Nicolas Claude René Amiot
Design and Synthesis of Conformationally Constrained Trisaccharides for Probing Protein-Carbohydrate Interactions
(Under the Direction of Professor G.J. Boons)

This dissertation first describes a computer modeling study aimed at understanding reported enthalpy/entropy compensation phenomena occurring upon binding of trisaccharide **51** with lectin Concanavalin A. The design of new cyclic trisaccharides **70**, **71** and **72**, assisted by computer modeling, is also presented in this dissertation. The study of these new compounds was intended to further understand the role of carbohydrate ligand conformational properties in protein-carbohydrate interactions.

A synthesis toward cyclic trisaccharides **70** and **71** is described. Their rigidity was increased by the introduction of a methyl group at C-6 of the Glc-NAc moiety**.** One of the challenging aspects of these syntheses was the introduction of the methyl moiety by converting a 6-hydroxyl to an aldehyde, followed by a Grignard reaction. The latter low-yielding step, performed on a suitably protected glucoseamine, was challenging but successful. The macrocyclization step, however, could not be completed through any of the attempted approaches.

My efforts were then focused on the synthesis and characterization of conformationally constrained trisaccharide **72**. Compound **72** was designed to be a much more rigid molecule than **51.** The synthesis of **72** presented two major challenges. First, one of the building blocks required the synthesis of a non-natural, non-commercially available 4-amino sugar. The second challenge came from the formation of a carbamate bond at the cyclization stage, which has never been used with oligosaccharides. Two different approaches yielding trisaccharide **72** are described.

In the last part of this dissertation, the synthesis of trisaccharide **74** and **75** is described. These compounds were specifically designed to conduct Biacore experiments aiming to study the kinetics of protein-carbohydrate interactions. They are similar to compound **51**, to which an amino-propyl linker was added. The synthesis had therefore to be modified accordingly, using the appropriate building blocks. A high-yielding synthesis route was also developed for the building block bearing the linker, granting yields of 80% or above. Compounds **74** and **75** were then assembled following a route similar to **51**.

INDEX WORDS**:** Oligosaccharide, Conformational Constraint, Synthesis, Protein-Carbohydrate interactions

DESIGN AND SYNTHESIS
OF CONFORMATIONALLY CONSTRAINED TRISACCHARIDES
FOR PROBING CARBOHYDRATE-PROTEIN INTERACTIONS

by

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DEDICATION

A Théo et Nathalie.

En mémoire d’Arnaud et de mon père Jean-Louis.

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ABBREVIATIONS

Abe	Abediose
Ac	Acetyl
Ac_2O	Acetic anhydride
AgOTf	Silver triflate
All	Allyl
AMG	Amyloglucosidase
$BF_3.OEt_2$	Boron trifluoro diethyl etherate
Bn	Benzyl
br	broad
CAN	Cerium ammonium nitrate
Con A	Concanavalin A
CSA	(±)-10-camphor sulfonic acid
CsOAc	Cesium acetate
DBU	1,8-diazobicyclo[5.4.0]undec-7-ene
DCE	Dichloroethane
DCM	Methylene chloride
DMF	*N*,*N*-dimethylformamide

DMSO	Dimethylsulfoxide
eq	Equivalent
Et	Ethyl
Et_2O	Diethyl ether
EtOH	Ethanol
EtSH	Ethanethiol
Fuc	Fucoside
Gal	Galactoside
gg	Gauche-gauche
GlcNAc	*N*-acetyl glucoseamine
gt	Gauche-trans
h	hour
HPLC	High Performance Liquid Chromatography
Hz	Hertz
IgG	Immunoglobulin G
K	Kelvin
K_d	Diassociation Constant
LacNAc	*N*-acetyl lactoseamine
Le^x	$Lewis^x$
LOLI	Lathyrus ochrus isolectin I
LSI	Liquid Secondary Ion
m.p.	Melting point

m/z	Mass to charge ratio
Man	Mannoside
Me	Methyl
MeOH	Methanol
Min	Minute
MIN	Minimum
mM	millimolar
mmol	millimole
MP	*p*-methoxy phenyl
Ms	Methane sulfonyl
MS	Molecular sieves
MTM	Methyl thiomethyl
NaOAc	Sodium acetate
NeuAc	Neuraminic acid
NIS	*N*-iodosuccinimide
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
PapG	*Echerichia coli* pilus
$Pd(OAc)_2$	Palladium (II) acetate
Ph	Phenyl
Phth	Phthalimido
PMB	*p*-methoxy benzyl

Ppm	Parts per million
*R*f	Retention factor
RU	Resonance Unit
SPR	Surface Plasmon Resonance
TBAI	Tetra-*n*-butylammonium iodide
TBDMS	*Tert*-butyl dimethylsilyl
Tf_2O	Triflic anhydride
TFA	Trifluoro acetic acid
TFAA	Trifluroacetic anhydride
TfN_3	Triflic azide
TfOH	Triflic acid
tg	Trans-gauche
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TmSEt	Trimethyl silyl ethyl
TMSOTf	Trimethylsilyl trifluoromethane sulfonate
Tr	Trityl
TR-NOE	Transfer- Nuclear Overhauser Effect

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Carbohydrates represent a large group of natural compounds and are involved in a wide range of biological systems.[1] They differ from the other classes of biomolecules in the sense that their constituents (monosaccharides) can be connected to one another by a great variety of linkage types. In addition, they can be highly branched, which results in an almost infinite number of structural variations. It is now well established that carbohydrates act as biological information carriers.[2] The decoding process of the existing information in oligosaccharide structures involves their recognition by other biomolecules. Carbohydrates are most often recognized by proteins and these interactions mediate a particular biological response. Hence, they are involved in a wide range of biological processes such as inflammation, fertilization, embryogenesis, neuronal development, blood group differentiation, cell-cell adhesion and cell proliferation and organization into tissues.[1] Oligosaccharides are also sites of attachment for viruses, bacteria and toxins and promote pathogen invasion resulting in disease.[3] They are also thought to play an important role in cancer and metastasis.[3] Therefore, the study of how oligosaccharides are recognized by the binding site of proteins such as lectins, enzymes and antibodies is of major interest. The understanding of the nature of

their interactions with protein receptors could indeed lead to new carbohydrate-based therapeutic agents.

Oligosaccharide-protein interactions are characterized by a weak binding affinity.[4,5] Dissociation constants (K_D) are usually in the millimolar range, and occasionally, in the micromolar range. However, it is generally accepted that recognition phenomena require at least nanomolar K_D's. In nature, low affinity for carbohydrates is overcome by the binding of multiple carbohydrate ligands to multiple receptors.[6] This affinity enhancement phenomenon is known as the glycoside cluster effect. Although multi-valent ligands could potentially be designed, their complex structures do not make them ideal drug candidates. A better alternative would be to enhance the binding affinity of smaller, less complex oligosaccharides. In that context, it becomes critical to develop a detailed understanding of the different factors that play a role in protein-carbohydrate interactions. One of the factors that have been proposed to be important is the loss of flexibility of carbohydrate ligands upon binding to a protein.[7] However, this effect still remains largely undetermined and further research in that area is urgently required.

Synthetically modified oligosaccharides have proven to be important tools to study carbohydrate-protein interactions. These efforts first focused on the synthesis of oligosaccharides that allow the mapping of a binding site by determining functionalities that are critical for binding. In more recent studies, chemical modifications were introduced to determine the role played by specific factors in protein-carbohydrate interactions. These factors include the flexibility of sugar ligands or the influence of the displacement of conserved water molecules in the binding site. Many groups have also worked in the area of oligosaccharide analog synthesis with the hopes of improving

binding affinities. Although chemical modifications generally result in only marginal improvements, a few exceptions have shown that it is possible to enhance the binding affinity of an oligosaccharide with a protein using this approach.

The research in this area resulted in the design and synthesis of anti-cancer,[8] antiviral[9] and anti-convulsant[10] agents. These encouraging results have paved the way for new inhibitors of carbohydrate-mediated biological processes to be further investigated.

1.2 Chemical Modifications

Research with chemically modified oligosaccharides has provided a wealth of information about protein-carbohydrates complex formation. It is now well established that hydrogen bonds and van der Waals interactions, which often include the stacking of the hydrophobic face of a sugar against aromatic amino acid side chains, play a key role in protein specificity.[6,11-15] In addition to these direct interactions, other molecules have also been shown to be involved in the formation of protein-carbohydrate complexes. Water molecules, located either directly in the binding site or at the surface of the protein, are also responsible for additional interactions, which, by helping to stabilize the complex, result in an overall higher selectivity.[6,11-13]

Several types of these modifications will be presented in subsequent paragraphs because of their interesting background for my research project. In a first instance, the implications of sugar hydroxyls replacement and substitution of an oxygen atom by a carbon atom in a glycosidic linkage will be discussed. Then, it will be presented how

sugar hydroxyls replacement and modification of anomeric configuration can be used to enhance binding affinities of specific oligosaccharides.

1.2.1 Replacement of Sugar Hydroxyls

Saccharide analogues, in which a hydrogen or a fluoride atom replaces a hydroxyl moiety, are commonly used to determine which hydroxyls are critical for complex formation. These hydroxyls have been termed key polar interactions (required hydrogen bonds for specificity).[16-18] The deoxygenation of a saccharide analogue results in the cancellation of hydrogen bonding, while fluorine substitution only allows acceptor hydrogen bonds. This procedure gives valuable information about the specificity of a protein for a given carbohydrate and, together with X-ray analysis, it provides a precise mapping of a protein binding site.

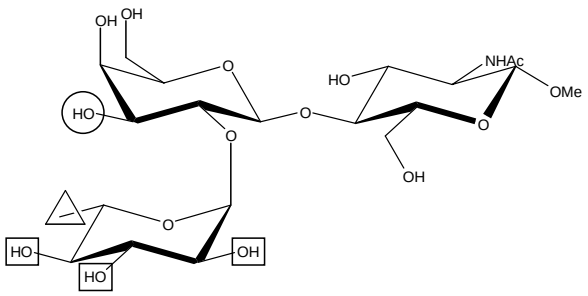


Figure 1.1: H-type 2 blood group determinant; squares: buried hydroxyl groups involved in hydrogen bonds deep in the binding site, triangles: essential hydrophobic interactions, circles: hydrogen bonds implicated at the periphery of the binding site. The study was conducted using Ulex europaeus I lectin.

As early as 1967, Goldstein and co-workers realized the mapping of the binding site of Concanavalin A (Con A). Using modified monosaccharides such as 3-deoxy-α-D-glucose, 4-deoxy-α-D-glucose and 6-O-methyl-α-D-glucose, it was found that hydroxyls C-3, C-4 and C-6 were key polar groups. Their removal resulted in the complete loss of

binding affinity. Another example is the more recent studies by Lemieux and co-workers, who realized the epitope mapping of blood determinant H-type 2 trisaccharide with three different lectins.[19-22] The mapping process revealed key polar interactions, van der Waals and peripheral interactions as depicted in Figure 1.1.

In order to study the implications of conserved water molecules in the binding sites of proteins, Boons and co-workers[23] achieved another type of chemical modification. A hydroxyl was replaced by a hydroxyethyl moiety to mimic a conserved water molecule in the binding site of the complex between trimannoside **1** (Figure 1.2) and Con A.

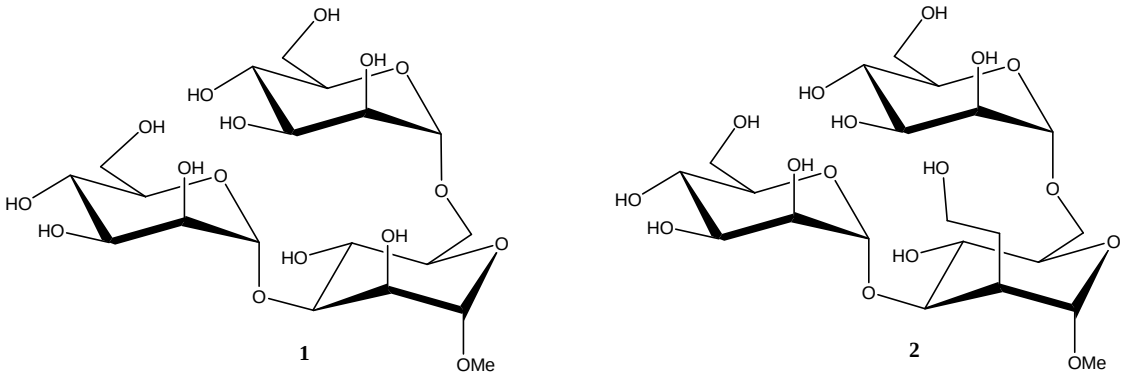


Figure 1.2

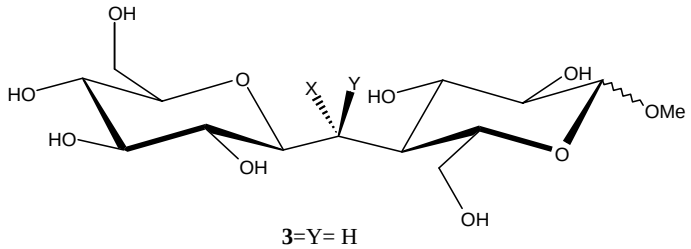
The affinity of modified analogue **2** was determined by Isothermal Calorimetric Titration (ITC) and it was found that, in spite of an expected gain in entropy, the affinity of binding was slightly lower than that of the parent trisaccharide. NMR and computer modeling structural studies were carried out to interpret this loss in enthalpy. It was found that, as expected, the hydroxyethyl displaced the conserved water molecule upon binding between **2** and Con A. Analogue **2** was also shown to bind the protein using

similar hydrogen bond pattern and in a similar low energy conformation. It was then concluded that indirect protein-saccharide interactions mediated by water molecules might be enthalpically more favorable because of a larger number of hydrogen bonds with higher occupancies in the binding site.

1.2.2 C-Type Glycosidic Linkage

One of the major concerns associated with the development of carbohydrate-based drugs is that they may be hydrolyzed by glycosidases. Their half-life is thus decreased and their potential as inhibitors of biological processes is weakened. Chemical modifications can be exploited to create sugar analogues, which, although having the same biological activity, would be resistant to enzymatic hydrolysis. One interesting approach is the replacement of a glycosidic linkage by a C-type glycosidic linkage. Schmidt and co-workers reported an example of such compounds.[24] They synthesized a series of carbon-bridged C-disaccharides **3, 4,** and **5** (Figure 1.3).

In some instances, C-type glycosidic linkages result in a loss of binding affinity, in which case others modifications to stabilize carbohydrate against enzymatic hydrolysis have to be found.



3=Y= H
4= H, Y= OH
5= OH, Y= H

Figure 1.3

1.2.3 Enhancement of Binding Affinity

A general characteristic of the mode of binding of oligosaccharides to proteins is schematically summarized in Figure 1.4, part A. Many protein-combining sites are cleft or groove-like and bind the oligosaccharide on one side. As depicted in Figure 1.4, some hydroxyls may not be involved in the binding. In principal, molecular structure could be added to the ligand through these OH groups, and if this structure formed favorable interactions with the protein surface, then the affinity of the ligand should be enhanced (Figure 1.4, part B).[5]

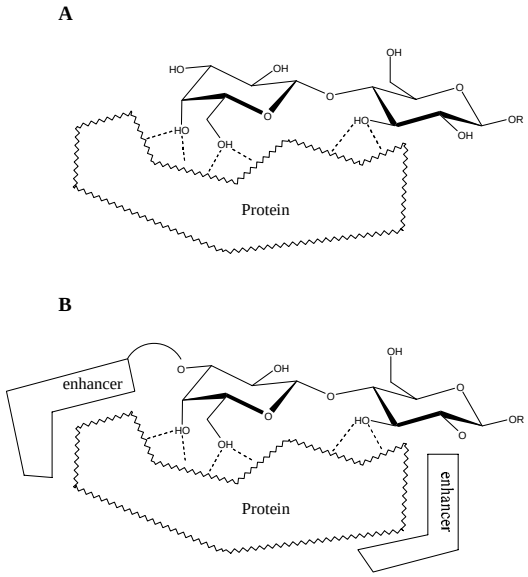


Figure 1.4

This approach was successfully used by von Itzstein and co-workers to design two potent sialidase-based inhibitors of influenza virus replication.[25] Chemical modifications were achieved at the C-4 position of 2-deoxy-2,3-di-dehydro-D-N-

acetylneuraminic acid (Neu5Ac2en) (**6**, Figure 1.5) an inhibitor of the influenza sialidase but that failed to demonstrate any beneficial effect in animal models of infection.

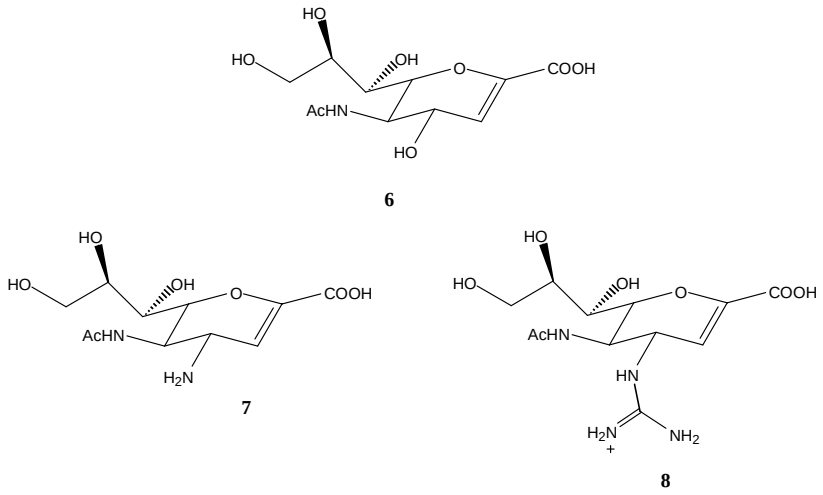


Figure 1.5

Computer modeling studies showed that the replacement of the hydroxyl at the C-4 position of Neu5Ac2en by an amino group (**7**, Figure 1.5) should result in an increase in the binding interactions due to salt formation with the side chain carboxylic group of Glu 119.[25] The replacement of the same hydroxyl with a guanidinyl group (**8**, Figure 1.5) was anticipated to produce an even tighter affinity as a result of lateral binding through the terminal nitrogens of the guanidino group with both Glu 119 and Glu 227. X-ray crystallography of 4-amino and 4-guanidino-Neu5Ac2en complexed to influenza virus sialidase confirmed the predicted binding mode for both analogues. These two analogs proved to be potent inhibitors of the virus influenza sialidase with inhibition constant (K_i) of 5×10^{-8} M for the 4-amino analog and 2×10^{-10} M for the guanidine analog.[25] These two compounds were also found to be more potent inhibitors than Neu5Ac2en which was

found to have an inhibition constant of 1×10^{-6} M. In the case of the 4-guanidino analog a 10000-fold increase in binding affinity was observed. Furthermore, where Neu5Ac2en failed to inhibit the influenza virus replication these two analogs were found to be excellent inhibitors.

Bundle and co-workers[26] found that another type of chemical modification could lead to enhancement of the binding affinity of certain oligosaccharides. In effect, the modification of the anomeric configuration of the galactose residue of trisaccharide Methyl 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-2-*O*-(α-D-galactopyranosyl)-α-D-mannopyranoside (**9**, Figure 1.6) had dramatic effects. A 100-fold increase in binding affinity for Methyl 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-2-*O*-(β-D-galactopyranosyl)-α-D-mannopyranoside (**10**, Figure 1.6) to the Salmonella monoclonal antibody Se155.4 was observed.[26] It was hypothesized that this increase in binding affinity could arise from new saccharide-protein contacts due to the different orientation of a β-Gal, as opposed to an α-Gal. A large entropy term determined during thermodynamic studies, which signifies a major loss of motional freedom, corroborated this hypothesis.

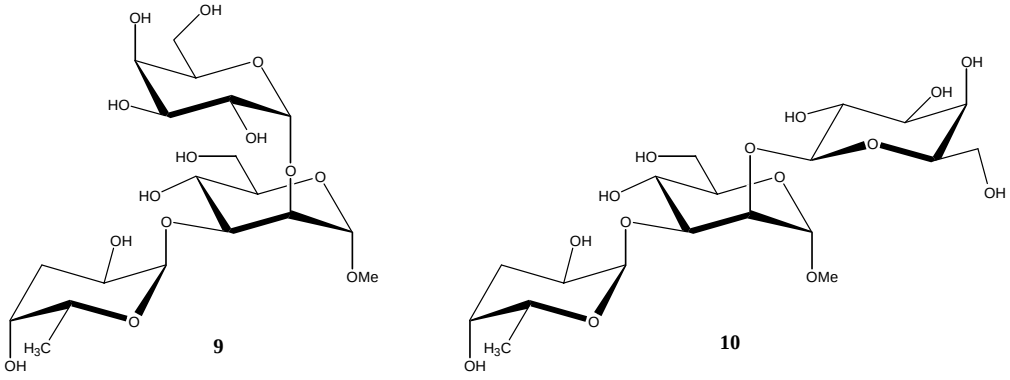


Figure 1.6

1.3. Conformationally Constrained Oligosaccharides

It is well established that carbohydrates have some degree of flexibility around their glycosidic linkages and that they can adopt several conformations of minimum energy in solution.[27,28] Although an endless number of conformations could theoretically be adopted by rotation around a glycosidic linkage, it has been found that only a limited number of these conformations are populated and certain conformers are preferred.

Oligosaccharides are flexible molecules that adopt different conformations by movement around glycosidic linkages. Two types of torsional rotations are generally accepted to explain their flexibility. The rotational freedom around the C5-C6 linkage in hexoses, defined by the torsional angle ω (O6-C6-C-5-O5 in IUPAC nomenclature), confers flexibility, especially if engaged in a glycosidic linkage. Rotation occurs between three energetically favorable, staggered conformations: synchiral, antichiral and antiperiplanar (IUPAC nomenclature) also known as gauche-gauche (gg), gauche-trans (gt) and trans-gauche (tg) (Figure 1.7).

