), 4.28 (m, 2H, H-6a, OCHHCH=CH_2), 4.07 (m, 2H, H-4, OCHHCH=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, $CDCl_3$): δ 150.4 (OCON), 150.0 (NCON), 136.4 (C_{ipso}), 132.3 (OCH_2CH=CH_2), 129.4, 128.4, 126.2 (C-Ar), 118.0 (OCH_2CH=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_2CH=CH_2). MALDI TOF-MS: calcd for

$C_{35}H_{36}N_2O_{13}$ (m/z), 692.22; 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-α-D-glucopyranos-2-yl)urea (14).** Compound **10** (44.7 mg, 64.6 μmol) was dissolved in 4:1 v/v dioxane/water (1.2 mL) and then treated with (±)-camphor-10-sulfonic acid (35.0 mg, 151 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; 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; H_2O); ^1H NMR (400 MHz, D_2O): δ 5.86 (m, 1H, $OCH_2CH=CH_2$), 5.54 (d, 1H, $J_{1,2}$ 2.9 Hz, H-1), 5.32 (dd, 1H, J_{vic} 17.3 Hz, J_{gem} 1.1 Hz, *trans* $OCH_2CH=CHH$), 5.25 (dd, 1H, J_{vic} 10.5 Hz, J_{gem} 0.8 Hz, *cis* $OCH_2CH=CHH$), 4.81 (dd, 1H, $J_{3,2}$ 12.0 Hz, $J_{3,4}$ 10.0 Hz, H-3), 4.30 (dd, 1H, J_{gem} 13.2 Hz, J_{vic} 5.0 Hz, $OCHHCH=CH_2$), 4.19 (dd, 1H, $J_{2,3}$ 12.0 Hz, $J_{2,1}$ 2.9 Hz, H-2), 4.11 (m, 2H, H-4, $OCHHCH=CH_2$), 3.86 (m, 2H, H-6a, H-6b), 3.73 (m, 1H, H-5); ^{13}C NMR (50 MHz, D_2O): δ 153.4 (OCON), 149.8 (NCON), 134.0 ($OCH_2CH=CH_2$), 118.8 ($OCH_2CH=CH_2$), 94.3 (C-1), 78.6 (C-3), 75.3 (C-5), 69.6 ($OCH_2CH=CH_2$), 67.9 (C-4), 60.6 (C-2), 60.4 (C-6). MALDI TOF-MS: calcd for $C_{21}H_{28}N_2O_{13}$ (m/z), 516.16; found, 539.32 $[M+Na]^+$. Anal Calcd for $C_{21}H_{28}N_2O_{13}$: C, 48.84; H, 5.46; N, 5.42. Found: C, 48.77; H, 5.28; N 5.35.

Allyl 4,6-*O*-benzylidene-2-deoxy-3-(*p*-nitro-phenoxycarbonyl)-2-(*p*-nitro-phenoxycarbonylamino)-α-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 CH_2Cl_2 (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. $[\alpha]_D$=-15 (*c* 0.9; CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, 2H, $J_{3',2'}$ 9.1 Hz, 2 H-3' *p*NO_2-Ar), 8.20 (d, 2H, $J_{3',2'}$ 9.1 Hz, 2 H-3' *p*NO_2-Ar), 7.49-7.39 (m, 5H, H-Ar benzylidene), 7.28 (d, 2H, $J_{2',3'}$ 9.1 Hz, 2 H-2' *p*NO_2-Ar), 7.27 (d, 2H, $J_{2',3'}$ 9.1 Hz, 2 H-2' *p*NO_2-Ar), 5.94 (m, 1H, OCH_2CH=CH_2), 5.61 (d, 1H, $J_{H,NH}$ 10.0 Hz, NH), 5.59 (s, 1H, CHPh), 5.37 (bd, 1H, J_{vic} 17.2 Hz, *trans* OCH_2CH=CHH), 5.32 (bd, 1H, J_{vic} 10.6 Hz, *cis* OCH_2CH=CHH), 5.27 (t, 1H, $J_{3,2}$=$J_{3,4}$ 9.9 Hz, H-3), 5.05 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.35 (dd, 1H, J_{gem} 10.3 Hz, $J_{6a,5}$ 4.8 Hz, H-6a), 4.28 (m, 2H, H-2, OCHHCH=CH_2), 4.09 (dd, 1H, J_{gem} 12.6 Hz, J_{vic} 6.5 Hz, OCHHCH=CH_2), 4.01 (dt, 1H, $J_{5,4}$=$J_{5,6b}$ 10.3 Hz, $J_{5,6a}$ 4.8 Hz, H-5), 3.88 (t, 1H, $J_{4,3}$=$J_{4,5}$ 10.3 Hz, H-4), 3.83 (t, 1H, J_{gem}=$J_{6b,5}$ 10.3 Hz, H-6b); ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.3, 155.2, 145.5, 144.8 (4 C_{ipso}), 152.9, 152.5 (NCOO, OCOO), 136.6 (C_{ipso} benzylidene), 132.7 (OCH_2CH=CH_2), 129.3-121.6 (C-Ar), 118.9 (OCH_2CH=CH_2), 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, OCH_2CH=CH_2). MALDI TOF-MS: unstable. Anal Calcd for $C_{30}H_{27}N_3O_{13}$: 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/CH_3CN (3.67 mL) and then cooled to 40°C. $NaHCO_3$ (317 mg, 3.77 mmol) and then a solution of 4-