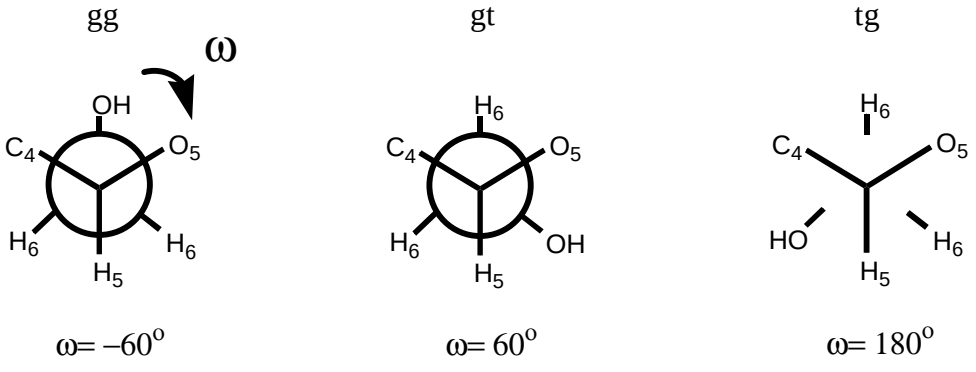


Figure 1.7

Flexibility of carbohydrate ligands also arises from rotations around other glycosidic linkages. The relative orientation of two monosaccharide units compared to each other can be described using two new torsional angles Φ and ψ (Figure 1.8). The torsional angle ψ represents the angle around the C-anomeric-OX' and is defined as H1-C1-Ox'-Cx'. Φ represents the angle around OX'-CX' bond and is defined as C1-OX'-CX'-HX'. Although the hexose ring is considered flexible - it can adopt several conformations (chair, boat and skew) - in most cases, the ring is considered fixed in the $^4C_1$ chair conformation.[29]

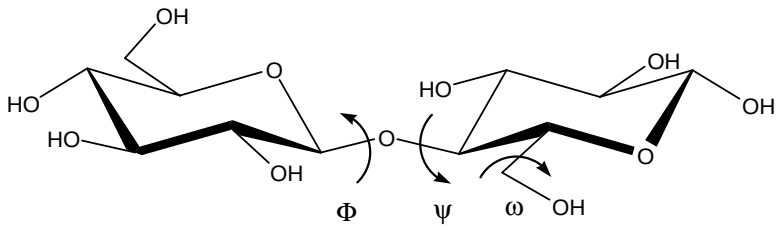


Figure 1.8

Conformational properties of an oligosaccharide can also be modulated by the addition or the removal of saccharide residues or its attachment to a protein or a lipid. These properties impact recognition phenomena based on the shape of ligands. For example, it was shown that disaccharide LacNAc is highly flexible and can adopt at least three different conformations in solution.[30] The attachment of a fucoside at the C-3 position of the galactoside of LacNAc, resulting in trisaccharide Lewis^X (Le^X), modified the conformational properties of the glycosidic linkage Gal-hexose. NMR spectroscopy and X-ray analysis showed that this trisaccharide was much more rigid and resulted in a single conformation family.

There is also evidence that when oligosaccharides are part of a macromolecular structure such as glycoproteins or glycolipids, their conformational properties differ. For example, the pentasaccharide of biantennary N-linked oligosaccharides of the Fc region of human immunoglobulin G (IgG) has different conformational properties compared with the same saccharide "free" in solution.[30] As an example, the galactoside residue of the (1-6) linkage undergoes extensive interactions with the protein, which result in torsional angles ϕ and ψ different from those predicted by NMR and theoretical calculations. These interactions may also result in a loss of flexibility around glycosidic linkages. In nature, glycosidic linkages have similar limited flexibility, which could result in increased affinity and selectivity.

The examination of structural properties of a wide range of saccharides and their complexes with proteins has revealed that both global and local minima can be complexed. It has also been shown that carbohydrate ligands lose some of their conformational freedom upon binding with a protein. Thermodynamic studies suggested that this loss of flexibility could introduce an unfavorable entropic term.[7] The resulting loss of entropy could then be one of the contributors of the low affinity of carbohydrates for proteins.

These observations have spearheaded the design of conformationally constrained oligosaccharides. Starting from naturally occurring sugars, chemical modifications were introduced to restrain conformational freedom around glycosidic linkages. Two types of conformational constraints have been studied. First restrictions targeted at constraining the ω-torsional angle of the (1-6) glycosidic linkage. Chemical modifications then focused on reducing conformational freedom around other types of glycosidic linkages.

In both cases, these modifications have been performed on small oligosaccharides in an attempt to enhance their biological activity.

The next sections present several examples of conformational constraints that have been applied to study the implications of oligosaccharide conformational properties on the affinity of binding.

1.3.1 Natural Conformationally Constrained Saccharides

Before discussing synthetic conformationally constrained oligosaccharides, it is interesting to note that the reduction in the rotational freedom is a well-known principle in nature. This phenomenon is frequently observed in peptides and macrolides, which results in an enhancement of their selectivity in either ion or receptor binding and their stability against enzymatic digestion. Several natural conformationally constrained oligosaccharides have been isolated. Calonyctin A (**11**, Figure 1.9) was the first plant cyclic oligosaccharide that was discovered.[31,32] Conformational studies have shown that its carbohydrate part is not very flexible. Its linker moiety, however, can adopt several conformations. This macrocyclic compound[31-35] acts as plant growth promoter and the role of the conformational constraint on its biological activity is largely undetermined.

Tricolorin A (**12**, Figure 1.9) is also a natural conformationally constrained oligosaccharide.[33-35] This compound is a selective plant growth inhibitor and has interesting anti-microbial potential and cytotoxic properties.

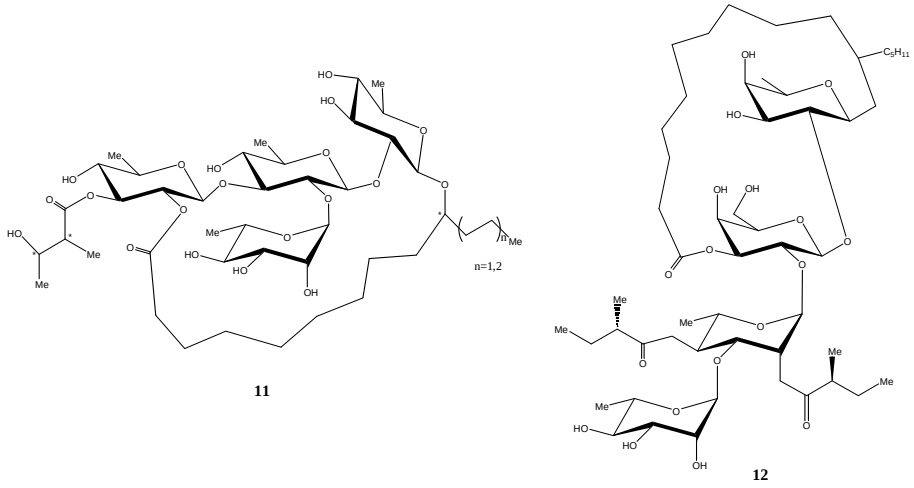


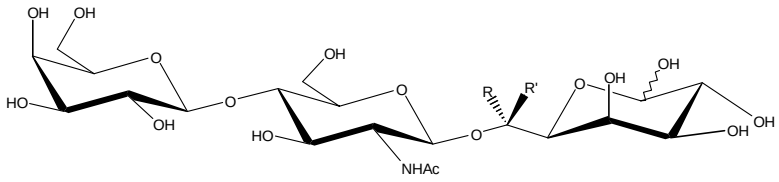
Figure 1.9

No studies that would link the biological activity of these compounds and their conformational restrained properties have been reported. Nature is, however, very efficient and it is reasonable to assume that these conformational constraints do indeed play a role. It is interesting to note that natural conformational constraints are encountered in small oligosaccharides that are not part of larger macromolecular structures. This last point has inspired organic chemists to create new small conformationally constrained carbohydrates, with the aim to create higher affinity ligands that could be a useful concept for the design of carbohydrate based drugs.

1.3.2 Conformational Constraints Around (1-6) Glycosidic Linkage

In order to study the conformational properties of oligosaccharides, with a focus on (1-6) glycosidic linkage, several chemical modifications were performed. A few examples of conformationally constrained oligosaccharides are discussed below.

Lemieux and co-workers[36,37] designed the first conformationally constrained oligosaccharide, which was derived from β-D-Gal(1→4)-β-D-GlcNAc(1→6)-D-Gal. Computer modeling energy calculations were performed to determine which of the three staggered conformations around the GlcNAc(1→6)-D-Gal of this trisaccharide was the most populated. It was found that this linkage could adopt all three conformations without encountering energy barriers greater than ± 0.5 kcal/mol and no conclusion could be drawn from this study. It was then decided to restrain the rotational freedom of this linkage by chemical modifications. The constraint was achieved by replacing one of the two hydrogen atoms at the C-6 position with a methyl group on the reducing galactose. It resulted in a rotational barrier that was high enough to severely restrict the rotation around the C5-C6 bond. Both R and S isomers (**13** and **14**, Figure 1.10) were synthesized.

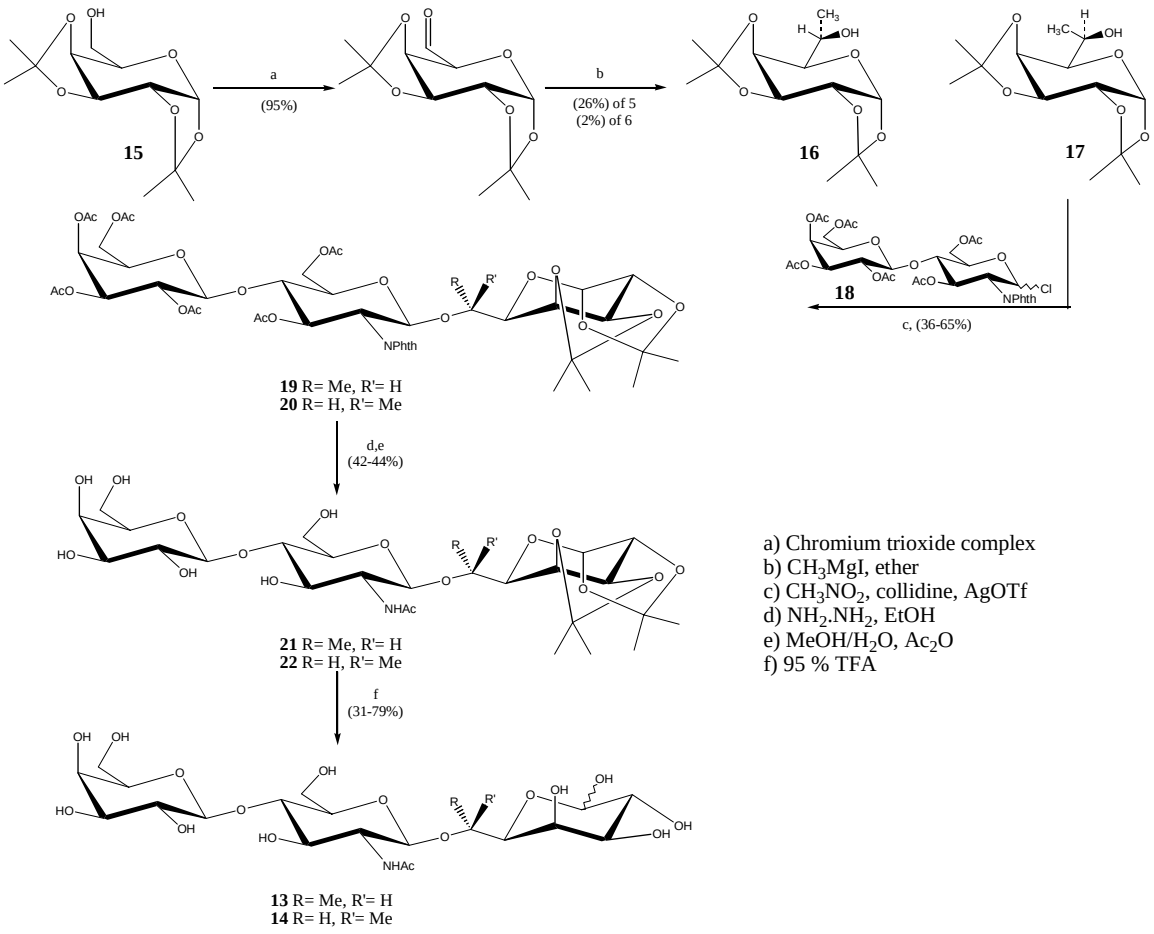


13 R= Me, R'= H
14 R= H, R'= Me

Figure 1.10

Starting from suitably protected building block **15**, oxidation using chromium trioxide pyridine complex to give an aldehyde, followed by a Grignard reaction with methyl magnesium iodide, afforded a mixture of diasteroisomer **16** and **17** (Scheme 1.1). Trisaccharides **19** and **20** were synthesized by condensation of **16** and **17** with

disaccharide **18**, using AgOTf as a promoter. The treatment of the latter trisaccharide with hydrazine monohydrate gave two amine intermediates that were acetylated to afford compounds **21** and **22**. Finally trisaccharides **13** and **14** were obtained after treatment with TFA to remove the isopropylidene protecting groups.



Scheme 1.1

Compounds **13** and **14** were tested for binding against the anti-I-Ma monoclonal antibody. Compound **13** was shown to bind with higher affinity to the antibody than its natural counterpart and diastereoisomer **14** showed much weaker binding. It was then hypothesized that these changes in free energy of binding were the direct consequence of

the conformational constraint. Because of interactions between the methyl and galactoside ring hydroxyls, the (1-6) glycosidic linkage of compound **13** was preorganized in the gt conformation ($\omega = +60^{o}$), which was determined to be the conformation required for binding. This preorganization resulted in a higher free energy of binding with anti-I Ma monoclonal antibody. It also pointed out that the loss of conformational freedom could have some implications on the thermodynamics of binding between carbohydrate and proteins.

In a similar way, Lemieux and co-workers[38] realized the synthesis of conformationally constrained isomaltosides **23** and **24** (Figure 1.11). Kinetic studies on the binding of saccharide analogues with the enzyme amyloglucosidase indicated that the *R*-diasteroisomer **23** is more strongly bound by the enzyme ($K_m = 0.9$ mM) than the parent isomaltosides ($K_m = 24.5$ mM). The binding affinity of the *R*-compound was also shown to be similar to the affinity of binding of maltose **25** ($K_m = 1.3$ mM), a structurally related saccharide. On the other hand, *S*-diastereoisomer **24** ($K_m = 90.0$ mM) showed the weakest binding affinity of all. This can be explained by comparing the structures of maltose, isomaltose and compound **23**. Because of its (1-4) glycosidic linkage, maltose has a well-defined conformational preference, while torsional freedom around the C5-C6 bond makes isomLatose conformationally ill-defined. It is well established that OH-3, OH-4' and OH-6' are intimately involved in the hydrolysis of maltoside. However, in the case of isomaltoside, the same enzyme recognizes different hydroxyls, OH-4, OH-4 'and OH-6'. Conformational analysis showed that chemically modified methyl 6*R*-C-methyl-α-isomaltoside **23** could energetically more readily achieve the preferred conformation of maltose, resulting in a better affinity of binding with amyloglucosidase. From these

observations, it was concluded that the ease of hydrolysis of a disaccharide by AMG is related to an ability to achieve a conformation in which the spatial arrangement of the key polar groups are in close correspondence to that of maltose. This example also emphasizes that conformational constraint strategies can be used to achieve better affinity ligands. Provided that a chemical modification can be identified to pre-organize an oligosaccharide towards a specific binding conformation, it is reasonable to assume that ligands with higher free energy of binding with proteins can be designed.

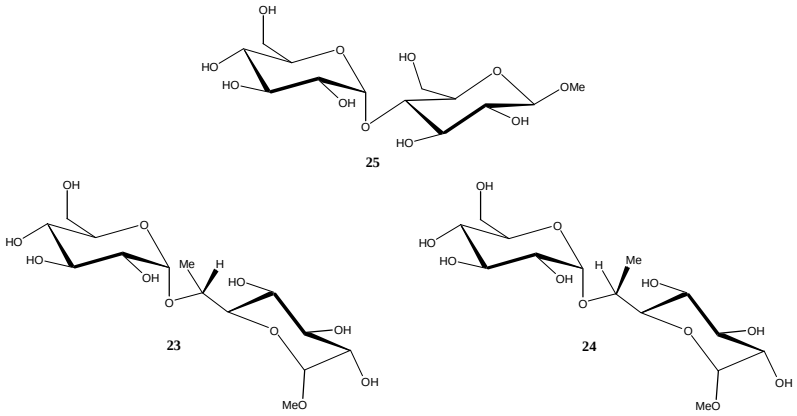
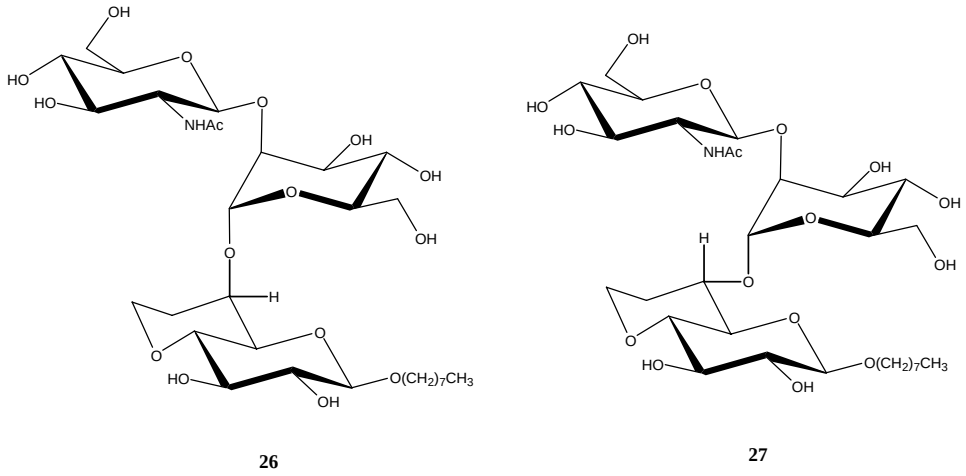


Figure 1.11

The role of the flexibility around C5-C6 bond was also studied by Hindsgaul and co-workers.[39,40] Conformationally restricted diastereoisomers **26** and **27** (Figure 1.12) were synthesized and investigated as acceptors for *N*-acetyl glucosaminyl transferase-V. Compound **27** having the C5-C6 bond sterically restricted toward the gt conformation was found to be twice as active as the parent saccharide. Diastereoisomer **26** sterically biased toward the gg conformation proved to be two-fold less active than its natural counterpart. This example emphasizes the role of the tertiary structure of

oligosaccharides. Although isomers **26** and **27** have the same primary structure, one displays a higher affinity of binding due to differences of conformational properties that are more desirable for the binding process. This factor is of crucial importance because of the role it plays in the selectivity of glycoconjugate biosynthesis. It explains why a glycosyl transferase transfers a glycosidic residue to a certain oligosaccharide depending on its conformation via a mechanism known as site-directed processing.[41]

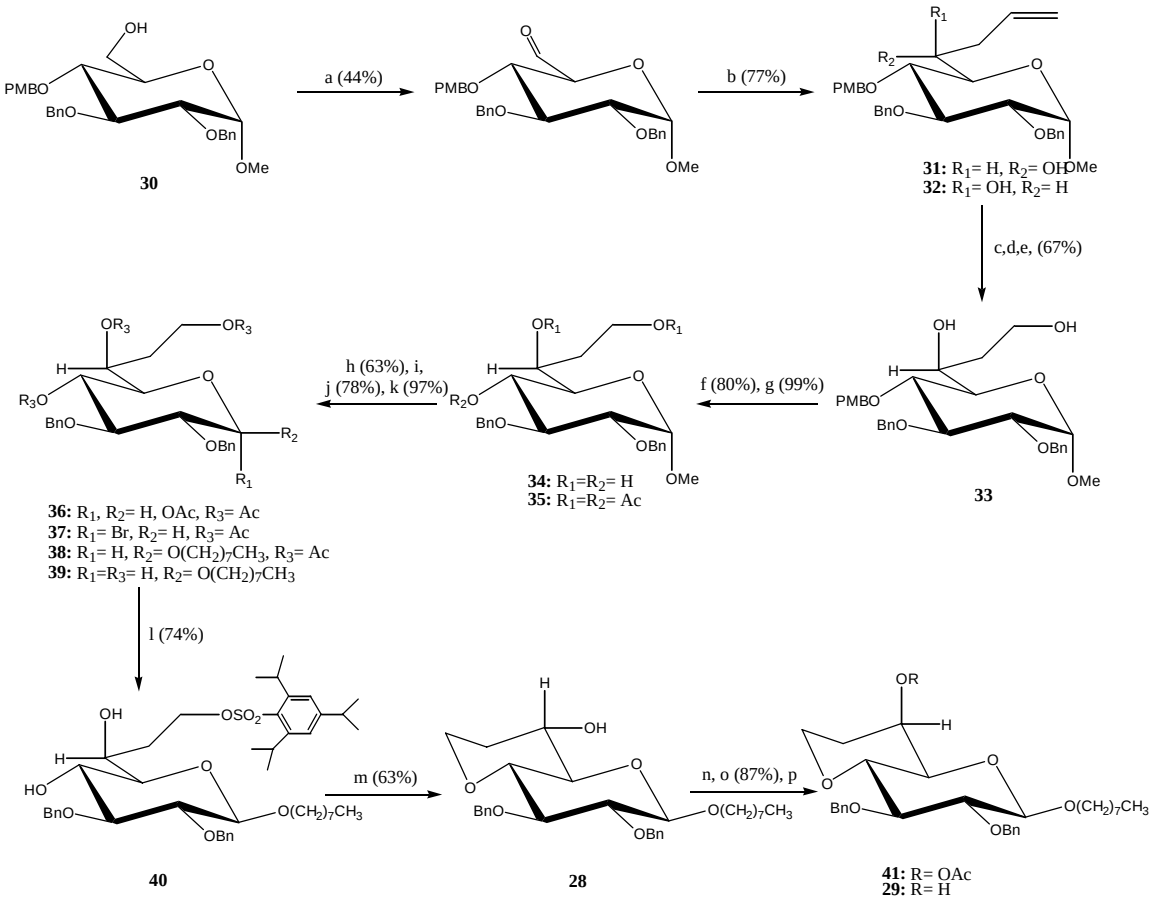


26 **27**

Figure 1.12

The pre-organization was accomplished by linking C-4 and C-6 hydroxyls with an ethylene bridge. The synthesis of required conformationally restricted building blocks **28** and **29** is discussed below (Scheme 1.2).[40,42] Swern oxidation of suitably protected methyl α-D-glucopyranoside (**26**) followed by a Grignard reaction using allyl magnesium chloride in THF afforded a 1:1 mixture of diastereoisomers **31** and **32** (Scheme 1.2). Ozonolysis of **32** followed by reduction yielded glucopyranoside **33**. The *p*-

methoxybenzyl of **33** was selectively removed and the obtained triol **34** was acetylated to afford compound **35**.