nitrophenyl chloroformate (750 mg, 3.97 mmol) in CH_3CN (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_2SO_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_2Cl_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, $CDCl_3$): δ 8.47 (d, 2H, $J_{3',2'}$ 7.2 Hz, 2 H-3' *p*NO_2-Ar), 7.51-7.38 (m, 7H, benzylidene, 2 H-2' *p*NO_2-Ar), 5.87 (m, 1H, OCH_2CH=CH_2), 5.73 (d, 1H, $J_{1,2}$ 3 Hz, H-1), 5.67 (s, 1H, C*H*Ph), 5.30 (dd, 1H, J_{vic} 18.9 Hz, J_{gem} 2.4 Hz *trans* OCH_2CH=C*H*H), 5.23 (d, 1H, J_{vic} 10.2 Hz, *cis* OCH_2CH=C*H*H), 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, OC*H*HCH=CH_2, H-6), 4.20-4.12 (m, 2H, H-6b, OC*H*HCH=CH_2), 3.76 (s, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.1, 145.8 (2 C_{ipso}), 150.3, 148.9 (2 N*C*OO), 136.8 (C_{ipso} benzylidene), 133.0 (OCH_2CH=CH_2), 129.3, 128.3, 126.1, 125.3, 122.1 (C-Ar), 118.4 (OCH_2CH=CH_2), 100.3 (*C*HPh), 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, O*C*H_2CH=CH_2). MALDI TOF-MS: unstable. Anal Calcd for $C_{24}H_{22}N_2O_{10}$: 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, $CDCl_3$): δ 7.56-7.18 (m, 5H, benzylidene, H-Ar), 5.87 (m, 1H, OCH_2CH=CH_2),

5.65 (s, 1H, C*H*Ph), 5.62 (d, 1H, $J_{1,2}$ 2.7 Hz, H-1), 5.28 (dd, 1H, J_{vic} 13.7 Hz, J_{gem} 0.8 Hz *trans* $OCH_2CH=CHH$), 5.18 (d, 1H, J_{vic} 8.4 Hz, J_{gem} 0.8 Hz, *cis* $OCH_2CH=CHH$), 4.91 (dd, 1H, $J_{2,3}$ 12.2 Hz, $J_{3,4}$ 2.4 Hz, H-3), 4.66-4.62 (m, 2H, H-2, H-4), 4.37 (dd, 1H, $J_{6,5}$ 1.12 Hz, J_{gem} 12.7 Hz, H-6a), 4.24 (dd, 1H, J_{vic} 5.2 Hz, J_{gem} 11.6 Hz, $OCHHCH=CH_2$), 4.16 (dd, 1H, $J_{6,5}$ 1.73 Hz, J_{gem} 12.7 Hz, H-6b), 4.10 (s, 1H, J_{vic} 5.8 Hz, J_{gem} 11.7 Hz, $OCHHCH=CH_2$), 3.72 (s, 1H, H-5); ^{13}C NMR (50 MHz, $CDCl_3$): δ 150.4 (O*C*ON), 150.0 (N*C*ON), 137.0 (C_{ipso}), 133.1 ($OCH_2CH=CH_2$), 129.4, 128.3, 126.2 (C-Ar), 117.0 ($OCH_2CH=CH_2$), 100.4 (*C*HPh), 95.2 (C-1), 74.0, 71.6, 69.8, 68.4, 69.4, 63.4, 55.5 (C-2, C-3, C-4, C-5, C-6, $OCH_2CH=CH_2$). MALDI TOF-MS: calcd for $C_{35}H_{36}N_2O_{13}$ (m/z), 692.22; 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, 60.44; H, 5.03; N 3.34.

According to the reaction conditions reported in Table 4.7 also compound **23** can be formed. Allyl 4,6-*O*-benzylidene-2,3-*N*,*O*-carbonyl-2-deoxy-α-D-galactoopyranoside **(23):** δ 7.52-7.36 (m, 5H, H-Ar), 5.91 (m, 1H, $OCH_2CH=CH_2$), 5.63 (s, 1H, C*H*Ph), 5.32 (dd, 1H, J_{vic} 17.1 Hz, J_{gem} 1.1 Hz, *trans* $OCH_2CH=CHH$), 5.28 (dd, 1H, J_{vic} 16.9 Hz, *cis* $OCH_2CH=CHH$), 4.82 (dd, 1H, $J_{3,2}$=12.1 Hz, $J_{3,4}$=2.3 Hz, H-3), 4.6 (bs, 1H, H-1) 4.39 (dd, 1H, $J_{3,4}$ 2.0 Hz, $J_{4,5}$ 12.1 Hz, H-4) 4.23-4.25 (m, 2H, H-6a, $OCHHCH=CH_2$), 4.16-4.06 (m, 2H, H-2, $OCHHCH=CH_2$), 3.90 (s, 1H, H-5). MALDI TOF-MS: calcd for $C_{17}H_{19}NO_6$ (m/z), 333.12; found, 334.16 $[M+H]^+$. Anal Calcd for $C_{17}H_{19}NO_6$: C, 61.26; H, 5.75; N, 4.20. Found: C, 61.13; H, 5.47; N 4.18.