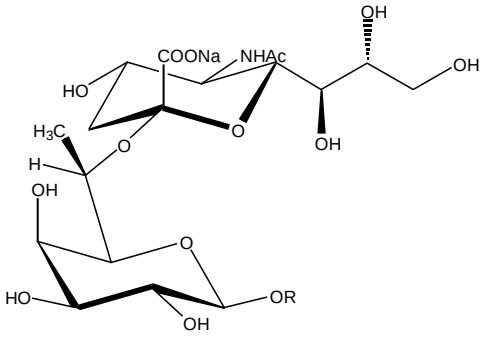
a) DMSO, CH_2Cl_2, oxalyl chloride. b) Allyl magnesium bromide, THF. c) MeOH, O_3, -78 ^oC, CH_2Cl_2. d) dimethyl sulfide. e) $NaBH_4$. f) CAN, CH_3CN/H_2O. g) Ac_2O, DMAP, pyridine. h) H_2SO_4, Ac_2O, -5 ^oC. i) HBr, CH_2Cl_2, 0 ^oC. j) 1-octanol, silver zeolite. k) NaOMe, MeOH. l) 2,4,6-triisopropylbensenesulfonyl chloride, CH_2Cl_2, 0-18 ^oC, DMAP. m) NaH, DMF. n) Tf_2O, CH_2Cl_2, -10 ^oC. o) Et_4NOAc, DMF. p) NaOMe, MeOH.

Scheme 1.2

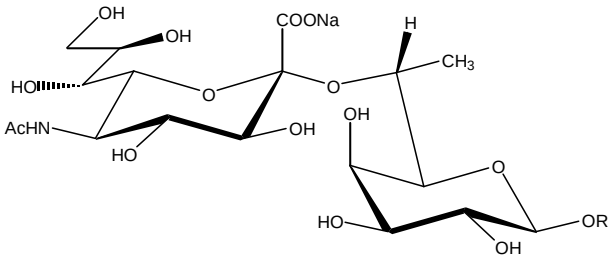
Acetolysis of **35** gave the anomeric acetate **36**, which was then treated with HBr to afford α-bromide **37**. A glycosylation was performed with 1-octanol and **37** using silver zeolite as promoter to give β-glycoside **38**. Deacetylation of **38** yielded diol **39**, which was then selectively activated at position C-6 using 2,4,6

triisopropylbenzenesulfonate to yield **40**. The 4,8-anhydro ring formation was achieved via S_N2 by treatment of **40** with sodium hydride to afford diastereoisomer **28**. In the last step, inversion of configuration consequently occurred at C-6. Compound **28** was then converted to a 6-*O*-trifluoromethylsulfonyl ester, which was treated with tetraethylammonium acetate to afford the second diastereoisomer **41**. Deacetylation of **41** yielded second building block **29**. The remainder of the synthesis entailed assembly and deprotection steps using common carbohydrate synthetic routes to obtain trisaccharides **26** and **27**.

As a final example, the two conformationally restricted analogues of NeuAc(2-6)-β-D-Gal **42** and **43** depicted in Figure 1.13 will be discussed. Sabesan and co-workers tested these compounds as substrates for influenza A neuraminidase.[43]



42



43

Figure 1.13

The *gt* rotamer analogue **42** was slowly hydrolyzed by neuraminidase, whereas the *tg* rotamer analogue **43** was hydrolyzed at a rate comparable to the natural disaccharide. It was thus concluded that the tg conformation of the (1-6) glycosidic linkage was required for binding.

1.3.3 Conformational Constraints Around Other Glycosidic Linkages

Since no modification can be performed on the glycosidic bond itself, a new type of chemical modification had to be introduced. In most cases, a covalent linker between two monosaccharide moieties is employed to reduce rotational freedom of a glycosidic linkage.

Bundle and co-workers studied the conformational implications of a series of conformationally restricted trisaccharide analogues **45-50** of 3-β-D-Abe-2-βD-Gal-α-D-Man (**44**) (Figure 1.14) a trisaccharide epitope recognized by the salmonella monoclonal antibody Se 155.4.[44] Binding studies of these analogues with antibody Se 155.4 revealed that compounds **45** and **46** were not recognized by the protein. Their conformations were shown to be distorted compared with the conformation of the parent compound **44**, which accounted for the loss in bioactivity. However, the conformational constraint impacted compounds **47** to **50** differently: these molecules were found to be pre-organized in conformations that were similar to the bound state conformation of natural compound **44**. The thermodynamic parameters of antibody-tethered oligo-saccharides interactions was determined. The free energy change was found to be smaller than ± 0.5 kcal/mol in spite of the pre-organization and reduced flexibility of the ligands. From these results, it was concluded that the inter-residue flexibility was not responsible for the weak affinity of oligosaccharide-protein interactions. It was also hypothesized that the lack of significant impact on enthalpy and entropy meant that oligosaccharides displayed a restricted range of conformations.

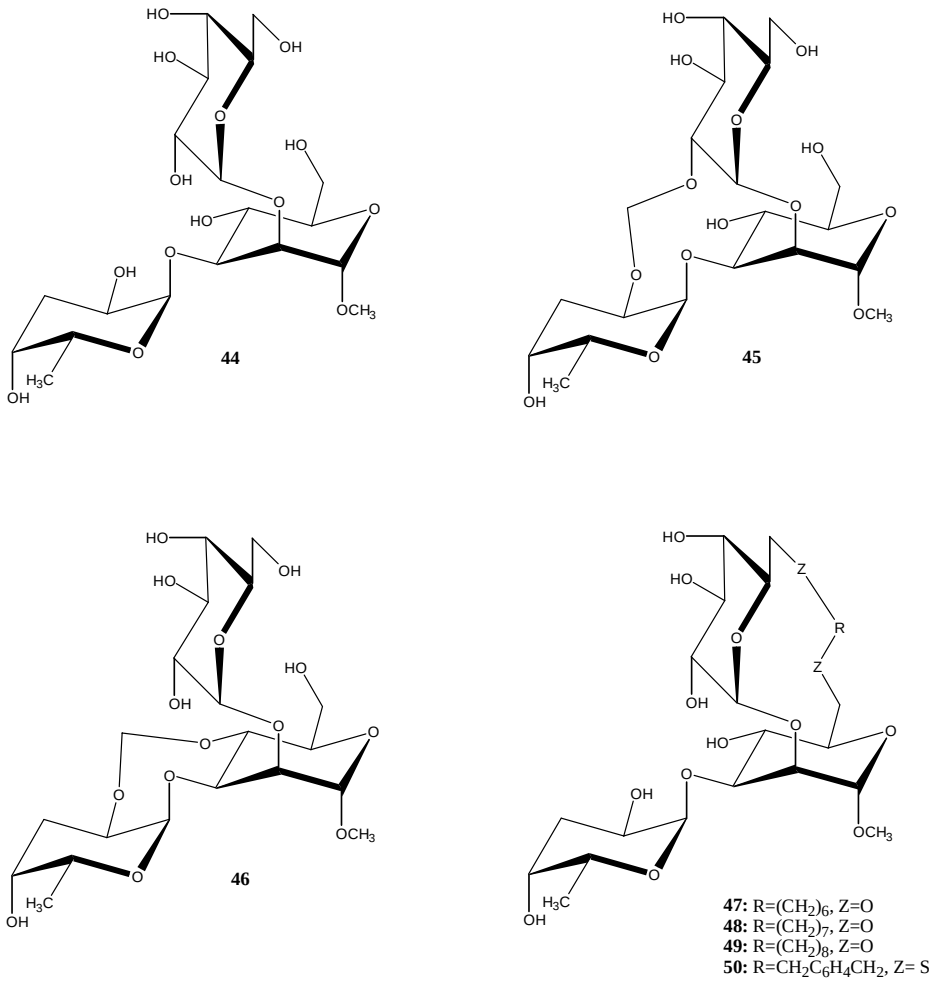


Figure 1.14

Boons and co-workers designed trisaccharides **51** and **52** (Figure 1.15) based on X-ray crystallography and molecular mechanics modeling data of oligosaccharides and Lathyrus Ochrus lectin complexes.[45] Two intramolecular hydrogen bonds between O2 and O6" and between O4 and O6" identified in the bound conformation of trisaccharide β-GlcNAc-(1→2)-α-Man-(1→3)-Man were replaced by methylene acetal bridges. It was expected that upon binding, conformationally constrained trisaccharides **51** and **52** would lose less conformational freedom than its natural linear counterpart, which would in turn result in a smaller entropic penalty.

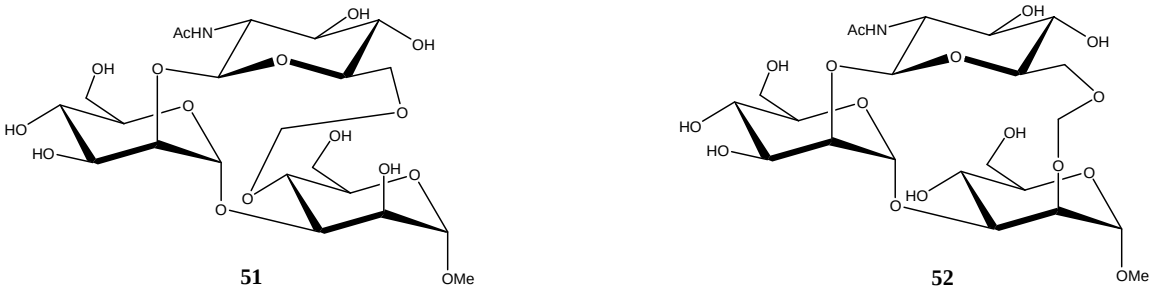
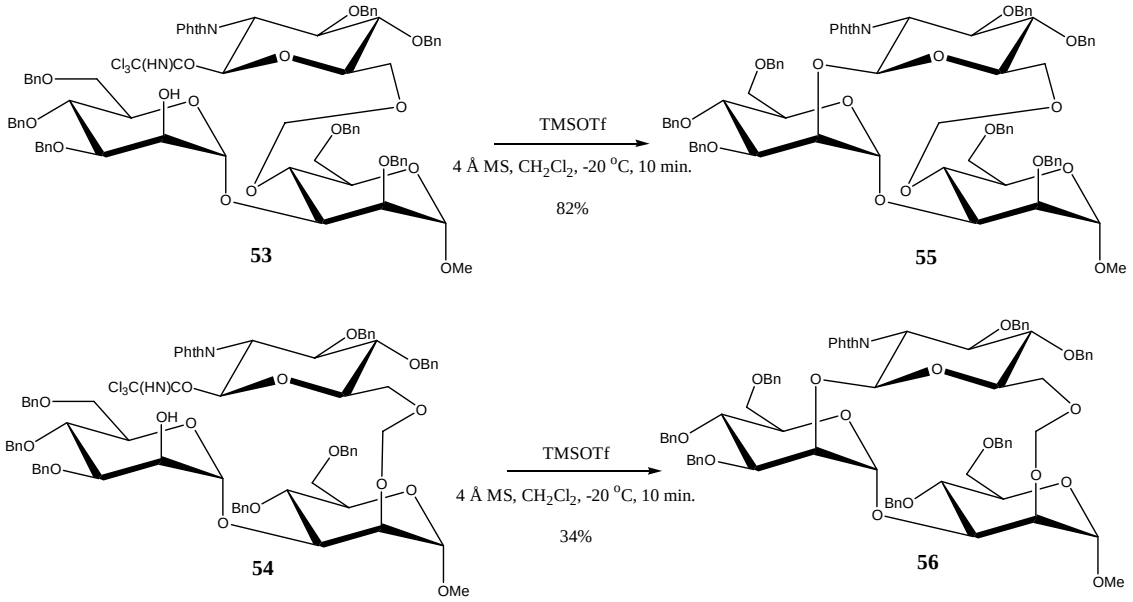


Figure 1.15

The synthesis of these constrained molecules (Scheme 1.3) was achieved by intramolecular glycosylation of methylene acetal linked linear trisaccharides **53** and **54** using trimethylsilyl trifluoromethanesulfonate as a promoter. These precursors were synthesized using standard protecting group interconversions and glycosylation strategies. The deprotection of cyclic molecules **55** and **56** was achieved by treatment with hydrazine to remove the phtalimido protecting group. An *N*-acetylation was then performed before catalytic hydrogenation to yield trisaccharides **51** and **52**.

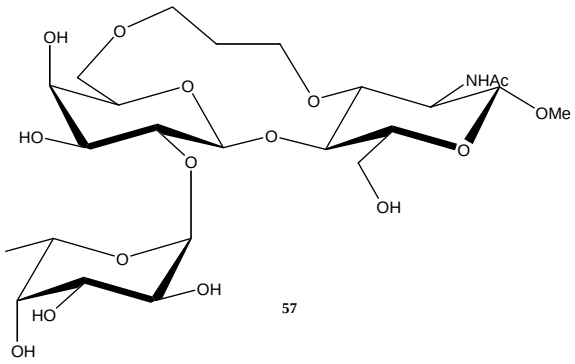


Scheme 1.3

Thermodynamic studies (ITC) were then conducted to evaluate the binding of **51** and **52** and ConA. As expected, the pre-organized compounds displayed a favorable entropic term of binding. However, this gain was surprisingly offset by an unfavorable enthalpic term, resulting in an overall similar free energy of binding for both the modified analogues and the natural linear trisaccharide. This enthalpic term remains largely unexplained and further studies on these compounds need to be performed to assess the origin of this enthalpy-entropy compensation. It is important to note that Quiocho and co-workers[46] recently identified similar enthalpy-entropy compensation in the complexation of a cyclic oligosaccharide with a maltodextrin-binding protein of *Escherichia coli*. Those sets of results contradict Bundle's observations that conformational constraints do not have a significant impact on the entropy and enthalpy of binding.

In another attempt to produce a high affinity carbohydrate ligand, Bundle and co-workers also reported the synthesis of conformationally constrained H-Type 2 blood group trisaccharide **57** (Figure 1.16).[47] A chemically modified analogue of Fuc-(1→2)-Gal*p*-(1→4)-Glc*N*Ac→OCH_3, where Gal O-6 and GlcNAc O-3 are linked via a three-carbon tether, was synthesized. Although computer modeling studies suggested that the trisaccharide could be tethered in bioactive conformation, this compound was tested against different lectins (*U europaeus* I and *P tetragonolobus* II lectins) without any increase in affinity of binding. In fact, a difference of +0.6 kcal/mol was observed for compound **57** compared to its natural counterpart. This example further confirms that the implications of ligand flexibility in thermodynamic parameters of oligosaccharide-protein interactions remain largely undetermined. It is also not clear whether or not pre-

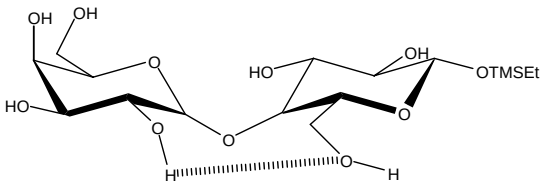
organization will lead to ligands with higher affinities of binding. New studies using a variety of conformational constraints will provide additional information required to fully understand these phenomena.



57

Figure 1.16

With the aim to enhance the resistance of galabiose (**58**) towards enzymatic hydrolysis, Wilsterman *et al*. used a methylene acetal bridge in compound **59** to replace an internal hydrogen bond between the 6- and 2'- hydroxyl groups of galabiose (Figure 1.17).[48]



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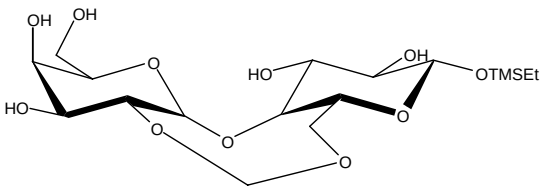
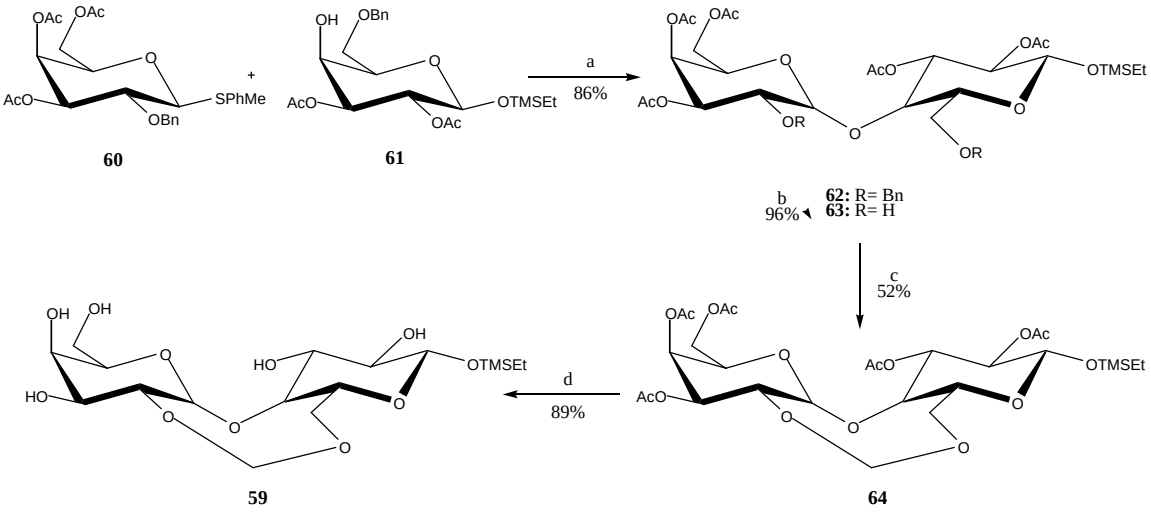


59

Figure 1.17

The synthesis of disaccharide **59** is depicted in Scheme 1.4. A glycosylation was performed in acetonitrile, using glycosyl donor **60** and acceptor **61** with NIS and triflic acid as promoters, to yield disaccharide **62**. Hydrogenolysis of the latter gave diol **63**, which was then used for methylidenation. Reaction of **63** with formaldehyde diphenylmercaptal, NIS, and triflic acid provided cyclic compound **64**, which was deprotected to yield the target compound **59**.



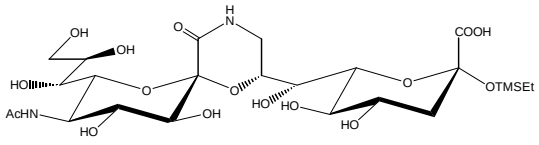
a) NIS, TfOH, MeCN. b) H_2, Pd/C, AcOH. c) $(PhS)_2CH_2$, NIS, TfOH, MeCN, CH_2Cl_2, -35 ^oC. d) NaOMe, MeOH.

Scheme 1.4

The affinity of binding of both parent and modified disaccharides were tested against three different bacterial proteins known to use galabiose-containing glycolipids as attachment points for the infectious process: the *Escherichia Coli* pilus protein PapG in complex with its chaperone PapD, Verotoxin, produced by enterotoxic *E. Coli*, and the *Streptococcus suis* bacterium. Both proteins, PapG/PapD complex and Verotoxin did not recognize modified disaccharide **59** because their binding specificity require 2-OH and

6’-OH free. However, 6’,2- methylene galabiose **59** showed reduced (compared to galabiose), but still significant binding affinity for the *Suis* bacteria. This difference in binding affinity was attributed to a slightly altered conformation caused by a different positioning of the key hydroxyl groups involved in the binding recognition.

Magnusson *et al.* synthesized the bis(sialic acid) 8,9-lactam **65** and compared its conformational properties with corresponding natural lactone **66** (Figure 1.18).[49]



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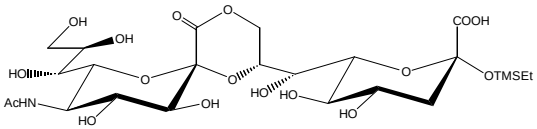


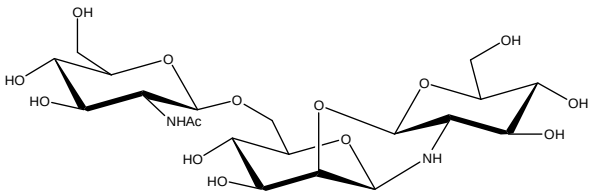
66

Figure 1.18

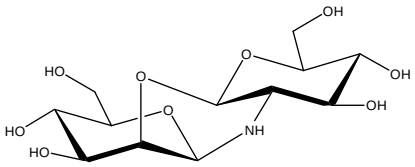
The 8,9-lactam **65** was found to adopt a conformation very similar to natural compound lactone **66**. This finding proves especially interesting since analogue **65** of the lactone **66** is considered to be more stable toward enzymatic hydrolysis. This observation becomes of prime importance since δ-lactones such as compound **66** are thought to be immunogens in the preparation of antiganglioside antibodies that would result in immunization against cancer. However, the hydrolytic labilities of these lactones reduce dramatically their potential as cancer vaccines.

As a final example, Goddat and co-workers reported the synthesis of two oligosaccharides with intramolecular NH-glycosidic linkage.[50] Trisaccharide **67** (Figure 1.19) was accidentally obtained when deprotecting a phtalimido protecting group using hydrazine hydrate in ethanol at 80 ^{o}C. Although unexpected, this result proved to be

interesting in terms of conformational restriction and disaccharide **68** was synthesized using the same chemistry.



67



68

Figure 1.19

1.4 Investigating Carbohydrate-Protein Interactions

1.4.1 Concanavalin A

Lectins belong to a class of proteins that bind carbohydrates but are devoid of catalytic activity. They are found in almost all living organisms without being confined to specific organs or tissues. Investigations of lectin-carbohydrate complexes are providing valuable information on the precise molecular mechanism of protein-carbohydrate interactions. Lectins serve then as an invaluable tool in biological and medical research and find many applications in areas as diverse as separation and characterization of glycoproteins and glycopeptides, histochemistry of cells and tissues, cell differentiation and tracing neuronal pathways.[51]

Concanavalin A (Con A) is a saccharide-binding lectin that was isolated from the jack bean in 1919[52] and was crystallized in 1936[53] by Sumner. Although the biological role of Con A in nature is unknown,[54] its specific saccharide binding properties make it

an ideal object for studying protein-carbohydrate interactions.[55,56] Con A, together with other related legume lectins, became a tool of choice in various domains of research, and even more so when studies targeted saccharide recognition. All legume lectins share high sequence homology that confer them similar tertiary structures.[51,57-59] Their quaternary structures however are not identical. In solution Con A adopts two different quaternary structures depending on the *p*H of the solution. This protein forms a dimer at *p*Hs ranging between 5.0 and 5.6, while two dimers associate to form a tetramer at *p*H 7 and above.[54,56] X-ray structures 1.2 Å, [55] then refined to 0.94 Å,[60] recently confirmed previously reported structures.[61-63] Each identical monomeric unit is composed of a polypeptide chain of 237 amino acids with a molecular weight of 25 kDa.[64] This subunit has been reported to be in the shape of a dome or gumdrop, approximately 42 Å-high, 40 Å-wide and 30 Å-thick. The monomeric subunit consists of three anti-parallel β-sheets: a 12-stranded β-sheet forms the back of the unit, a 7-stranded β-sheet passes through its center and finally, a 5-stranded β-sheet holds the two others together (Figure 1.20).[54-56,62,63] 50% of the amino acids of a subunit are involved in this type of structures, the others have been located in loops and/or turns joining these β-strands. Each monomer has a saccharide-binding site situated in a shallow pocket at the surface of the protein. An important feature of each subunit is the presence of two cation binding sites, each accommodating a calcium cation (Ca^{2+}) and a transition metal manganese cation (Mn^{2+}).[55,62,65,66]

Figure 1.20

A schematic representation of the quaternary structure mentioned above is given in Figure 1.21.[54,62] The two dimers consist of subunits A and B on the one side, and subunits C and D on the other. The stability of these structures is secured by hydrogen bonds bridging water molecules and van der Waals contacts. The tetramer is formed through interactions between subunits A and C and subunits B and D. There are no contacts between subunits A and D, or subunits B and C.

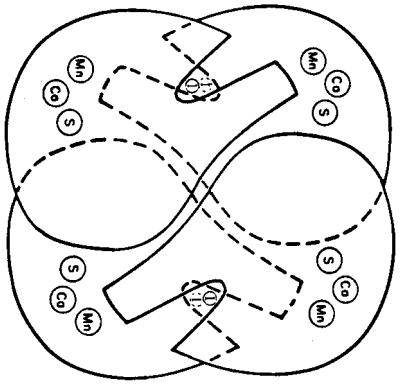


Figure 1.21

One of the characteristics of legume lectins is that their ability to bind saccharides is related to the two cations, Ca^{2+} and Mn^{2+}.[62] This property is due to the geometry of the binding site and is illustrated in Figure 1.22, where methyl α-mannopyranoside is depicted when binding with ConA.[62] In such binding events, the calcium-ion binds with three of the four loops forming the binding site. The absence of calcium would most likely cause one, or even several of the loops to move, which would in turn prevent carbohydrate binding activity. Mn^{2+} is involved in the stabilization of the saccharide binding site. However, due to indirect contact with the calcium binding site, Mn^{2+} role is less critical than Ca^{2+}.

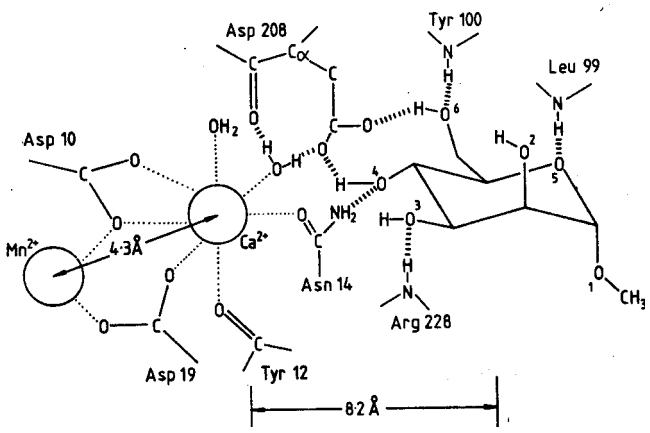


Figure 1.22

Con A was first described as α-glucoside and α-mannoside specific,[17] however, methyl α-mannoside has proven to be a better ligand.[18] Extensive studies about the binding specificity of Con-A were conducted. It was demonstrated that OH-3, OH-4 and OH-6 formed key polar interactions with carbohydrates. The region defined by O3, O4, C6, and O6 was also revealed to be subject to extensive van der Waals contacts.[62]

Several disaccharide, trisaccharide and oligosaccharide complexes were identified, provided that a α-mannoside was present in the structure.[45,67-69] As an example, the first crystal structure of a trimannoside, the methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyrannoside was determined (Figure 1.23).[69] The α-mannoside is buried deeply in the binding site and the other sugars interact with peripheral protein side chains. The presence of additional hydroxyl groups, which were able to create favorable contacts at the periphery of the protein, was reported to enhance the binding affinity of the protein.

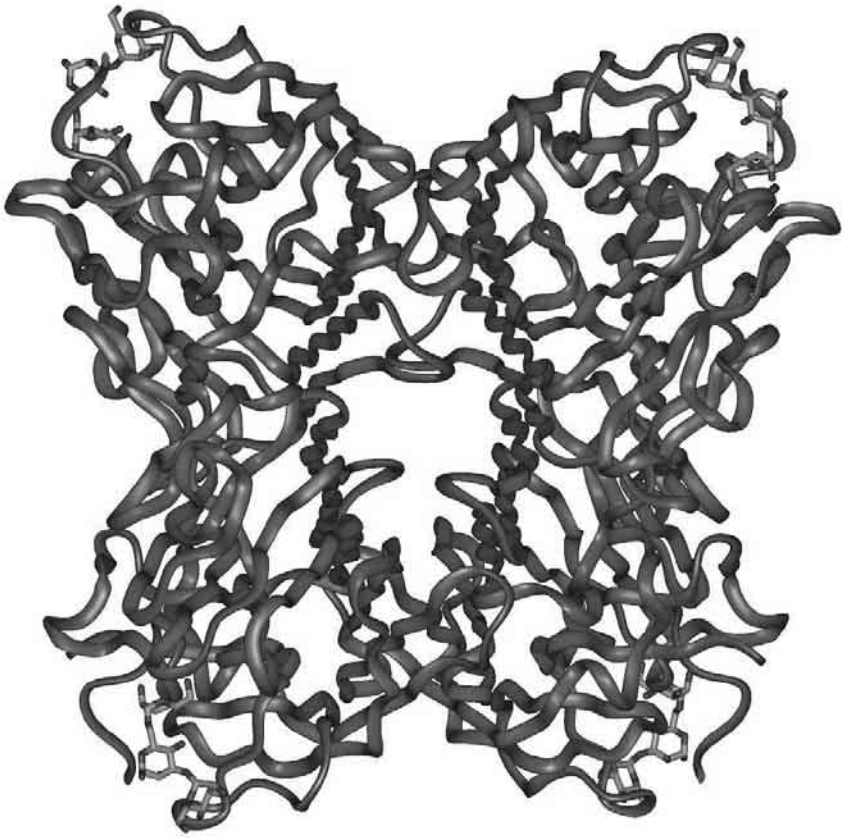


Figure 1.23

1.4.2 Nuclear Magnetic Resonance (NMR)

To understand the molecular basis of oligosaccharide-mediated recognition phenomena, some knowledge of the structure of free carbohydrate ligands and ligand-receptor complexes is needed. Since most carbohydrates fail to crystallize, which prevent the use of X-ray crystallography, Nuclear Magnetic Resonance (NMR) is a good alternative to study their structure.

The first part of a structural study by NMR consists in the assignment of the oligosaccharide spectrum. To do so, chemists and biochemists can nowadays rely on a wide range of experiments. However, it is still not an easy task because carbohydrate NMR spectra become very rapidly complicated by a lot of signal overlapping. Simple carbohydrates like monosaccharides can be assigned from 1D experiments such as ^{1}H and ^{13}C. Because of their relation to the chemical environment, the proton and carbon chemical shifts provide a lot of useful data. The deshielding effect of an acetate moiety on a geminal proton is a good illustration of the type of data obtained by NMR. The carbohydrate chemist often uses this characteristic to check the stereoselectivity of a given reaction. Three-bond coupling constants ($^{3}J_{nn}$)[29] provide another useful type of information. Karplus showed that an empirical relationship of the form $^{3}J_{nn} = A \cos 2\theta + B \sin 2\theta + C$ (where A, B and C are constants) can be used to derive angular information from three-bond coupling.[29] Table 1.1 shows typical coupling constants for common monosaccharides. In most cases, the anomeric selectivity can be determined from well-resolved signals. Signals corresponding to β-anomers typically show a coupling constant of 7 or 8 Hz, whereas small coupling constants of 1 to 4 Hz are characteristic of α-anomers. The splitting of the signals resulting from this three-bond coupling can also be

used to identify other saccharide protons. When analyzing more complex oligo-saccharides, the spectrum rapidly becomes crowded and at that point, other experiments, such as 2D, COSY, HSQC, and TOCSY, have to be considered to overcome signal overlapping. As for monosaccharides, important information can still be extracted from 1D experiments, but precise proton assignments require 2D experiments.

Table 1.1: Observed coupling constants (Hz) in aldohexopyranoses

Monosaccharide	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
α-D-Glc-(1→	3.8	10.0	9.1	9.9	5.1	2.1	12.1
β-D-Glc-(1→	8.0	9.4	8.9	9.7	5.8	2.2	12.3
α-D-Man-(1→	1.8	3.8	10.0	9.8	5.7	2.0	12.7
β-D-Man-(1→	1.5	3.8	10.0	9.8	5.0	2.0	10.0
α-D-Gal-(1→	3.6	10.0	3.4	0.8	6.4	6.4	12.3
β-D-Gal-(1→	7.9	9.9	3.5	1.1	7.9	4.3	11.7
β-D-GalNAc-(1→	8.2	10.4	8.5	9.5	6.2	2.3	12.0
α-D-GalNAc-(1→	4.1	11.3	3.1	1.2	7.0	5.4	11.7
α-D-Fuc-(1→	3.9	9.5	3.4	1.0	6.5	-	-

When a precise assignment of the 1D spectrum is available, conformational analysis can be attempted. Through-space connectivity (<5 Å) can be established using Nuclear Overhauser Effect (NOE), which ideally permits to determine three-dimensional structures. However, conformational analyses are complicated by several factors: in

addition to spectral overlap, small NOE magnitudes tend to fade away rapidly because of an $1/r^6$ dependence on the distance between sender and receiver protons. The number of interresidual contacts in oligosaccharides is also generally very small. Another complication arises from the fact that oligosaccharides exist in dynamic equilibria involving many conformers. The resulting rapid conformational exchange occurring on the NMR timescale can reveal a virtual statistical conformation deducted from NOE data.[70] To overcome this problem, this type of experiment is often used in conjunction with energetic calculation by computer modeling simulations.

Long-range three bond couplings $^3J_{CH}$ are also used to probe carbohydrate glycosidic linkage conformations. Coupling constant $^3J_{CH}$ between the anomeric proton and the aglyconic carbon on the one hand, and the anomeric carbon and the aglyconic proton on the other hand, provides information on ϕ and ψ respectively via the appropriate Karplus relationship.[30,71] These couplings cannot always be measured in oligosaccharides because ^{13}C is naturally abundant at 1%, which requires samples of several milligrams to accommodate the method sensitivity.

Recent advances in technology have promoted new NMR techniques using ^{13}C-enriched material, enabling ^{13}C-^{13}C coupling constants to be used for conformational analysis purposes.[29] Homo- and hetero-nuclear *trans*-glycosidic $^3J_{HCOC}$, $^3J_{CCOC}$, and $^2J_{COC}$ coupling constants can provide direct measures of glycosidic dihedral angles ϕ and ψ.

Although sometimes complicated, NMR spectroscopy proved useful to determine structural aspects of oligosaccharides in solution state. It also found an application in the study of the bound state based on the transfer NOE (TR-NOE) effect.[11,72] The TR-NOE

is a regular NOESY experiment, but it is applied to a protein-ligand system in a dynamic system where the ligand is present in excess. In complexes involving large molecules, cross relaxation rates of the bound compound are of opposite sign to those of the free compound, which results in negative NOEs. Binding activity can then be easily deducted by visual inspection, since NOEs for small molecules are positive.

Although a lot of information can be generated by NMR spectroscopy, it often proves insufficient to fully describe conformational properties of oligosaccharides in solution and bound states. To this end, NMR spectroscopy is often associated with computer modeling simulations to obtain a complete description of all carbohydrate-protein interaction phenomena.

1.4.3 Computer Modeling

As emphasized before, understanding conformational properties of carbohydrates in solution and upon binding are of prime importance when studying the molecular basis of saccharide recognition. X-ray crystallography and NMR spectroscopy are commonly used to determine conformations of saccharides but both techniques present major drawbacks. X-ray crystal structures are rare because carbohydrates and protein generally fail to crystallize. As discussed above, NMR is a valuable tool but its use is limited by problems arising from the conformational flexibility of carbohydrates. In that context, computational chemistry proved ideal to complement NMR and X-ray crystallography-based studies.[27]

In the last decade, computational chemistry became more and more popular in the field of protein-carbohydrate interactions because of the ever-ending development of

hardware and software. Increasingly complex calculations could be performed in a relatively short time. Depending on the type of system studied, simulations using physical concepts such as quantum mechanics, molecular mechanics and molecular dynamics can be applied. The last two concepts are usually the preferred options when calculations have to be performed on oligosaccharides. Molecular mechanics and molecular dynamics are indeed more suited for macromolecules, such as proteins and DNA, compared with quantum mechanics based *ab initio* methods that give the best results when applied to small systems.[73]

Molecular mechanics simulations have been developed to study the conformational properties of oligosaccharides in solution and upon binding. This method is based on the calculation of the difference of energy from one conformation to another. Molecular mechanics simulations are based on the assumption that the penalty associated with a particular motion, like the stretching of a carbon-carbon single bond, will be the same for every molecule. Molecular mechanics simulations use a series of simple equations, known as force fields, to calculate the energy of a compound.[73] Simple calculations can then be applied to very large molecular systems. In molecular mechanics simulations, a grid search is carried out. In a carbohydrate, the two bonds involved in glycosidic linkage are defined as torsional angles, while the sugar rings are considered fixed. Each torsional angle is rotated in 10^{o} increments and the energy of each obtained conformation is calculated after minimization steps before the next rotation.[27,28,74,75] The final results of these calculations are three or two-dimensional maps known as adiabatic or relaxed conformational energy maps. After energy cut-offs have been applied, an energy level is plotted according to ϕ and ψ values for each

glycosidic linkage. Minimum energy conformations and occupied conformational space are typical information obtained from molecular mechanic simulations.

Several software packages were developed to model a wide range of systems. As an example, force fields such as AMBER,[76] CHARMM[77,78] and the MM series[79-82] are commonly used to model proteins and nucleic acids. Protein-carbohydrate interactions can be simulated by using an additional carbohydrate-specific parameterization.[27] As an example, Imberty *et al*. realized the modeling in solution and upon binding of different saccharides with proteins such as lectins Con A or LOLI.[74,75] Using their own set of parameters, they were able to model the interactions between α-mannoside and ConA, which further validated the credibility of their technique against X-ray and NMR. Their studies also revealed that large oligosaccharides could be modeled in solution and upon binding by studying the complexes of biantennary glycans with lentil lectin.[83] Finally, they demonstrated that modeling could be used even in instances when no protein crystal structure was available. For example, the *Ulex europaeus* lectin I was modeled based on other lectins X-ray data, and that before studying its interactions with fucose.[84]

Although there is a good correlation between experimental and modeled data, molecular mechanics simulations have some limitations. Molecular dynamics simulations introduce a “time” factor enabling the determination of the motional properties of a molecule. Molecular dynamics calculations describe then the motional behavior of a system of atoms at a certain temperature, using differential equations of motions in the context of a force field. The alterations of location by molecular motions, known as trajectories, can be visualized as a series of snapshots of a molecule after a

specific period of time. One advantage of these simulations is the better parameterization of the solvent molecules.[85] The role of the solvent is directly taken into account because simulation can be conducted in a box of water.[85,86] The drawbacks of this method is that it requires much more computer time utilization and a lot of parameters have to be manually entered, making the use of molecular dynamics softwares less user-friendly than molecular mechanics.[73] However, combinations of the molecular mechanics and dynamics simulations can provide valuable insights on the positioning of the low-energy conformations in the ϕ, ψ-maps and the frequency of transitions between such conformations.[71]

Woods and co-workers[87] were able to demonstrate the strength of molecular dynamics simulations by computing relative energies of binding of antibody-carbohydrate complexes. They modeled the interactions of several trisaccharides with a fragment of the monoclonal anti-salmonella antibody Se 155.4. NMR, X-ray and thermodynamic data further corroborated their results.

1.4.4 Isothermal Titration Calorimetry (ITC)

ITC has long been recognized as a useful tool to evaluate binding constants. In the early 1990s, the appearance of commercially available titration microcalorimeters has generalized thermodynamic studies of protein-carbohydrate interactions. This technique measures the heat liberated by the gradual addition of a ligand aliquot in a cell containing a protein and in equilibrium with a reference cell. The enthalpy resulting from each injection is then determined and integration over time yields the plot of enthalpy per injection versus ligand concentration. Using a mathematical treatment, a binding curve is

obtained from this graph, and data such as stoichiometry of binding, binding constant (K_{eq}) and binding enthalpy (ΔH) are determined. The free energy of binding (ΔG) (Equation 1.1) can then be calculated by using the above experimental data.

$$\Delta G = -RT \ln K_{eq} \qquad \textbf{Equation 1.1}$$

The entropy of binding can then be determined by subtraction of ΔH from ΔG. Calculations also lead to the constant pressure heat capacity ΔC_p ($\Delta\Delta H/T$).

Titration microcalorimetry can determine thermodynamic parameters for systems with binding constants ranging from 10^3 to 10^7 M^{-1}. Low affinity protein-carbohydrate interactions, whose binding constants typically range in millimolars, can therefore be studied. However, several limiting factors have to be considered. The binding constant determines required levels of both sugar and protein concentration. Millimolar binding constants demand high protein and sugar concentrations, which may be a concern for the protein solubility. The availability of the studied oligosaccharide constitutes also a limiting factor since several milligrams are required to conduct a single ITC experiment.

Over the years, several ITC applications have been developed. Studies concerning the specificity and the binding site of a protein can be supported by ITC, since free energy of binding can be evaluated using this method.[88,89] The same technique has been used to explain the factors involved in the thermodynamics of binding. As a first example, ITC allows the quantification of the impact of solvent reorganization on the thermodynamics of binding.[4,90,91] The differential in enthalpy of binding in light and heavy water, called the thermodynamic solvent isotope effect, was shown to be a direct measurement of water molecules rearrangement upon binding.[91] A second example is

found in the estimation of solvation entropy. $\Delta C_{p,}$ obtained from previous experimental data, allows the calculation of the entropy of solvation, using the following equation:[4]

$$\Delta S_{sol} = \Delta S^{*}_{sol} + \Delta C_p(\ln T/T^{*})$$

T is the temperature at which the entropy of binding was measured and T* is the temperature at which all solvation-associated entropies approach zero. ΔS^{*}_{sol} includes entropy contributions from proton transfer and electrostatic effects; for most protein-carbohydrate interactions, ΔS^{*}_{sol} is negligible. The quantification of the fraction arising from solvation of the entropy of binding can then be determined. Depending on the design of the experiment, ITC can be used to evaluate additional data. For example, Mandal *et al.* used this technique to assess the role of conserved water molecules.[90] During the last decade, ITC proved to be a major tool in the study of carbohydrate recognition. However, the intrinsic lack of motion of the system and its limitations suggest the use of other analysis techniques to complement the assessment of studied phenomena.

1.4.5 Biacore

Biacore is a technique that has become a standard method in studying biomolecular interactions since its commercial introduction in 1990. This system was designed to study the kinetics of interaction between biomolecules,[92,93] and was naturally applied to the field of protein-carbohydrate interactions.[94-98] The detection principle of Biacore relies on the physical phenomenon known as Surface Plasmon Resonance (SPR).[99] The binding of one partner to another immobilized on the matrix

side of a flat sensor chip results in a change in refractive index, which is detected in real-time and indicated immediately. The operating system of Biacore is depicted in Figure 1.24.

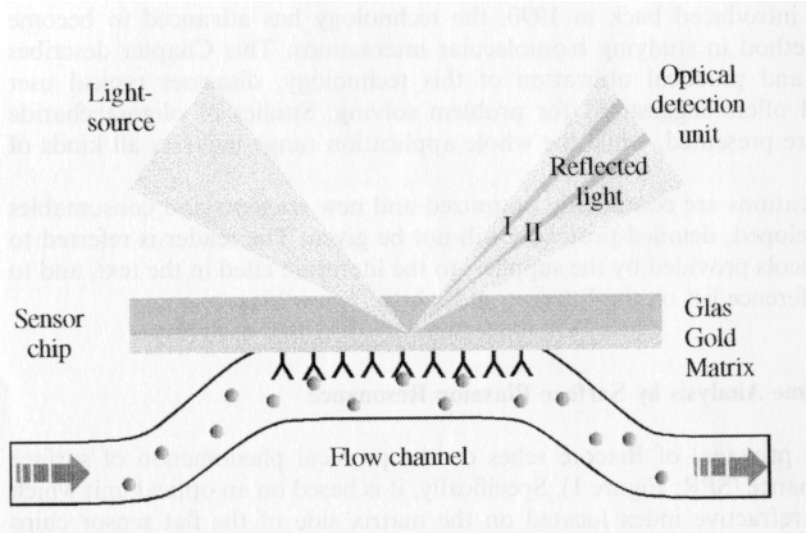


Figure 1.24:[99] The sensor chip is composed of a glass carrier coated with a thin gold layer on which a biocompatible matrix is attached. An evanescent field is generated when light under the condition of total internal reflection is directed to the glass side. This field extends about 300 nm into the solution on the matrix side and interacts with the refractive index of the solution close to the gold surface. The electromagnetic phenomenon of surface plasmon resonance (SPR) arises in the gold film, resulting in the extinction of the reflected light at a specific angle. The angle of minimum reflected intensity (the resonance angle) varies with refractive index on the matrix side. Changes the resonance angle are directly proportional to changes in mass concentration due to binding or dissociation of biomolecules. The signal is presented in resonance units (RU), where 1 RU corresponds approximately to 0.8 pg carbohydrate or 1 pg protein bound per mm^2.

Depending on the design of the experiment, the dynamics of an interaction, characterized by the speed of complex building and decay, are reflected by the constant of association (K_{ass}) and dissociation (K_{diss}). The raw data is then processed using a software package from Biacore Inc. and the above constants can be determined as a result of this operation. The affinity of binding can also be determined using Biacore: different concentrations of ligands are used until saturation is reached.[99] The ligand concentration leading to 50% surface saturation corresponds to affinity dissociation constant (K_D).

Comparison of kinetics data and affinity (K_D=K_{diss}/K_{ass}) ensures the consistency of obtained results.