***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-α-D-glucopyranos-2-yl)-urea (26a).**

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 Na_2SO_4 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. $[\alpha]_D$ +81.4 (*c* 1.0; CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (d, 1H, $J_{H,NH}$ 8.7 Hz, NH), 7.51-7.36 (m, 10H, H-Ar), 5.90 (m, 2H, 2 OCH_2CH=CH_2), 5.76 (d, 1H, $J_{1,2}$ 2.8 Hz, H-1_A), 5.62 (s, 1H, C*H*Ph), 5.56 (s, 1H, C*H*Ph), 5.32 (dd, 1H, J_{vic} 17.2 Hz, J_{gem} 1.5 Hz, *trans* OCH_2CH=C*H*H), 5.29 (dd, 1H, J_{vic} 17.2 Hz, J_{gem} 1.5 Hz, *trans* OCH_2CH=C*H*H), 5.24 (dd, 1H, J_{vic} 10.3 Hz, J_{gem} 1.5 Hz, *cis* OCH_2CH=C*H*H), 5.22 (dd, 1H, J_{vic} 10.5 Hz, J_{gem} 1.5 Hz, *cis* OCH_2CH=C*H*H), 4.91 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1_B), 4.83 (dd, 1H, $J_{3,2}$ 11.7 Hz, $J_{3,4}$ 10.1 Hz, H-3_A), 4.26 (m, 4H, H-$6a_A$, 3 OC*H*HCH=CH_2), 4.16 (m, 2H, H-2_A, OC*H*HCH=CH_2), 4.04 (m, 3H, H-5_A, H-5_B, H-$6a_B$), 3.91 (m, 4H, H-2_B, H-3_A, H-4_A, H-4_B), 3.76 (d, 1H, J_{gem}=$J_{6,5}$ 10.3 Hz, H-$6b_A$), 3.59 (d, 1H, J_{gem}=$J_{6,5}$ 9.9 Hz, H-$6b_B$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.9 (O*C*ON), 152.5 (N*C*ON), 137.0, 136.4 (2 C_{ipso}), 133.2, 133.1 (2 OCH_2CH=CH_2), 129.3-126.1 (C-Ar), 118.4, 118.3 (2 OCH_2CH=CH_2), 102.0, 101.4 (2 *C*HPh), 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-2_A, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, C-6_A, C-6_B, 2 O*C*H_2CH=CH_2), 54.7 (C-2_B). MALDI TOF-MS: calcd for $C_{34}H_{38}N_2O_{12}$ (m/z), 666.24; found, 689.39 $[M+Na]^+$. Anal Calcd for $C_{34}H_{38}N_2O_{12}$: 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. $[\alpha]_D$ +32 (*c* 0.5; CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50-7.34 (m, 5H, H-Ar), 5.91 (m, 1H, OCH_2CH=CH_2), 5.55 (s, 1H, C*H*Ph), 5.32 (bd, 1H, J_{vic} 17.2 Hz, *trans* OCH_2CH=C*H*H), 5.24 (bd, 1H, J_{vic} 10.4 Hz, *cis* OCH_2CH=C*H*H), 4.88 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1), 4.26 (dd, 1H, J_{gem} 9.9 Hz, $J_{6a,5}$ 4.5 Hz, H-6a), 4.20 (dd, 1H, J_{gem} 12.8 Hz, J_{vic} 5.2 Hz, OC*H*HCH=CH_2), 4.01 (dd, 1H, J_{gem} 12.8 Hz, J_{vic} 5.2 Hz, OC*H*HCH=CH_2), 3.93 (m, 2H, H-2, H-3), 3.83 (dt, 1H, $J_{5,4}$=$J_{5,6b}$ 9.9 Hz, $J_{5,6a}$ 4.5 Hz, H-5), 3.74 (t, 1H, J_{gem}=$J_{6b,5}$ 9.9 Hz, H-6b), 3.56 (t, 1H, $J_{4,3}$=$J_{4,5}$ 9.9 Hz, H-4); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.1 (N*C*ON), 137.2 (C_{ipso}), 133.4 (OCH_2CH=CH_2), 129.1, 128.2, 126.4 (C-Ar), 118.2 (OCH_2CH=CH_2), 102.0 (*C*HPh), 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, O*C*H_2CH=CH_2). MALDI TOF-MS: calcd for $C_{33}H_{40}N_2O_{11}$ (m/z), 640.26; found, 641.39 $[M+H]^+$. Anal Calcd for $C_{33}H_{40}N_2O_{11}$: 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-methoxycarbonylα-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 CH_2Cl_2 (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. NH_4Cl (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 Na_2SO_4, 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. $[\alpha]_D$ +66 (*c* 0.5; CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (d, 1H, $J_{H,NH}$ 9.5 Hz, NH), 7.49-7.34 (m, 10H, H-Ar), 5.90 (m, 2H, 2 OCH_2CH=CH_2), 5.75 (d, 1H, $J_{1,2}$ 2.8 Hz, H-1_A), 5.62 (s, 1H, C*H*Ph), 5.52 (s, 1H, C*H*Ph), 5.33 (dd, 1H, J_{vic} 17.2 Hz, J_{gem} 1.5 Hz, *trans* OCH_2CH=C*H*H), 5.30 (dd, 1H, J_{vic} 17.2 Hz, J_{gem} 1.5 Hz, *trans* OCH_2CH=C*H*H), 5.23 (m, 3H, H-3_B, 2 *cis* OCH_2CH=C*H*H), 4.91 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1_B), 4.80 (dd, 1H, $J_{3,2}$ 11.7 Hz, $J_{3,4}$ 10.1 Hz, H-3_A), 4.36-4.21 (m, 6H, H-2_B, H-$6a_A$, H-$6a_B$, H-$6b_A$, 2 OC*H*HCH=CH_2), 4.15 (dd, 1H, J_{gem} 12.8 Hz, J_{vic} 5.9 Hz, OC*H*HCH=CH_2), 4.05 (m, 2H, H-4_A, OC*H*HCH=CH_2), 3.98 (td, 1H, $J_{5,6a}$= $J_{5,6b}$ 10.3 Hz, $J_{5,4}$ 9.4 Hz, H-5_B), 3.90 (d, 1H, $J_{5,4}$ 6.8 Hz, H-5_A), 3.87 (dd, 1H, $J_{2,3}$ 11.7 Hz, $J_{2,1}$ 2.8 Hz, H-2_A), 3.78 (t, 1H, $J_{6,5}$=J_{gem} 10.3 Hz, H-$6b_B$), 3.76 (s, 3H, OCH_3), 3.74 (t, 1H, $J_{4,3}$=$J_{4,5}$ 9.4 Hz, H-4_B); ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.3, 154.7, 151.8 (*C*$OOCH_3$, N*C*ON, O*C*ON), 136.9, 136.4 (2 C_{ipso}), 133.1, 133.0 (2 OCH_2CH=CH_2), 129.3, 129.1, 128.3, 128.2, 126.2, 126.1 (C-Ar), 118.5, 118.3 (2 OCH_2CH=CH_2), 101.6, 101.4 (2 *C*HPh), 97.0, 96.3 (C-1_A, C-1_B), 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_A, C-2_B, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, C-6_A, C-6_B, 2 O*C*H_2CH=CH_2, OCH_3). MALDI TOF-MS: calcd for $C_{36}H_{40}N_2O_{14}$ (m/z), 724.25; found, 747.11 $[M+Na]^+$. Anal Calcd for $C_{36}H_{40}N_2O_{14}$: C, 59.66; H, 5.56; N, 3.87. Found: C, 59.48; H, 5.47; N 3.80.