Studying biomolecule interactions by Biacore is interesting for several reasons. First, the chip surface mimics the cell surface. It also reflects the in vivo environment and its dynamic flow, which is thought to play a role in the interactions between macromolecules.[99-101] Biacore, for which a dynamic flow is part of the experimental conditions, may be a better mimic of the physiological conditions than an assay free in solution. Unlike ITC, Biacore experiments only require small quantities of the studied compounds to perform a set of analyses. Finally, another advantage of this technique is its user-friendliness and, provided a signal can be obtained upon injection, the experiment can be conducted in a much shorter time compared to other bioassays. Screening for biological activity of large libraries of new biomolecules can thus be completed in a relatively short period of time.[102]

Biacore now has several applications in the field of protein-carbohydrate binding, mainly because kinetics is thought to play a key role in such interactions. Different types of Biacore experiments have been reported. The general protocol to study small carbohydrates is to attach the sugar to the matrix chip and to dissolve the protein in the running buffer.[95,96,98] In most cases, they are immobilized on the solid phase by biotinylation at the reducing end of the carbohydrate.[98] In the case of glycoproteins, the receptor protein is attached to the chip.[97,103] Another alternative is to carry out competitive binding assays: both the sugar and the protein are equilibrated in the buffer and injected onto the matrix, where one is recognized in a competitive manner after dissociation of the former complex.[94,104]

1.5 Research Background

For the last three decades, it has become clear that carbohydrate-protein interactions were involved in cell recognition phenomena. In that context, research focused on understanding the molecular basis of interactions between protein receptors and carbohydrate ligands. While the interpretation of highly refined X-ray crystal structures has revealed important features, other implications of those complex interactions remain largely undetermined. Among those, the role of carbohydrate flexibility and the conformation adopted upon binding – and the impact of those two factors on binding affinity – seem to be of crucial importance in the field of glycobiology.

Bourne and co-workers[105,106] have provided valuable information on those last two points in their study on the crystal structure of a biantennary octasaccharide - *Lathyrus ochrus* isolectin I (LOL1) complex refined at 2.3 Å resolution level. The octasaccharide (Figure 1.25) is a glycan derived from the main N-acetyllactosaminic type biantennary glycan found in the human lactotransferrin.

The study of the sugar-protein complex revealed that specificity arose from both hydrogen bonding and van der Waals interactions, as typically encountered in protein-carbohydrate interactions. The complex is stabilized by 23 hydrogen bonds, 14 of which occur directly between the saccharide and the protein, and seven are mediated by water. In addition, 14 water molecules interact indirectly to link the octasaccharide to the lectin or to itself. 68 van der Waals contacts, 27 of which involve aromatic residues, further stabilize the complex. The tightest saccharide-protein interaction occurs with Man 3 (Figure 1.25) in a pocket located near the Ca^{2+} cation. This pocket has clearly been identified as the monosaccharide binding site.

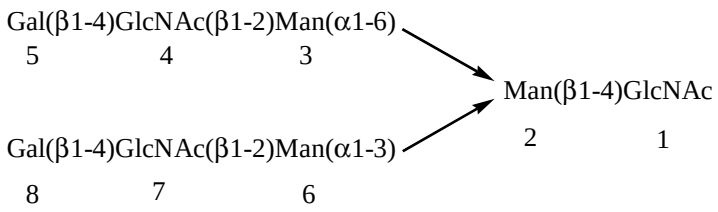
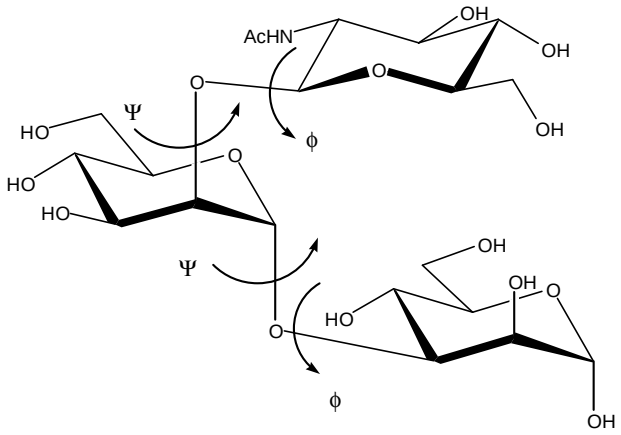


Figure 1.25

The most interesting observation is that the conformation adopted by the oligosaccharide, where five of the seven ϕ-ψ angles fall into main minima on calculated ϕ-ψ energy charts, is not a minimum energy conformation. This binding conformation is indeed a local minimum energy conformation, where the junction Man-α-(1-3) is located on a pass between energy minima.

Based on this observation, Imberty and co-workers[107] realized the computer modeling study of mannose-containing trisaccharide β-D-GlcNAc*p*-(1-2)-α-D-Man*p*-(1-3)-D-Man (Figure 1.26), bound to lectins LOL1 and Con A. This trisaccharide is a fragment of the previously mentioned biantennary octasaccharide and the obtained results were analyzed in the light of its X-ray crystallographic data.



69

Figure 1.26

Conformation analyses were conducted on trisaccharide **69** after central mannose had been buried in the binding site of LOL1 and ConA. 237 possible conformations were retained within an energy window of up to 12 kcal/mol above the absolute energy minimum. These conformations were subjected to family analysis and depending on orientations around glycosidic linkages, were classified in three different groups - LOL1-Min1, LOL1-Min2 and LOL1-Min3. Interconversions between families proved impossible, even with an energy cut off set at 20 kcal/mol. LOL1-Min1 is the conformation of minimum energy and differs from LOL1-Min2 because of a different orientation around β-D-GlcNAc*p*-(1-2)-α-D-Man*p* bond: ($-150^{o} < \phi < -130^{o}$, $\psi = 120^{o}$) for LOL1-Min1 and ($60^{o} < \phi < 70^{o}$, $150^{o} < \psi < 160^{o}$) for LOL1-Min2. In both cases, the number of conformations is much larger around α-D-Man*p*-(1-3)-D-Man linkage than β-D-GlcNAc*p*-(1-2)-α-D-Man*p*. A third family was identified around minimum LOL1-Min3, which is higher in energy. However, after full optimization, this minimum revealed an unexpectedly favorable energy and that was identical to the trisaccharide in the crystal

structure of the octasaccharide previously discussed. Optimized structures of the three complexes are shown in Figure 1.27. The trisaccharide has a tendency to adopt folded conformations with inter-residue hydrogen bonds. It was established that minimum LOL1-Min2 was stabilized by an internal hydrogen bond between OH-4 of the reducing end mannose and OH-6"of glucopyranoside. In a similar way, minimum LOL1-Min3 was found to be stabilized by an internal hydrogen bond between OH-2 of the reducing end mannose and OH-6"of the *N*-acetyl glucosamine.

Docking studies were also achieved on Con A, a lectin with high sequence homology but different saccharide specificity. Similar results were obtained: after the central mannose was buried in the binding site of Con A, 383 possible conformations were retained within an energy window of up to 12 kcal/mol above the minimum energy. Although the energy cut-off had to be lowered to 6 kcal/mol, it was possible to determine 3 families - Con A-Min1, Con A-Min2 and Con A-Min3. After full optimization, Con A-Min2 was found to have the lowest energy because of a strong intramolecular hydrogen bond resulting from a fold in the conformation. Optimized structures of the three complexes are shown in Figure 1.27.

It is generally accepted that the enthalpy of binding is determined by hydrogen bonds and van der Waals contacts, whereas entropy terms are the result of water molecule rearrangement and loss of flexibility of the carbohydrate ligand during the process of binding. In order to assess the contribution of the loss of flexibility upon binding, the entropy of binding was calculated for both complexes between trisaccharide **69** and LOL1 and Con A. It was shown that the entropy barrier did not play a role in the binding of the α-D-Man*p*-(1-3)-D-Man linkage. However, in the case of β-D-GlcNAc*p*-

(1-2)-α-D-Man*p* glycosidic linkage, a completely different thermodynamic behavior was observed and the entropic contribution was estimated to 2-4 kcal/mol (T = 298 K) in TΔS. This result can be interpreted as follows: the α-D-Man*p*-(1-3)-D-Man linkage hardly loses any conformational freedom during binding, whereas a considerable amount of conformational freedom is lost in the case of β-D-GlcNAcp-(1-2)-α-D-Manp linkage. Although similar trends were reported for both lectins, the sugar/LOL1 complex showed a higher entropy barrier than the sugar/ConA complex.

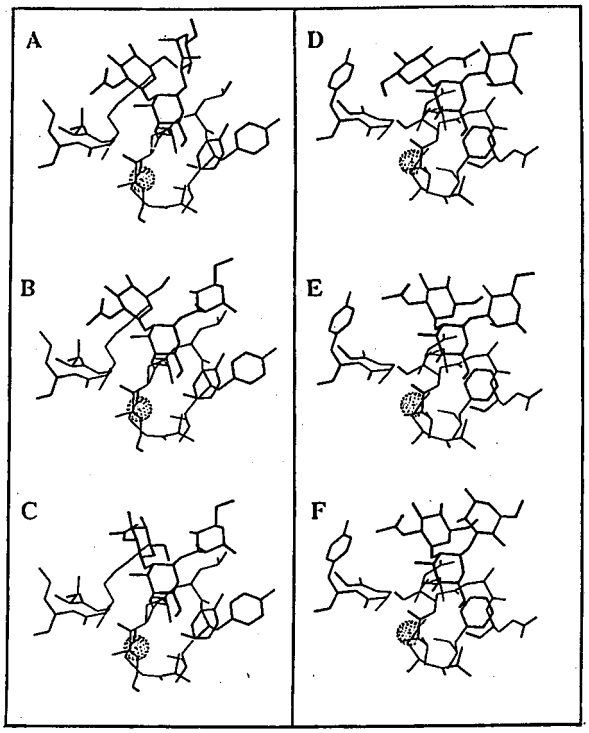


Figure 1.27: Representation of the lowest energy conformation of each family of β-D-GlcNAc*p*-(1-2)-α-D-Man*p*-(1-3)-D-Man trisaccharide interacting with lectins after full optimization. (A) Con A-Min1, (B) Con A-Min2, (C) Con A-Min3, (D) LOL1-Min1, (E) LOL1-Min2, (F) LOL1-Min3.

Based on these findings, Boons and co-workers[45] designed and synthesized two conformationally constrained trisaccharides **51** and **52** (Figure 1.28), where the internal

hydrogen bonds encountered in complex LOLI-Min2 and LOLI-Min3 or Con A-Min2 were replaced by methylene acetals (Figure 1.28). It was anticipated that the complexation entropy barrier of the conformationally constrained compound with Con A would be smaller because such compounds have shown to lose less conformational flexibility during binding. The interactions of both trisaccharides with Con A were studied using microcalorimetry (ITC). As expected, a more favorable entropy term was observed but it was surprisingly offset by a loss in enthalpy, which resulted in a similar affinity of binding for all three compounds. The origin of this enthalpy-entropy compensation still remains to be explained. Possible options include computer modeling simulations, and/or design of new conformationally constrained oligosaccharides in various conformations and flexibility.

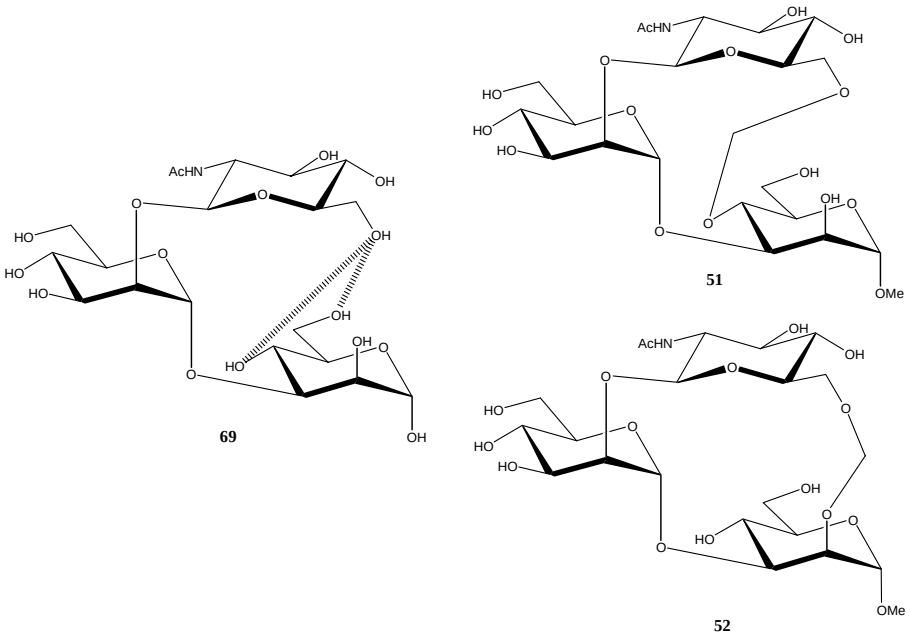


Figure 1.28

1.6 Research Project

The first part of my project focused on conducting computer modeling studies of Con A-trisaccharide **51** and Con A-trisaccharide **52** to better understand the enthalpy-entropy compensation that occurred upon binding. Starting from modeled structures in solution, a docking procedure in the binding site of Con A was realized. The obtained information concerning conformational behaviors and binding thermodynamics of those cyclic trisaccharides are presented in this dissertation. Computer modeling was also used to design new cyclic trisaccharides with characteristics such as preorganization toward a determined conformation or high rigidity.

Based on computer modeling studies, target compounds **70**, **71**, and **72** (Figure 1.29), were proposed. They are thought to be good candidates to study the role of conformational constraints in oligosaccharides and thus assess the flexibility implications in the phenomenon of saccharide recognition.

Trisaccharides **70** and **71** were designed to be preorganized towards specific conformations. Their synthesis was successful up to the linear trisaccharide stage, after which several cyclization steps failed to produce the final compounds. However, because of the implications on the rest of my research project, the successful part of the synthesis is discussed in this dissertation.

The design of trisaccharide **72** was based on the introduction of a carbamate bond to add a cyclic character to the molecule. It was anticipated that, compared to the methylene acetal bridge used for compounds **51** and **52,** an amide bond in the bridge would bring some rigidity to the structure. The challenging synthesis of **72** was achieved via two different routes. The cyclization was first carried out by intramolecular

glycosilation. However, the poor yield obtained for some of the synthesis steps lead us to rethink the initial strategy. The synthesis was achieved by intramolecular formation of the carbamate bond, which afforded much better yields. The synthesis of parent trisaccharide **73** is also presented, since this compound will be used as a reference when testing compound **72** for biological activity.

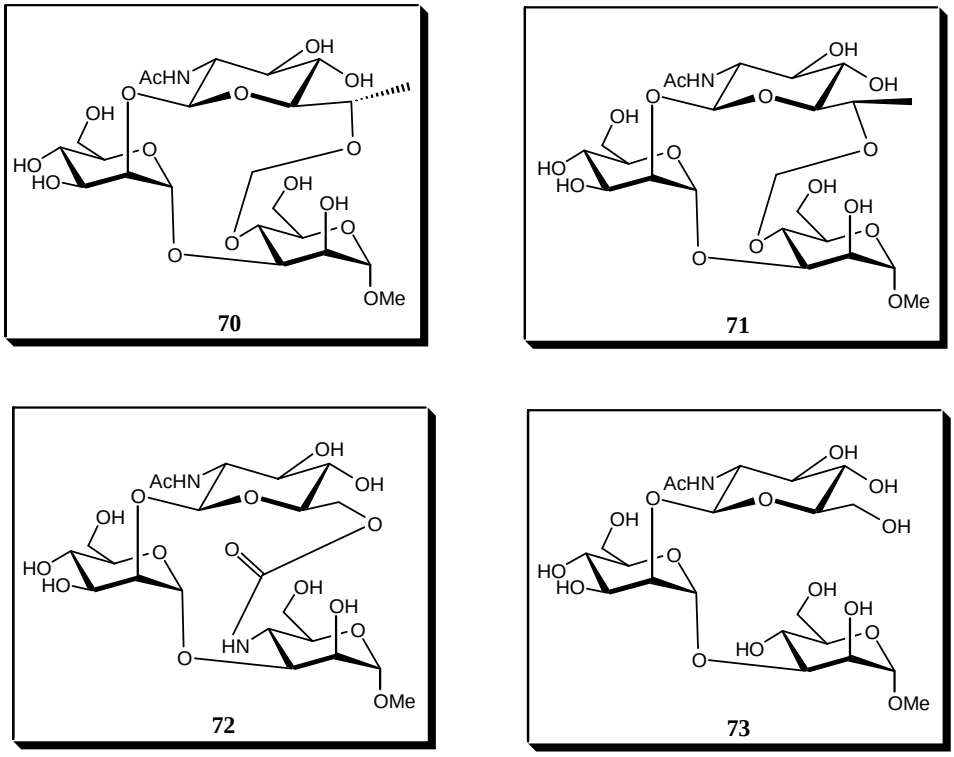


Figure 1.29

Target compounds **72** and **73** are actually studied for their complexation behavior with Con A, which should lead to the determination of valuable thermodynamic parameters. A conformational analysis by NMR spectroscopy will be performed in the near future. The results will be reported in a future publication.

In a second part of the project, two new trisaccharides **74** and **75** (Figure 1.30), which were specifically designed to study the kinetics of carbohydrate-protein interactions, were synthesized. The obtained trisaccharides will later be attached to a biosensor through an amino-propyl linker located at their reducing end. This linker will be used to study the kinetics of these two compounds with the Biacore technology. The successful synthesis of this "linker' building block follows the description of the synthesis of the two target molecules. These two new trisaccharides will later be tested on Biacore and results will be presented in a future publication.

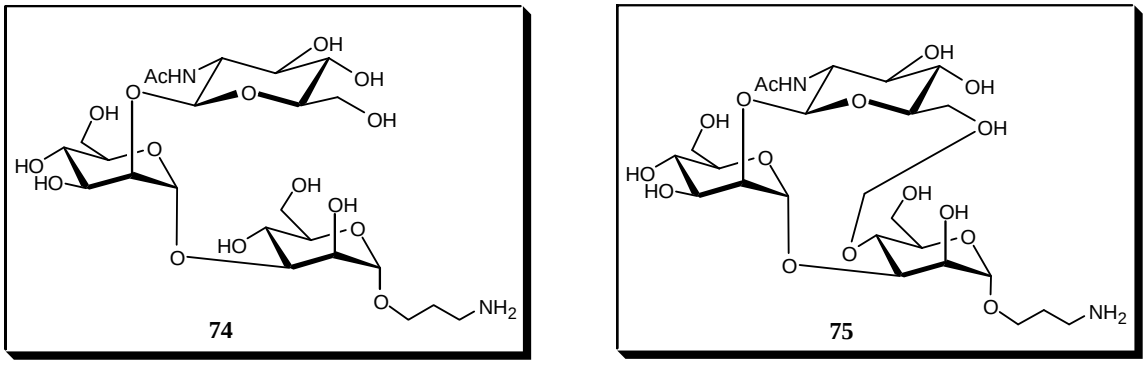


Figure 1.30

CHAPTER 2

RESULTS AND DISCUSSION

This section focuses first on computer modeling studies conducted on trisaccharides (2-acetimido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (**73**) and (2-acetimido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6”-methylidene acetal (**50**) (Figure 2.1). The main purpose of those studies was to understand the origin of the enthalpy-entropy compensation observed during the binding of the above oligosaccharides with ConA.

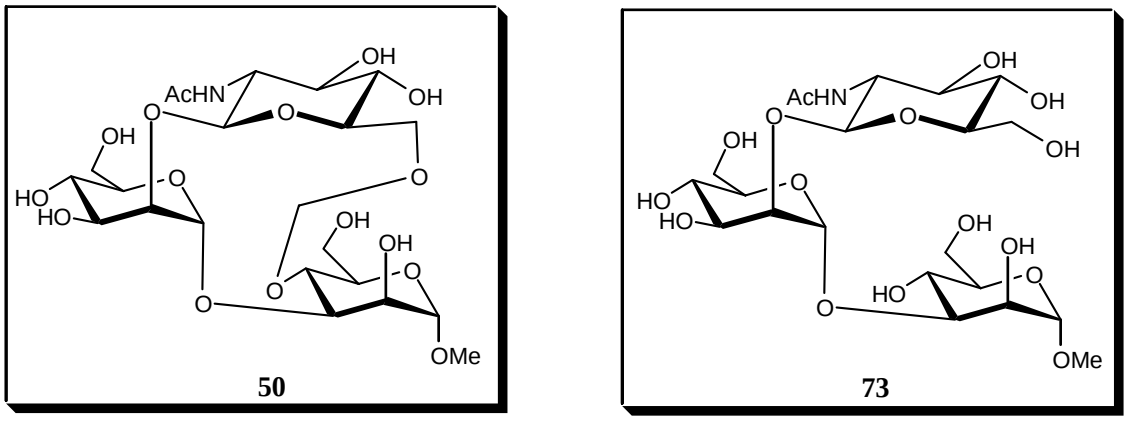


Figure 2.1

The design of new conformationally constrained trisaccharides **70**, **71** and **72** (Figure 2.2), also assisted by molecular modeling, will also be discussed in this section.

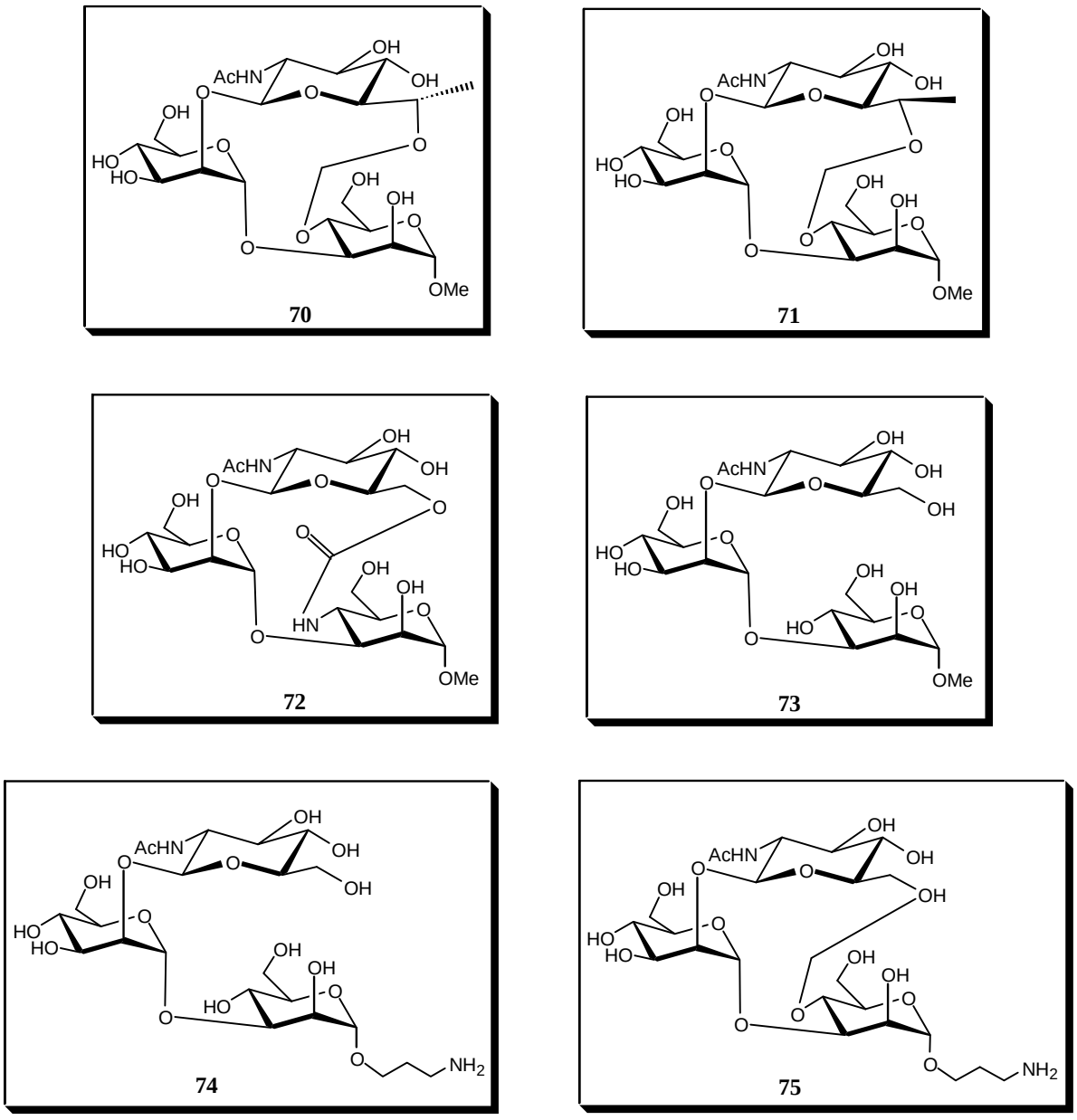


Figure 2.2

The second part of this chapter first describes and discusses the multi-step synthesis of trisaccharides **70**, **71, 72** and **73** (Figure 2.2). The synthesis of trisaccharides **74** and **75** (Figure 2.2), two related molecules with different applications, are then presented. For

each of these compounds, challenges, and where appropriate, solutions and/or alternative routes, are highlighted.

2.1 Molecular Modeling of (2-*N*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (**73**) and (2-*N*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6"-methylidene acetal (**50**) Interactions with Lectin Con A.

Cyclic trisaccharide (2-*N*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6"-methylidene acetal (**50**) (Figure 2.1) was synthesized and studied to assess the role of the conformational freedom of oligosaccharides in protein-carbohydrate interactions. This compound was designed to be a high affinity ligand. The loss of flexibility in its structure was supposed to introduce a favorable entropy term in the thermodynamics of binding, resulting in an overall higher affinity. Microcalorimetry titration (ITC) analyses of trisaccharide **50** in complex with Con A revealed, as anticipated, a gain in entropy, which was however offset by a loss in enthalpy and resulted in similar affinity of binding. To understand the enthalpy-entropy compensation occurring in this specific example, the binding of trisaccharide **50** and linear trisaccharide **73** (Figure 2.1) with Con A was studied by molecular modeling.

2.1.1 Molecular Modeling of Uncomplexed Trisaccharides

All computer modeling studies described below were carried out using the SYBYL software package, with the molecular mechanics Tripos force field adapted to carbohydrates (for method and procedure see Section 4.2).[27,74]

A four-dimensional systematic conformational search performed on torsional angles ϕ and ψ of βGlcNAc(1→2)αMan(1→3)αMan trisaccharide **73** identified 26712 possible conformations within a 10 kcal.mol^{-1} energy window. A further cut-off of 5 kcal.mol-1 resulted in 8798 conformations that were classified in seven families using the family analysis option of SYBYL. Each family was classified as Linear_XY, where X and Y corresponded to a range of torsional angles ϕ and ψ for glycosidic linkages βGlcNAc(1→2)αMan and Man(1→3)αMan respectively. Only the lowest energy conformation for each family was fully optimized. These low energy conformations are described in Table 2.1.

Table 2.1: Low energy conformations of linear trisaccharide **73** and structural characteristics (Torsion angle values in ° and relative energy value in kcal/mol).

Family	$\Phi_{1\text{-}2}$	Ψ_{1-2}	$\Phi_{1\text{-}3}$	Ψ_{1-3}	ΔE
Linear_AA	-50.7	-171.7	68.7	99.4	0.0
Linear_AB	-51.8	-174.5	81.8	-44.6	0.8
Linear_BC	44.4	143.7	164.6	174.5	1.6
Linear_BA	44.1	146.7	68.9	104.3	2.0
Linear_BB	46.4	149.5	92.5	-39.9	2.5
Linear_BD	56.7	159.1	133.6	66.2	3.3
Linear_DC	-104.0	71.4	152.9	160.1	3.6

The results were validated by comparison with experimental data. For example, the conformation Linear_AA matched the minimum energy conformation predicted by NMR spectroscopy. The two conformations Linear_BA and Linear_BB were found in the

crystal structures of N-glycans fragments when co-crystallized with LOL1, which further confirmed the validity of the above modeling technique.

The flexibility of the trisaccharide can be assessed when reporting the ϕ and ψ values for each conformation on energy maps corresponding to the studied linkages βGlcNAc(1→2)αMan and αMan(1→3)αMan (Figure 2.3). The occurrence of a conformation, whose torsion angles greatly differs (identified as B in our case) from the absolute minimum (identified as A) is characteristic of a high flexibility compound. Furthermore, comparing minimum energies for each family of conformations reveals very close energy barriers between conformations, resulting in the possible occurrence of very different conformation structures.

Dr. van Oijen achieved the conformational analysis of cyclic trisaccharide **50** and the results are discussed in appendix A. The obtained data was used to study the complex between compound **50** and Con A by computer modeling. 23 possible conformations were obtained using the same treatment and method as the linear trisaccharide. The identified conformations where classified in six families. Cyclic_AA, a similar conformation compared to the Linear_AA, was found to be the minimum energy conformation. The studies also revealed that the methylene bridge moved freely and that the constraints from cyclization did not force the molecule to adopt higher energy conformations. As depicted in Figure 2.3, both linkages βGlcNAc(1→2)αMan and Man(1→3)αMan could adopt several conformations. It was concluded that trisaccharide **50** was still fairly flexible, although a significant reduction in torsional freedom had been observed compared to the linear compound.

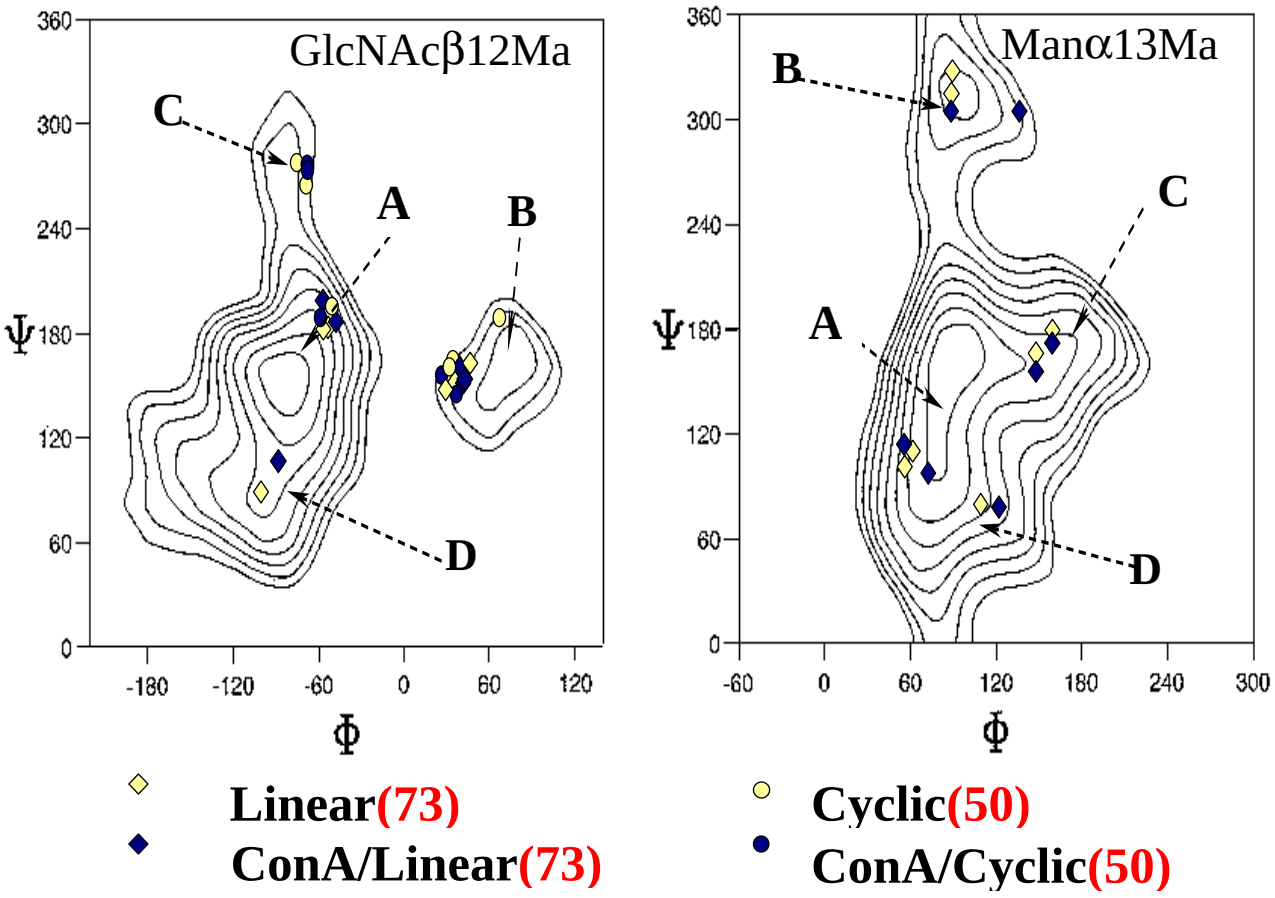


Figure 2.3

2.1.2 Molecular Modeling of Con A/ Trisaccharide Complexes

The minimum energy conformations for each of the seven families of the linear trisaccharide were docked in the Con A binding site using a protocol described by Imberty and co-workers (Cf section 4.2).[27,74] Except for Linear_BD, all conformation could be accommodated in the binding site without major changes in their geometries. Using a three-step minimization protocol, six complexes were obtained as depicted in Table 2.2. ConA-Linear_BA was identified as the lowest energy conformation. B

conformations were identified as preferred conformations for glycosidic linkage βGlcNAc(1→2)αMan, since the three lowest energy docking modes happened to have their same orientation. The conformation of this linkage impacts the orientation of the central mannoside in the binding site. When conformation B is adopted around βGlcNAc(1→2)αMan linkage, the central mannose is buried deeply into the binding site and the observed hydrogen bond pattern is similar to the ConA-αMan complex. When conformation A is adopted around the same linkage, the central mannose is not so deeply involved, which could be explained by steric interactions between GlcNAc and the protein surface. A third type of conformation was identified for βGlcNAc(1→2)αMan, the D conformations. In this conformation, the central mannose is not very deep, but many existing hydrogen bonds are preserved and additional ones are established between GlcNAc and protein side chains and backbone.

Table 2.2: Low energy conformations of linear trisaccharide I, when docked in the binding site of ConA (Torsion angle values in ° and relative energy value in kcal/mol).

Family	$\Phi_{1\text{-}2}$	$\Psi_{1\text{–}2}$	$\Phi_{1\text{-}3}$	$\Psi_{1\text{–}3}$	ΔE
ConA_linear_BA	52.2	151.7	71.0	102.3	0.0
ConA_linear_BC	51.5	150.5	161.7	166.1	2.6
ConA_linear_BB	54.3	146.5	139.3	-69.8	2.9
ConA_linear_AA	-55.8	-166.6	89.9	87.2	6.5
ConA_linear_AB	-52.6	-158.2	83.9	-68.6	6.8
ConA_linear_DC	-82.8	99.5	152.6	145.7	11.6
ConA_linear_BD	55.7	154.3	139.7	61.1	40.3

The 23 low energy conformations of cyclic trisaccharide **50** were used in a docking study with Con A. Using the same protocol, the central mannose residue was fitted in the binding site of the protein. The obtained complexes were optimized using specific energy parameters and 20 conformers were stored in an energy window of 15 kcal.mol^{-1}. The other conformers were rejected either because of their high energy or their convergence towards existing conformation. The remaining 20 conformations were subjected to family analysis and classified in six clusters based as previously on the conformation of βGlcNAc(1→2)αMan and Man(1→3)αMan glycosidic linkages (Table 2.3). The results of the docking study made obvious the fact that the cyclic trisaccharide could adopt several different conformations. However, conformation B appeared to be energetically favored in the bound state of linkage βGlcNAc(1→2)αMan. In a way similar to the linear compound, ConA-Cyclic_BA was identified as the minimum energy conformation. Both cyclic trisaccharides exhibit then the same conformational behaviors upon interaction with Con A.

Table 2.3: (Torsion angle values in ° and relative energy value in kcal/mol).

Family	Φ_{1-2}	Ψ_{1-2}	Φ_{1-3}	Ψ_{1-3}	ω_1	ω_2	ω_3	ω_4	ω_5	ΔE
ConA_cyclic_BA	48.8	148.9	70.7	88.9	-58.8	146.1	-84.1	-120.2	-86.5	0.0
	49.1	149.6	82.0	111.9	-60.0	111.7	-145.8	173.8	55.3	0.3
	46.2	147.0	92.8	85.7	-73.2	61.0	55.6	147.4	-103.5	0.5
	51.3	155.3	75.8	109.7	-59.3	118.4	-148.9	168.0	52.8	2.6
	49.3	142.0	71.5	130.4	-63.8	69.6	158.1	-178.7	136.4	3.2
	65.6	165.3	71.6	34.5	-47.1	98.3	-163.1	4.2	-105.5	16.4
ConA_cyclic_BD	52.9	149.1	141.3	55.7	-67.5	78.2	-96.2	-151.8	66.5	2.9
ConA_cyclic_AA	-55.9	-154.7	100.5	90.1	34.6	54.7	-134.8	-162.3	48.9	5.8
	-51.7	-159.8	124.7	100.2	46.3	-92.8	139.2	51.2	36.8	6.3
	-51.5	-166.4	94.1	83.1	68.2	-64.1	109.8	145.1	-95.3	6.9
	-38.4	-169.5	88.8	143.2	54.4	-83.8	-166.6	-61.5	111.5	7.1
	-50.1	-162.8	73.1	84.5	48.0	84.1	-104.3	-103.3	-83.2	7.4
	-31.8	175.1	69.3	133.3	68.2	-45.6	-172.1	175.1	166.2	7.7

Family	Φ_{1-2}	Ψ_{1-2}	Φ_{1-3}	Ψ_{1-3}	ω_1	ω_2	ω_3	ω_4	ω_5	ΔE
ConA_cyclic_BB	57.3	147.1	156.6	-20.2	-49.2	99.5	-155.1	28.7	-102.4	8.1
ConA_cyclic_CA	-62.1	-83.1	60.3	61.3	-68.2	146.4	-95.4	-108.0	-90.8	11.6
	-68.0	-116.0	57.7	62.8	38.3	106.6	-106.5	-102.6	-88.2	11.9
	-57.3	-74.8	64.5	68.7	-139.2	83.2	49.8	-178.9	-112.5	15.2.
	-56.8	-70.6	61.8	75.3	-37.4	-133.2	95.8	112.8	61.8	16.9
	-62.3	-81.6	53.4	73.6	-67.3	40.1	146.2	159.0	-171.6	25.5
ConA_cyclic_CD	-42.6	-156.8	128.4	71.6	-46.7	104.4	-38.8	172.5	-33.9	14.9

Figure 2.4 shows a superposition of the minimum energy conformation of both complexes, where a very similar binding behavior can be seen for both cyclic and linear trisaccharide. Figure 2.5 shows a detailed representation of both linear and cyclic trisaccharides in the binding site of Con A for minimum energy conformation BA. Nearly identical hydrogen bonding patterns was determined in both complexes.

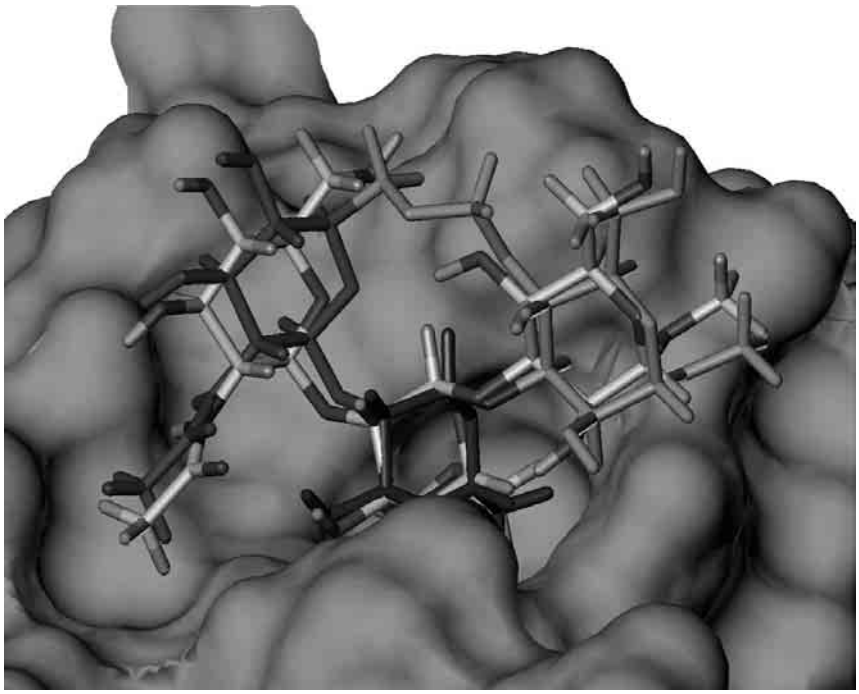


Figure 2.4

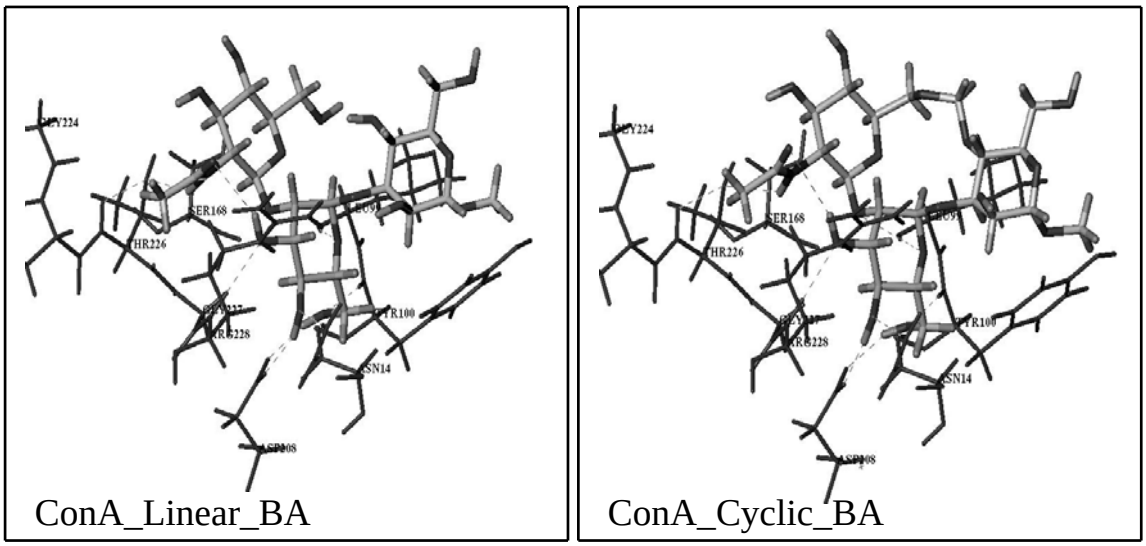


Figure 2.5

2.1.3 Conclusion

The conformational study has shown that the methylene acetal bridge does not distort the conformation of trisaccharide **50**, but reduces its conformational flexibility as intended. Molecular modeling of both complexes have demonstrated that both trisaccharides bind in similar secondary minimum energy conformations and have similar interactions with the protein. Although this study does not help to determine the origin of the entropy-enthalpy compensation, several hypotheses can be proposed. The first explanation is related to the conformational change from conformation AA to conformation BA, which is required for binding. This change introduces an unfavorable enthalpic term and this term may well be larger for the constrained molecule, due to its increased rigidity. It is also speculated that the enthalpy-entropy compensation could arise from solvation effects. The water molecules at the surface of the cyclic trisaccharide are expected to be more ordered due to a reduced flexibility. Upon binding,

the enthalpy of desolvation may then be high enough to account for this compensative phenomenon.

2.2 Design of New Conformationally Constrained Trisaccharides

The different studies performed on conformationally constrained trisaccharide **50** suggested that the enthalpy-entropy compensation might originate from the conformational change to adopt conformation BA. In order to assess this hypothesis, the design of a conformational constrained trisaccharide bearing the conformation BA in the uncomplexed form seemed to be a logical step. Such a ligand would have the advantage to be preorganized towards the binding conformation, which would be enthalpically favorable. It also would introduce a favorable entropic term in the thermodynamics of binding resulting in an overall high affinity ligand. This ideal ligand was designed by modification of cyclic trisaccharide **50** using molecular modeling.

Compound **50** was also found to be fairly flexible, which allowed it to adopt several conformations. A more rigid compound would help to further understand the role of flexibility in carbohydrate. In that context, a very rigid ligand was designed to test the limit of conformational constraints.

2.2.1 Design of R and S (2-N-acetyl-2-deoxy-6-C-methyl-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6"-methylidene acetal

It has been shown that the torsional freedom of a (X-6) glycosidic linkage can be reduced by the substitution of a hydrogen atom at the C-6 position by a methyl moiety. The resulting steric interactions between the methyl group and the sugar hydroxyl

decrease then the number of possible conformations around the C-5-C-6 linkage. The overall result is the preorganization of the oligosaccharide toward a specific conformation. Trisaccharides **70** and **71** were designed based on this observation (Figure 2.6).