Second eluted compound **26b** (12.1 mg, 34%) was recovered as a white powder. $[\alpha]_D$ +84.1 (*c* 1.5; CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46-7.35 (m, 5H, H-Ar), 5.87 (m, 1H, OCH_2CH=CH_2), 5.52 (s, 1H, C*H*Ph), 5.30 (bd, 1H, J_{vic} 17.2 Hz, *trans* OCH_2CH=C*H*H), 5.23 (bd, 1H, J_{vic} 10.3 Hz, *cis* OCH_2CH=C*H*H), 5.09 (t, 1H, $J_{3,2}$=$J_{3,4}$

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, OC*H*HCH=CH_2), 4.00 (dd, 1H, J_{gem} 12.8 Hz, J_{vic} 6.1 Hz, OC*H*HCH=CH_2), 3.91 (dt, 1H, $J_{5,4}$=$J_{5,6b}$ 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, $CDCl_3$): δ 156.2, 155.9 (*C*OOCH_3, N*C*ON), 137.0 (C_{ipso}), 133.2 (OCH_2*C*H=CH_2), 129.1, 128.3, 126.2 (C-Ar), 118.2 (OCH_2CH=*C*H_2), 101.6 (*C*HPh), 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, O*C*H_2CH=CH_2, OCH_3). MALDI TOF-MS: calcd for $C_{37}H_{44}N_2O_{15}$ (m/z), 756.27; found, 779.38 $[M+Na]^+$. Anal Calcd for $C_{35}H_{36}N_2O_{13}$: 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-α-D-glucopyranos-2-yl]-*N'*-(1-*O*-allyl-4,6-*O*-benzylidene-2,3-*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)

was then added. The mixture was stirred for 75 min at -30°C. Few drops of Et_3N 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. $[\alpha]_D$ +23.1 (*c* 1.7; CH_2Cl_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_2CH=CH_2), 5.69 (m, 2H, H-1_A, OCH_2CH=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_2CH=CHH), 5.22 (dd, 1H, J_{vic} 10.5 Hz, J_{gem} 1.5 Hz, *cis* OCH_2CH=CHH), 5.09 (dd, 1H, J_{vic} 17.0 Hz, J_{gem} 1.5 Hz, *trans* OCH_2CH=CH_2), 5.06 (dd, 1H, J_{vic} 10.5 Hz, J_{gem} 1.5 Hz, *cis* OCH_2CH=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_A), 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, $J_{2,1}$ 3.5 Hz, H-2_B), 4.31-4.17 (m, 5H, H-2_A, H-$6a_A$, H-$6b_A$, OCH_2CH=CH_2), 4.12 (q, 1H, $J_{5,6}$=6.4 Hz, H-5_C), 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-$6a_B$, H-$6b_B$, OCH_2CH=CH_2), 3.49 (d, 1H, $J_{4,3}$ 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_{ipso} Bn, 2 C_{ipso} benzylidene), 133.4, 133.0 (2 OCH_2CH=CH_2), 129.3-125.9 (C-Ar), 118.0, 117.9 (2 OCH_2CH=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_2Ph, 2 OCH_2CH=CH_2), 16.3 (C-6_C). MALDI TOF-MS: calcd for $C_{61}H_{66}N_2O_{16}$ (m/z), 1082.44; found, 1105.21 $[M+Na]^+$. Anal Calcd for $C_{61}H_{66}N_2O_{16}$: C, 67.64; H, 6.14; N, 2.59. Found: C, 67.48; H, 6.00; N 2.50.