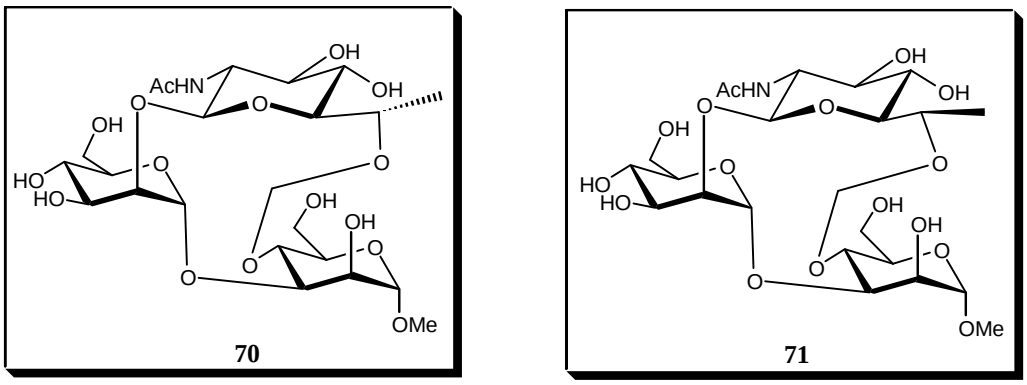


Figure 2.6

A methyl moiety was added at the C-6 position of the GlcNAc of cyclic trisaccharide **50**. The effect of this modification on the conformation of the two unbound compounds was studied by molecular modeling, using SYBYL and the carbohydrate-parameterized version of the Tripos force filed. A systematic conformational search was run on nine of the 13 torsion angles of cyclic trisaccharides **70** and **71**. More than 20,000 possible conformations were obtained. Time constraints - treating such a volume of data requires at least a week of computer time utilization per compound – led to consider another strategy to model these two molecules. Since the described modification is supposed to reduce flexibility, the 23 minimum energy conformations of uncomplexed trisaccharide **50** were assumed to be a good starting point for the modeling simulation. In each of these conformations, a R-methyl of a S-methyl was added and a minimization step was performed. The obtained conformations were subjected to family analysis. The

conformations corresponding to the R-isomer were classified in 8 families (Table 2.3) and Methyl_R_AA was identified as the minimum energy conformation. The R isomer appeared to be fairly flexible and has conformational behaviors similar to the parent trisaccharide.

Table 2.3

	E	Ψ1,2	Φ1,2	Ψ1,3	Φ1,3	ω 1	ω2	ω3	ω4	ω5	ΔE
AA											
Methyl_R_1	9.3	-44.9	-169.0	73.1	90.6	75.4	-72.9	133.4	161.7	-125.5	0.0
Methyl_R_3	10.5	-43.8	-168.4	61.7	83.0	57.8	81.3	-122.6	-82.1	-97.7	1.3
Methyl_R_5	12.5	-49.8	-163.1	78.6	116.5	40.9	71.6	-164.6	165.5	57.4	3.2
Methyl_R_13	13.1	-38.6	175.3	67.7	137.6	73.5	-44.5	-175.5	178.5	155.9	3.8
Methyl_R_11	14.0	-38.3	-170.7	76.9	146.5	60.4	-103.4	-157.3	-70.9	126.4	4.8
Methyl_R_12	14.6	-29.8	-172.5	96.6	85.9	-43.3	78.7	23.9	155.3	-104.7	5.4
BA											
Methyl_R_2	10.4	43.4	150.3	73.6	95.3	-73.4	73.5	57.9	157.1	-130.3	1.1
Methyl_R_7	12.9	66.6	-171.7	57.4	75.5	-76.2	82.5	-124.4	-169.4	65.5	3.6
Methyl_R_4	13.0	48.9	149.7	78.0	114.5	-58.4	107.1	-148.0	174.7	58.7	3.7
Methyl_R_10	13.5	45.5	147.3	68.0	93.7	-60.4	145.4	-78.0	-125.9	-89.8	4.2
Methyl_R_9	13.9	47.1	140.7	69.9	135.9	-60.0	61.9	152.4	-173.1	136.4	4.6
BD											
Methyl_R_6	12.8	54.6	153.7	140.7	47.9	-69.3	68.6	-84.0	-150.6	68.3	3.5
CA											
Methyl_R_8	13.2	-61.4	-86.3	54.0	54.7	67.3	-133.1	61.9	141.2	53.0	3.9
Methyl_R_14	15.6	-56.2	-83.8	54.1	47.8	-52.5	63.2	49.4	160.8	-102.3	6.3
Methyl_R_19	16.0	-51.8	-77.0	51.8	64.3	-46.0	125.0	-134.8	164.2	58.1	6.7
Methyl_R_21	16.7	-55.2	-84.4	52.2	48.1	-45.8	141.5	-57.1	-134.7	-81.7	7.5
CB											
Methyl_R_16	14.8	-68.9	-96.5	74.4	0.8	49.6	76.9	-155.0	-23.9	-98.6	5.5
AD											
Methyl_R_15	15.7	-48.3	-166.2	123.1	107.5	45.5	-92.9	150.4	39.4	43.5	6.4
Methyl_R_22	18.0	-38.8	-166.1	122.8	82.2	-43.6	116.0	-53.0	174.5	-35.5	8.7
Methyl_R_23	18.1	-39.9	-156.1	143.5	60.9	-46.2	111.2	-88.2	171.7	38.9	8.9
AB											
Methyl_R_1	16.1	66.0	-161.0	97.4	-29.8	-57.7	167.2	-164.7	-40.1	-102.2	6.8
BB											
Methyl_R_1	16.4	68.8	180.0	125.1	-32.1	-65.4	99.6	-161.5	42.6	-122.4	7.1
Methyl_R_1	16.5	65.3	160.6	141.2	-26.6	-58.8	105.2	-154.8	30.6	-107.8	7.2

The conformations corresponding to the S-isomer were classified in 6 families (Table 2.4), with Methyl_S_BA being the minimum energy conformation. Although conformation AA was identified as the first secondary minimum energy conformation, the modification preorganized the structure, as expected, towards a different conformation compared to the parent trisaccharide. The preorganized structure being in a BA conformation, the required conformation for binding makes this molecule an ideal candidate to study the effect of conformational constraints in protein-carbohydrate interactions.

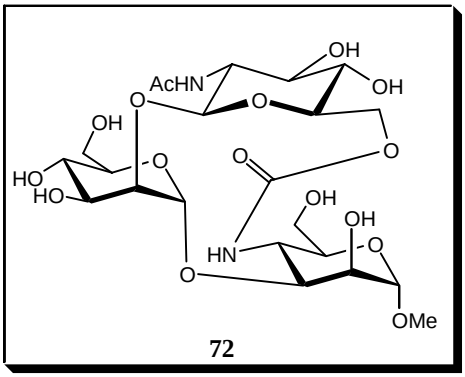
Table 2.4

	E	**Ψ1,2**	**Φ1,2**	**Ψ1,3**	**Φ1,3**	**ω 1**	**ω2**	**ω3**	**ω4**	**ω5**	**ΔE**
BB											
Methyl_S_12	9.7	46.4	150.8	74.8	92.5	-71.3	62.6	66.9	155.9	-126.5	0.0
Methyl_S_2	9.8	45.7	149.7	73.0	95.4	-70.8	64.5	66.9	156.8	-131.6	0.0
Methyl_S_4	12.3	47.1	150.2	77.9	115.0	-59.8	115.0	-149.6	171.8	57.6	2.5
Methyl_S_7	13.4	66.3	-172.7	57.2	76.3	-79.2	90.8	-127.8	-170.9	64.1	3.6
Methyl_S_10	13.9	46.5	148.1	67.3	94.6	-59.2	148.9	-78.1	-127.8	-94.5	4.1
Methyl_S_9	14.0	46.0	141.1	70.3	134.8	-61.6	70.1	150.2	-176.5	134.8	4.2
AA											
Methyl_S_1	10.6	-42.8	-170.5	72.6	91.1	76.8	-83.5	141.2	165.3	-126.9	0.9
Methyl_S_3	12.1	-40.9	-170.3	61.4	81.5	59.7	75.4	-128.6	-74.4	-99.4	2.4
Methyl_S_5	12.3	-51.0	-162.5	76.5	116.8	42.5	73.3	-165.5	163.6	58.6	2.6
Methyl_S_15	14.1	-46.5	-166.7	124.8	106.5	45.7	-99.7	149.7	45.3	43.1	4.4
Methyl_S_11	14.6	-41.1	-169.5	77.4	145.5	61.3	-89.5	-164.9	-78.0	124.5	4.9
Methyl_S_13	14.6	-39.0	174.1	69.1	137.5	75.6	-49.3	-176.4	178.5	158.3	4.9
Methyl_S_22	18.9	-37.9	-166.0	123.1	82.7	-40.9	110.3	-55.2	168.7	-28.9	9.2
Methyl_S_23	19.5	-35.9	-160.2	145.6	60.0	-43.9	102.2	-81.9	172.5	36.6	9.7
BD											
Methyl_S_6	13.0	52.0	148.4	140.9	54.2	-68.1	76.4	-94.6	-147.3	66.5	3.3
CA											
Methyl_S_8	13.1	-59.9	-86.3	54.3	54.0	67.9	-136.3	61.4	140.1	54.2	3.3
Methyl_S_14	14.4	-56.6	-80.1	52.5	44.7	-59.3	78.8	42.3	167.6	-103.3	4.7
Methyl_S_19	18.3	-52.3	-82.0	55.7	68.1	-37.2	113.0	-144.7	167.8	62.4	8.5
Methyl_S_21	18.5	-55.2	-86.7	52.9	50.5	-37.6	136.7	-65.5	-135.2	-82.1	8.8

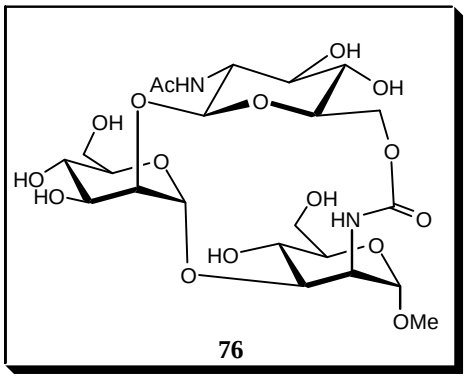
	E	Ψ1,2	Φ1,2	Ψ1,3	Φ1,3	ω 1	ω2	ω3	ω4	ω5	ΔE
CB											
Methyl_S_16	15.5	-68.4	-96.0	74.8	1.0	49.7	74.4	-155.4	-23.1	-98.8	5.8
BB											
Methyl_S_17	15.7	68.7	-167.1	87.1	-16.0	-64.0	123.1	-150.5	-9.2	-99.0	6.0
Methyl_S_18	16.3	68.5	178.2	125.9	-31.2	-66.8	106.8	-162.2	37.6	-120.0	6.5
Methyl_S_20	16.4	68.8	179.8	125.2	-32.0	-65.3	99.1	-161.2	42.7	-122.0	6.6

2.2.2 Design of Carbamate Compounds

In the quest for a very rigid molecule, two approaches were adopted. The first one started from the structure of compound **50**, for which it was proposed to modify the chemical structure of the methylene acetal bridge. The introduction of a more rigid bridge would result in an overall less flexible molecule. Trisaccharides **72** and **76** were designed so that a carbamate bridge would replace the methylene acetal moiety. The planar characteristic of the amide bond was expected to rigidify the structure.



72



76

Figure 2.7

A second approach consisted in removing one atom in the methylene acetal bridge, would yield a smaller and thus more rigid 12-membered ring. An oxygen atom was removed from the structure of trisaccharides **77** and **78** to confer more rigidity to the ring (Figure 2.8).

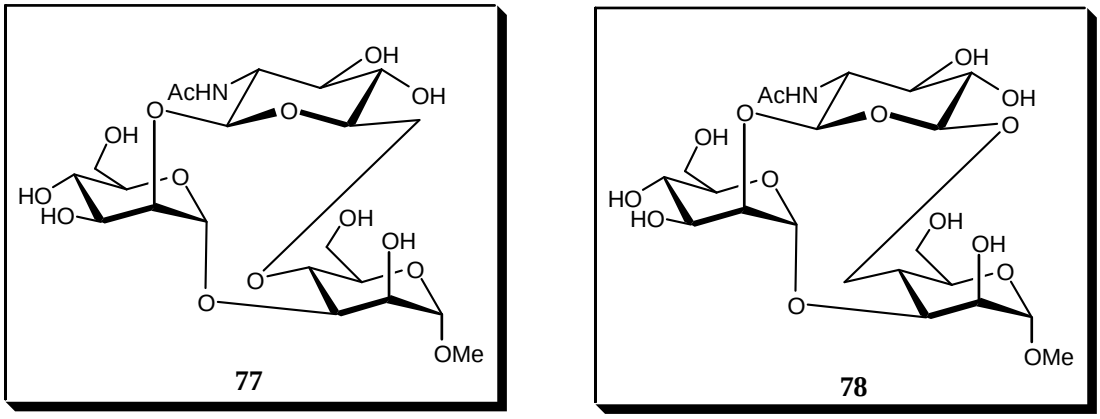


Figure 2.8

Trisaccharides **72** and **76** were subjected to a conformational search on nine of their torsional angles. Both were found to be much more rigid than trisaccharide **50**. In spite of their similar structures, their conformational behaviors were nevertheless different. After systematic search, molecules **72, 76** and **50** yielded 2,284, 2,106 and 26,716 possible conformations respectively. After ring chirality and torsional angle checks and an energy cut-off set at 20 kcal/mol, molecules **72** and **76** showed different characteristics. For Trisaccharide **72**, possible conformations dropped to 1,448 and to only 265 for compound **76**. This extremely low number of conformations eliminated Trisaccharide **76** as a possible candidate, being far too rigid to successfully achieve the cyclization step. It was indeed considered too risky to start a 30 or 40-step synthesis, not

knowing if the difficulties associated with the final cyclization step would ever be overcome. The minimum energy conformation for both saccharides is AB, which accounted for more than 97.7% of the obtained conformations. From these observations, it was concluded that trisaccharide **72** was the only suitable candidate for the synthesis. This saccharide appears to be interesting, first because it is very rigid and also locked in a conformation different from the one required for binding. This type of molecule should provide valuable information about the role of oligosaccharide flexibility upon binding with a protein.

Trisaccharides **77** and **78** were also subjected to a similar systematic conformational analysis, although on only seven of the torsional angles of the structure. Both molecules were found to be very rigid with 2,700 and 2,707 possible conformations respectively. Following the same procedure as for Trisaccharides **72** and **76**, the number of conformations respectively dropped to 795 for Trisaccharide **77** and 178 for Trisaccharide **78**. Not only were both molecules extremely rigid, several sugar residues appeared to be affected by ring distortion (Figure 2.9).

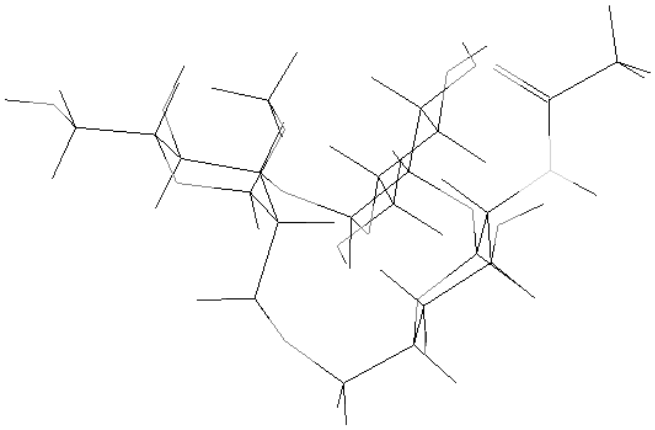


Figure 2.9

Although potentially difficult to synthesize, trisaccharide **77** revealed interesting conformational behaviors. The 795 possible conformations were classified in two families, with 99.7% of the conformations in a BB orientation. This meant that trisaccharide **77** was locked in the BB conformation and was therefore very unlikely to adopt another conformation.

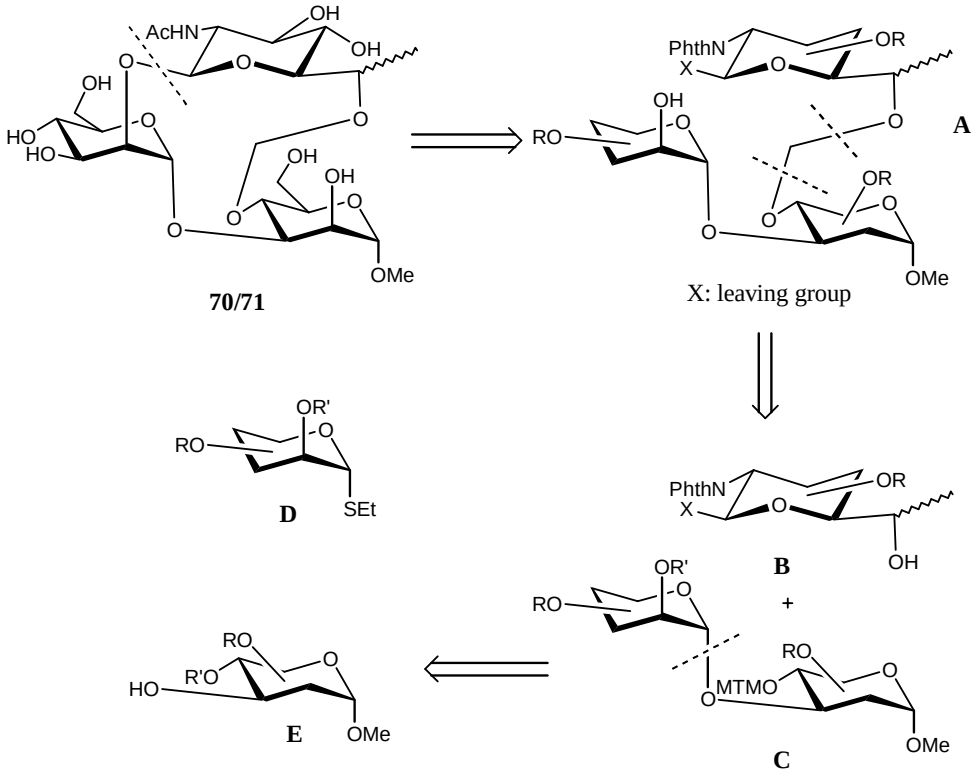
2.2.3 Conclusion

Thanks to assisted computer modeling, three new compounds were found to be promising for their estimated properties. It was decided to synthesize trisaccharides **70** and **71**, for which hydrogen atoms were replaced by methyl groups at the C-6 position of GlcNAc. These two molecules should help to determine the origin of the enthalpy term induced by necessary conformational changes occurring upon binding of flexible oligosaccharides with proteins. Trisaccharide **72** will also be synthesized, since it appears to be an excellent candidate to test the limits of conformational constraints on carbohydrates. The synthesis of trisaccharides with a shorter linkage to close the ring will not be attempted for now, since they may be too rigid to be successfully cyclized.

2.3. Synthesis of (2-*N*-acetyl-2-deoxy-6R-C-methyl-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6”-methylene acetal (**70**) and (2-*N*-acetyl-2-deoxy-6S-C-methyl-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3) -α-D-mannopyranoside-4,6”-methylene acetal (**71**)

2.3.1 Intramolecular Glycosylation Approach

A retrosynthetic analysis of the synthesis of cyclic trisaccharides **70** and **71** is shown in Scheme 2.1.



Scheme 2.1

Based on existing synthesis for similar cyclic trisaccharides, it was proposed to achieve the cyclization of linear trisaccharide isomers **A** and **A’** of compound **70** and **71**

using an intramolecular glycosylation.[45] Thioethyl or trichloroacetimidate could be used as leaving groups to perform this reaction on the free hydroxyl group at the C-3 of the second mannoside. Precursors **A** and **A'** could be obtained by methylene acetal bridge formation between building blocks **B** and **B'** and disaccharide **C**. The formation of such a bond can be achieved, by reaction of methyl thiomethyl moiety at O-4' of disaccharide **C** and the free hydroxyl at the C-6 position of building blocks **B** and **B'**.[108] The coupling of thioglycoside building block **D** with monosaccharide **E**, and the introduction of the methyl thiomethyl on the resulting disaccharide should lead to intermediate **C**.

To achieve the synthesis of both trisaccharides **70** and **71**, thioglycoside **79**, glycosyl acceptors **80**, **81** and **82** (Figure 2.10) were synthesized using standard protecting group interconversion methodologies.