***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); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40-7.27 (m, 25H, H-Ar), 5.88 (m, 1H, OCH_2CH=CH_2), 5.78 (m, 1H, OCH_2CH=CH_2), 5.54 (s, 2H, 2 C*H*Ph), 5.30 (dd, 1H, J_{vic} 17.2 Hz, J_{gem} 1.4 Hz, *trans* OCH_2CH=C*H*H), 5.28 (d, 2H, $J_{1,2}$ 3.1 Hz, H-1_A, H-1_B), 5.23 (dd, 1H, J_{vic} 10.4 Hz, J_{gem} 1.4 Hz, *cis* OCH_2CH=C*H*H), 5.17 (dd, 1H, J_{vic} 17.2 Hz, J_{gem} 1.4 Hz, *trans* OCH_2CH=CH_2), 5.14 (dd, 1H, J_{vic} 10.4 Hz, J_{gem} 1.4 Hz, *cis* OCH_2CH=C*H*H), 5.05 (bs, 2H, 2 NH), 5.01 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1_C), 4.89 (d, 1H, J_{gem} 11.5 Hz, OC*H*HPh), 4.82 (d, 1H, J_{gem} 11.9 Hz, OC*H*HPh), 4.75 (s, 2H, OCH_2Ph), 4.65 (d, 1H, J_{gem} 11.5 Hz, OC*H*HPh), 4.57 (d, 1H, J_{gem} 11.9 Hz, OC*H*HPh), 4.28 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{2,1}$ 3.5 Hz, H-2_A), 4.23 (dd, 1H, $J_{2,3}$ 10.0 Hz, $J_{2,1}$ 3.5 Hz, H-2_B), 4.16 (dd, 1H, J_{gem} 13.4 Hz, J_{vic} 6.8 Hz, OC*H*HCH=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 OC*H*HCH=CH_2), 3.53 (d, 1H, $J_{4,3}$ 2.0 Hz, H-4_C), 0.96 (d, 3H, $J_{6,5}$ 6.4 Hz, H-6_C); ^{13}C NMR (50 MHz, $CDCl_3$): δ 158.9 (N*C*ON), 138.7, 138.5, 137.6, 137.3, 137.1 (3 C_{ipso} Bn, 2 C_{ipso} benzylidene), 133.7, 133.2 (2 OCH_2CH=CH_2), 129.0-125.9 (C-Ar), 118.4, 117.9 (2 OCH_2CH=CH_2), 101.7, 101.1 (2 *C*HPh), 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,

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 O*C*H_2Ph, 2 O*C*H_2CH=CH_2), 16.5 (C-6_C). MALDI TOF-MS: calcd for $C_{60}H_{68}N_2O_{15}$ (m/z), 1056.46; found, 1078.61 $[M+Na]^+$. Anal Calcd for $C_{60}H_{68}N_2O_{15}$: C, 68.17; H, 6.48; N, 2.65. Found: C, 68.08; H, 6.42; N 2.60.

***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 $[\alpha]_D$ +37 (*c* 0.3; H_2O); ^{1}H NMR (400 MHz, D_2O): δ 5.04 (d. 1H, $J_{1,2}$ 3.9 Hz, H-1), 4.89 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1), 4.82 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 4.34 (q, 1H, $J_{5,6}$ 6.6 Hz, H-5_C), 3.88-3.40 (m, 19H, H-2_A, H-2_B, H-2_C, H-3_A, H-3_B, H-3_C, H-4_A, H-4_B, H-4_C, H-5_A, H-5_B, H-6_A, H-6_B, 2 O*C*H_2CH_2CH_3), 1.62 (m, 4H, 2 OCH_2C*H*$_2$CH_3), 1.16 (d, 3H, $J_{6,5}$ 6.6 Hz, H-6_C), 0.93 (t, 6H, J_{vic} 7.4 Hz, 2 OCH_2CH_2C*H*$_3$); ^{13}C NMR (100 MHz, D_2O): δ 160.1 (N*C*ON), 100.0, 98.3 (C-1_A, C-1_B, C-1_C), 79.0, 72.6, 72.5, 70.9, 70.8, 70.7, 69.3, 68.9, 67.8, 61.4 (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, O*C*H_2CH_2CH_3), 55.0, 54.7 (C-2_A, C-2_B), 22.8 (OCH_2*C*H_2CH_3), 16.0 (C-6_C), 10.7 (OCH_2CH_2*C*H_3). MALDI TOF-MS: calcd for $C_{25}H_{46}N_2O_{15}$ (m/z), 614.29; found, 637.10 $[M+Na]^+$. Anal Calcd for $C_{25}H_{46}N_2O_{15}$: 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_2SO_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. $[\alpha]_D$ +32 (*c* 0.9; CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49-7.33 (m, 5H, H-Ar), 5.85 (m, 1H, OCH_2CH=CH_2), 5.52 (s, 1H, C*H*Ph), 5.28 (m, 3H, H-3, OCH_2CH=CH_2), 5.00 (d, 1H, $J_{H,NH}$ 9.8 Hz, NH), 4.92 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.29 (dd, 1H, J_{gem} 10.2 Hz, $J_{6a,5}$ 4.8 Hz, H-6a), 4.20 (dd, 1H, J_{gem} 12.5 Hz, J_{vic} 5.4 Hz, OC*H*HCH=CH_2), 4.07 (dt, 1H, $J_{2,3}$=$J_{H,NH}$ 9.8 Hz, $J_{2,1}$ 3.8 Hz, H-2), 3.99 (dd, 1H, J_{gem} 12.5 Hz, J_{vic} 5.4 Hz, OC*H*HCH=CH_2), 3.94 (ddd, 1H, $J_{5,6b}$ 10.3 Hz, $J_{5,4}$ 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 (O*C*ON), 136.8 (C_{ipso}), 132.7 (OCH_2CH=CH_2), 129.2, 128.2, 126.3 (C-Ar), 119.2 (OCH_2CH=CH_2), 101.8 (*C*HPh), 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, O*C*H_2CH=CH_2). MALDI TOF-MS: calcd for $C_{34}H_{38}N_2O_{12}$ (m/z), 666.24; found, 689.37 $[M+Na]^+$. Anal Calcd for $C_{34}H_{38}N_2O_{12}$: 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. $[\alpha]_D$ +104 (*c* 0.9; D_2O); ^{1}H NMR (400 MHz, D_2O): δ 6.00

(m, 1H, OCH_2CH=CH_2), 5.38 (d, 1H, J_{vic} 17.2 Hz, *trans* OCH_2CH=C*H*H), 5.28 (d, 1H, $J_{1,2}$ 3.7 Hz, *cis* OCH_2CH=C*H*H), 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, OC*H*HCH=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, OC*H*HCH=CH_2), 3.91-3.73 (m, 4H, H-4, H-5, H-6a, H-6b); ^{13}C NMR (50 MHz, D_2O): δ 158.6 (O*C*ON), 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 $C_{20}H_{30}N_2O_{12}$ (m/z), 490.18; found, 513.00 $[M+Na]^+$. Anal Calcd for $C_{20}H_{30}N_2O_{12}$: 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-α-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 μL). 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, $CDCl_3$): δ 7.50-7.31 (m, 5H, H-Ar), 5.83 (m, 1H, OCH_2CH=CH_2), 5.37 (s, 1H, C*H*Ph), 5.28 (bd, 1H, NH), 5.23 (bd, 1H, J_{vic} 17.3 Hz, *trans* OCH_2CH=C*H*H), 5.10(bd, 1H, J_{vic} 10.3 Hz, *cis* OCH_2CH=C*H*H), 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, OC*H*HCH=CH_2), 4.11 (dd, 1H, J_{gem} 12.9 Hz, J_{vic} 4.9 Hz,), 4.13-4.08 (m, 2H, H-2, OC*H*HCH=CH_2), 3.98-3.94 (m, 2H, H-6b, OC*H*HCH=CH_2), 3.83 (bd, 1H, H-3), 3.58 (s, 1H, H-5); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.5 (N*C*ON), 137.8 (C_{ipso}), 133.6 (OCH_2CH=CH_2), 128.9, 128.0, 126.4 (C-Ar), 117.6 (OCH_2CH=CH_2), 101.1 (*C*HPh), 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 $C_{33}H_{40}N_2O_{11}$ (m/z), 640.26; found, 641.44 $[M+H]^+$. Anal Calcd for $C_{33}H_{40}N_2O_{11}$: C, 61.86; H, 6.29; N, 4.37. Found: C, 62.12; H, 6.36; N 4.48.