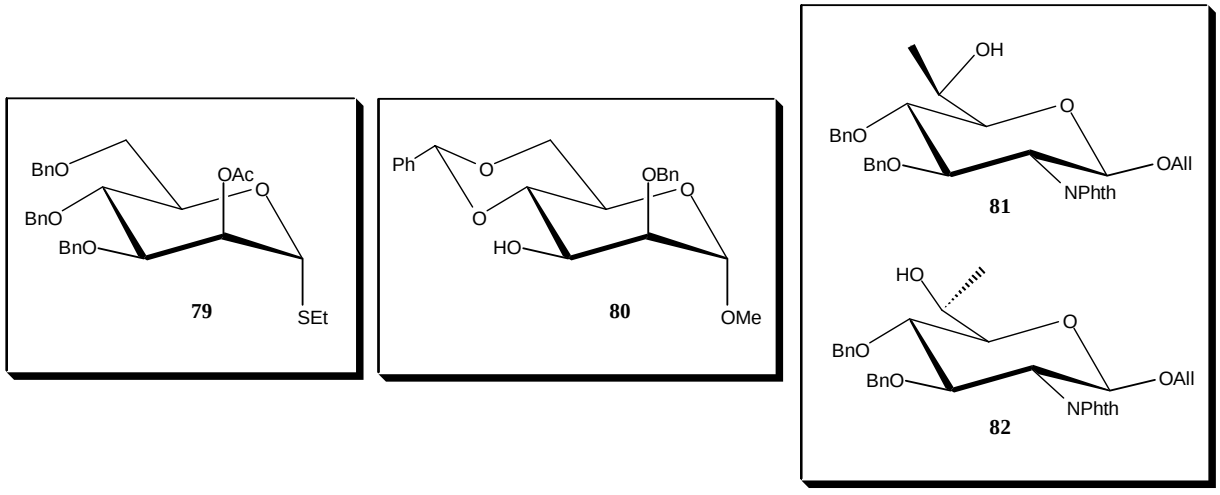
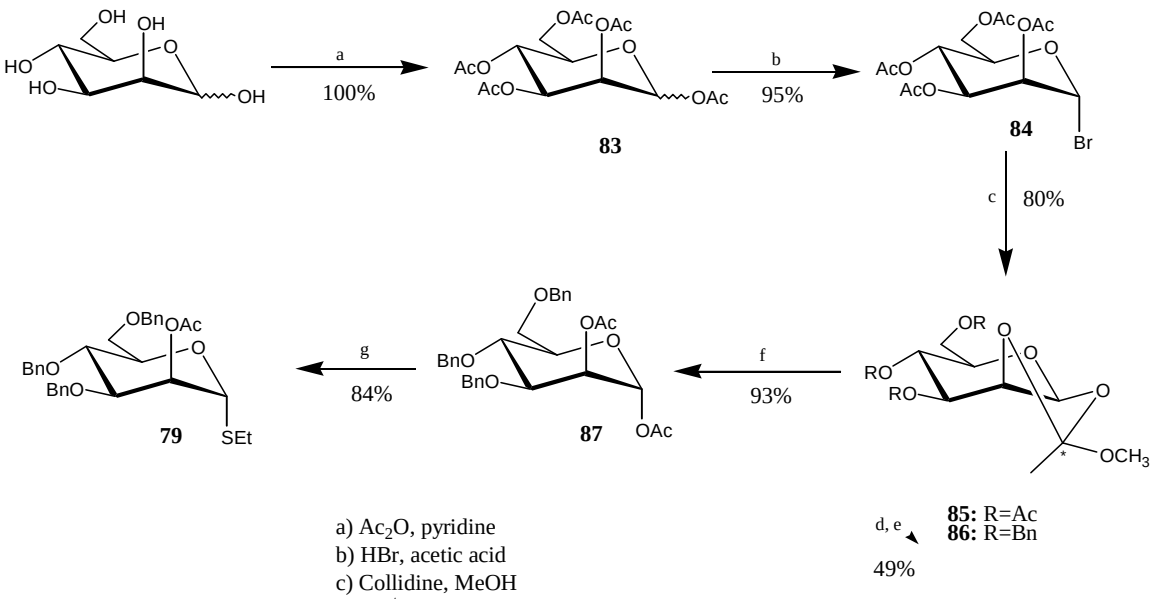


Figure 2.10

The first building block to be synthesized was thioglycoside **79**, which features an acetyl protecting group at C-2, three permanent benzyl protecting groups at C-3, C-4, C-6 and a thioethyl group at the anomeric center. The synthesis of **79** is depicted in Scheme

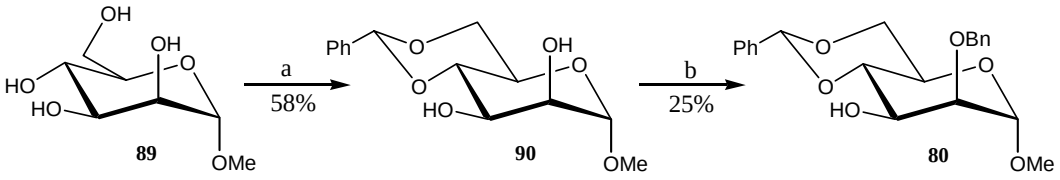
2.2. D-mannose was fully acetylated by treatment with acetic anhydride in dry pyridine to yield compound **83** as an anomeric mixture (α/β, 4/1) in quantitative yield. *Per*-acetylated mannose **83** was treated with 33% HBr in acetic acid to afford compound **84** in 95% yield. Unstable bromide **84** was immediately treated with collidine in a mixture of dry methanol and dry methylene chloride to obtain 1,2-ortho ester **85**.[109,110] After recrystallization of the crude product, **85** was obtained as a white solid in 80% yield. ^{1}H NMR revealed the presence of the ortho ester with two singlets at 3.28 and 1.75 ppm, which are characteristic of the methoxy and the methyl groups of the ortho ester, respectively. The next step consisted in the deacetylation of **85** by treatment with potassium *tert*-butoxide in methanol. The desired deacetylated compound was obtained in quantitative yield and was benzylated using sodium hydride and benzyl bromide to afford **86** as a white solid in 49% yield over 2 steps. The ortho ester of **86** was cleaved by treatment with acetic anhydride in glacial acetic acid for 18 hours to yield di-acetylated compound **87** as a pale yellow oil. The ethyl thio moiety was introduced at the anomeric center by reacting **87** with ethantiol in dry methylene chloride in the presence trimethylsilyl trifluoromethane sulfonate (TMSOTf) as the Lewis acid.[111] After column chromatography purification, thioglycoside **79** was obtained as a colorless oil in 84% yield.



a) Ac_2O, pyridine
b) HBr, acetic acid
c) Collidine, MeOH
d) KO^{t}Bu, MeOH
e) NaH, DMF, BnBr
f) AcOH, Ac_2O
g) HSEt, CH_2Cl_2, TMSOTf, MS 4 Å

Scheme 2.2

The second building block, glycosyl acceptor **80**, has two permanent protecting groups, benzyl at C-2 and methyl at the anomeric center. C-4 and C-6 are masked by a benzylidene acetal, an easily modulated protecting group. The synthesis of compound **80** can be achieved in a two-step reaction sequence as shown in Scheme 2.3.



a) Benzaldehyde dimethyl acetal, tetrafluoroboric acid 52% W/V in ether. b) Ag_2O, BnBr, DMF.

Scheme 2.3

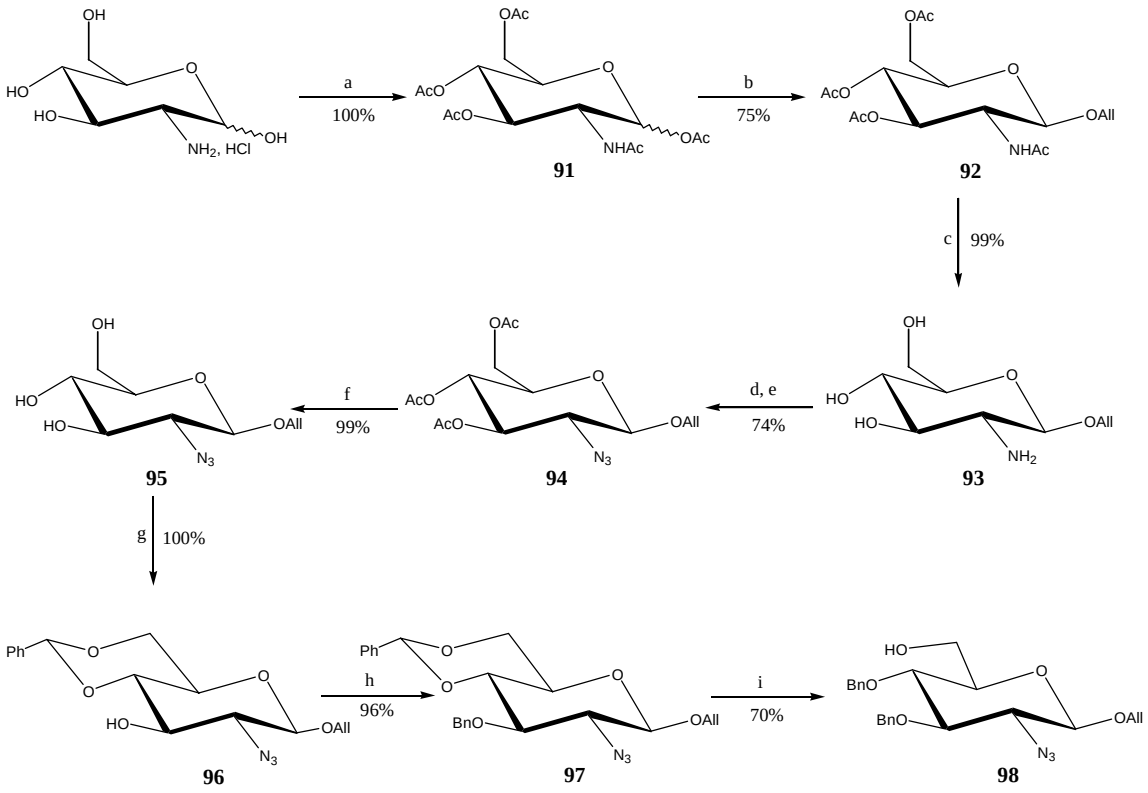
The first step of the synthesis is the selective benzylidene introduction to protect hydroxyls at the C-4 and C-6 positions. This step was achieved in 58% yield by treatment of methyl α-D-Mannose (**89**) with benzaldehyde dimethyl acetal and *tetra*-fluoroboric acid (52% w/v in diethyl ether) to afford **90**.[112] When performing this reaction in the mannoside series, 2,3 benzylidene is also formed. Although 2,3 benzylidene acetal is more sensitive to acidic conditions than 4,6 acetal, the formation of a side product was observed, which produced a di-benzylidene protected disaccharide in a rather low yield.[113-116]

The second step in the synthesis is the selective benzylation of monosaccharide **90** at the C-2 position.[117,118] Although associated with a poor 25% yield, this step can be performed using benzyl bromide and silver oxide as catalysts in DMF.[117] The term of stereoselectivity is abusive since the OH-2 and OH-3 in the case of 4,6-di-*O*-benzylidene-α-D-mannopyranoside display similar reactivity, resulting in the formation of non-desired side products such as dibenzyl and 3-O-benzyl and an overall poor yield. The consequence on the route of this very low yield is not dramatic it is completed in two short steps requiring relatively inexpensive reagents.

The last two building blocks **81** and **82** are the more challenging in terms of synthesis. A methyl group has to be introduced at the C-6 position of a glucosamine. The proposed route to synthesize these unusual carbohydrates is to oxidize the 6-hydroxyl into a ketone, which can then be used in a Grignard reaction with methyl magnesium bromide. The amino group had then to be protected with a suitable protecting group. For example, the phthalimido required later in the synthesis, is not

compatible with the Grignard conditions and had to be replaced by an azido functionality. It was then reintroduced later in the synthesis.

The first steps of synthesis are common for both building blocks **81** and **82** as depicted in Scheme 2.4.



a) Ac_2O, Pyridine. b) TMSOTf, AllOH, DCE, 4 Å MS. c) $BaOH.8H_2O$, H_20, 90 ^oC. d) TfN_3, DMAP, MeOH. e) Ac_2O, pyridine. f) NaOMe, MeOH. g) benzaldehyde dimethyl acetal, CSA, CH_3CN. h) BnBr, NaH, TBAI, DMF. i) Borane trimethyl amine complex, $AlCl_3$, DCM/Ether, 4 Å MS

Scheme 2.4

The first step was the quantitative acetylation of commercially available D-glucosamine hydrochloride in a pyridine and acetic anhydride mixture to afford compound **91** as a white solid.[119] Using TMSOTf as a promoter, the *per*-acetylated glucosamine **91** was glycosylated at the anomeric position with allyl alcohol to afford β-

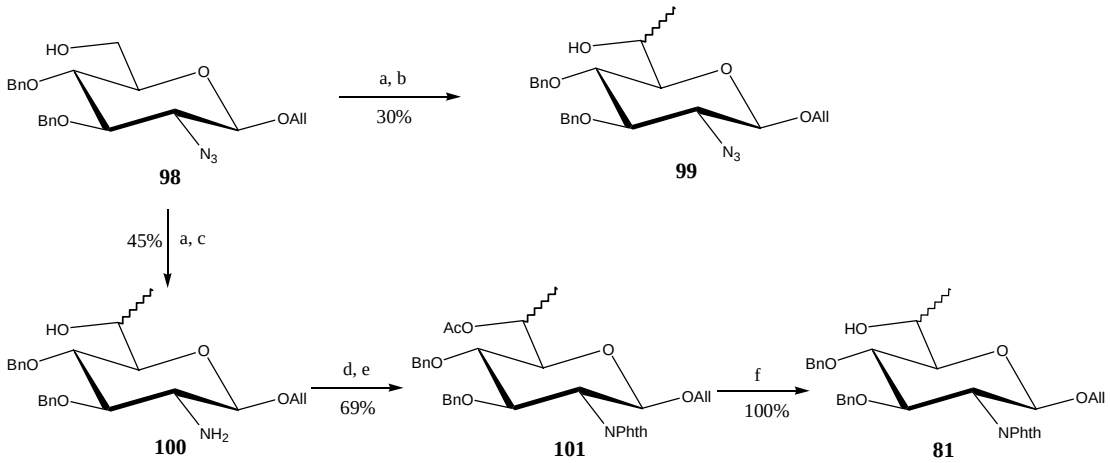
allyl building block **92** in 75% yield.[120] The formation of an oxazoline upon treatment with Lewis acid explains the β selectivity of the reaction. The α-face being blocked by the oxazoline, glycosylation can only take place towards the β-face.

Compound **92** was then fully deacetylated with barium hydroxide in water at 90°C in a quantitative manner leading to **93**.[121] The introduction of the azido functionality at the C-2 position was then achieved by treatment of **93** with freshly prepared triflic azide in the presence of DMAP.[122,123] It is important to notice that, at this stage, the purity of **93** before reaction is crucial, since it greatly affects the reaction time and the amount of triflic azide necessary to perform the reaction. When it reaches completion, the amount of solvent is reduced to a minimum for safety reasons, before being acetylated using acetic anhydride and pyridine. This last operation allows the destruction of the excess of explosive triflic azide. The obtained monosaccharide **94** was then deacetylated, using conventional methodology such as sodium methoxide in methanol, to yield compound **95** in quantitative yield. The next three steps of the synthesis consisted in the introduction of two permanent benzyl-protecting groups at the C-3 and C-4 positions. To do so, it was envisaged to selectively block the C-6 position with a trityl or TBDMS protecting group. The low reactivity of this position resulted in the failure of this approach. A benzylidene was then introduced onto OH-4 and OH-6, affording compound **96** in a surprisingly quantitative yield. The last free hydroxyl was then benzylated with benzyl bromide and sodium hydride in DMF, using TBAI as a catalyst to afford **97**. The last part of this synthesis consisted in the selective benzylidene ring opening toward the C-4 position of the glucosamine. This transformation was performed using borane-

trimethyl amine complex and aluminium chloride in a mixture of methylene chloride and diethyl ether to afford building block **98** as a single isomer in 70% yield.[124]

Starting from compound **98**, the introduction of the methyl at the C-6 position of the glucosamine was attempted. A Grignard reaction was performed after oxidation of the OH-6 into an aldehyde. Monosaccharide **98** was subjected to Swern oxidation,[125-127] using DMSO, oxalyl chloride and triethylamine in methylene chloride (Scheme 2.5).[128] The temperature is a determining factor in the success of this reaction, since it must be kept under –65 ^{o}C until triethylamine is added. The solvent of the previous reaction was removed and the resulting instable aldehyde was immediately treated with methyl magnesium bromide in ether at 0 ^{o}C.[128-130] Surprisingly, the outcome of the reaction depended on what scale the reaction was performed. When working with less than 250 mg of sugar, only one isomer **99** was obtained in 30% yield after column chromatography. Such a yield for a Grignard reaction applied to carbohydrates is not unusual, although some yields around the 50% mark have been reported for similar reactions.[43,128-133] Furthermore, although it is theoretically possible to obtain two isomers, a single isomer result is far from being an isolated scenario.[129,130] Lemieux[130] reported 39% of one isomer and 2% only for the other one for a similar reaction. A fraction composed of a least three other inseparable molecules was also obtained, none of which were the other isomer (Different masses were determined by Maldi-Tof). These compounds may be side products from the Grignard reaction, where elimination can occur. Varying the experimental conditions did not improve the yield of this reaction. Upon scaling up, compound **99** failed to be obtained and was substituted for a complex mixture of molecules characterized by a long polar trace on a TLC plate.

After a tedious chromatography column, impure amine **100** was obtained in 45% yield. As a single diastereoisomer the configuration of **100** could not be determined with certitude, since both isomers are normally required to identify configurations R and S. An inversion of configuration by S_N2 mechanism was attempted at the C-6 position of the compound, but it failed to produce the other diastereoisomer. For example, triflic anhydride in pyridine was used to form a triflate leaving group that was then treated with cesium acetate in DMF,[134,135] but it unfortunately did not yield the desired compound. In that context, we decided to move forward with the single isomer, so that the feasibility of the synthesis of a cyclic trisaccharide bearing such modification could be tested.



a) DMSO, oxalyl chloride, Et_3N, DCM, -65 ^oC. b) MeMgBr, Et_2O, 0 ^oC, (scale up to 250 mg). c) MeMgBr, Et_2O, 0 ^oC, (scale > 250 mg). d) phtalic anhydride, toluene, reflux. e) Ac_2O, pyridine. f) NaOMe, MeOH.

Scheme 2.5

Although the reduction of the amine was a side reaction it proved to be rather efficient, this step would have been the next step in the reaction sequence. Furthermore, most of

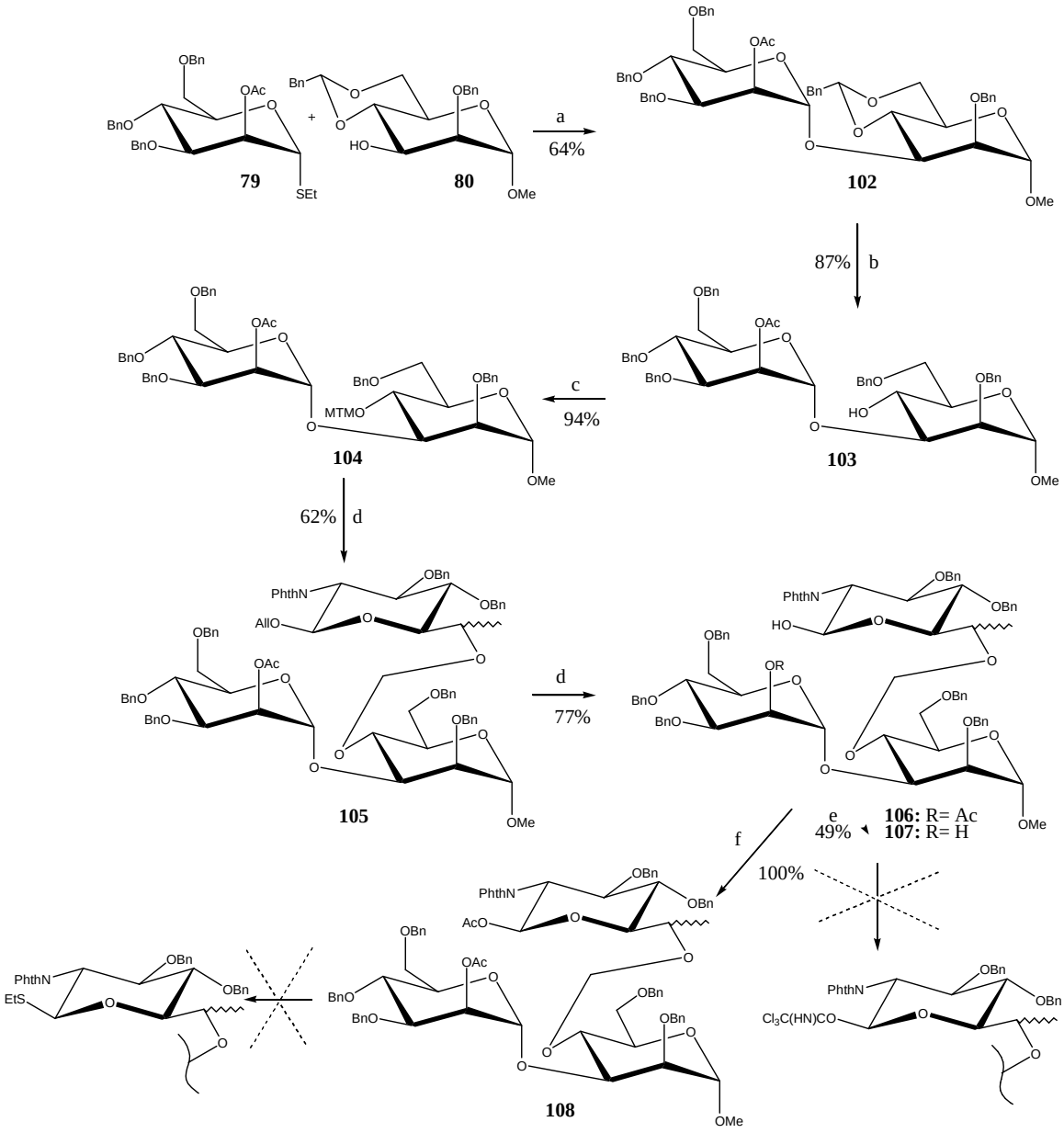
the time, azide functionalities are difficult to reduce with methods other than hydrogenations.

The next step in the synthesis was the protection of the amine with a phthalimido group. **100** was treated with phthalic anhydride in toluene under reflux for 2 hours.[136] To make sure that the phthalimido ring was completely closed, the residue of the previous reaction was acetylated using conventional method to yield **101**. This operation is commonly used to close a phthalimido ring when only one of its the two arms is attached to the nitrogen atom. The removal of the acetyl-protecting group yielded building block **81**.

With three building blocks **79**, **80** and **81** in hand, we started the assembly of the target cyclic trisaccharide (Scheme 2.6).

Condensation between donor building block **79** and saccharide acceptor **80** afforded disaccharide **102** using NIS and TMSOTf as the promoter system in a mixture of CH_2Cl_2/Et_2O (6/4, v/v).[137] It is interesting to note that the use of diethyl ether is not necessary but it prevents the formation of undesirable β-anomers that often form when methylene chloride is used as the only solvent. Selective opening of the benzylidene leading to benzylated 6-OH was accomplished by treatment of compound **102** with sodium cyanoborohydride and hydrogen chloride in THF to afford disaccharide **103**.[138]

A methyl thiomethyl moiety was introduced on the free hydroxyl group located at the C-4 position of the reducing mannose. Disaccharide **103** was then treated in acetonitrile with dimethyl sulfide, to which benzoyl peroxide was gradually added in dark.[139] After conventional work up and purification by column chromatography, compound **104** was obtained in an excellent yield of 94%.



a) NIS, TMSOTf, DCM/Et_2O, 6/4, v/v, 4 ÅMS, rt . b) $NaBH_3CN$, THF, HCl/Et_2O. c) Dimethyl sulfide, Benzoyl peroxide, CH_3CN. d) **73**, NIS, TfOH, DCE/THF. d) $PdCl_2$, NaOAc, Bu_3SnH, AcOH/H_2O. e) NaOMe, MeOH. f) Ac_2O, Pyridine.

Scheme 2.6

This donor disaccharide was engaged in the next step with monosaccharide **81** as the acceptor. The reaction was performed in a solvent mixture of DCE and THF and with NIS and Triflic acid as the promoter system to afford linear trisaccharide **105** in 62%

yield.[48,140] The next two steps of the synthesis are the deprotection of the two ends of compound **105**. After several unsatisfactory deallylation attempts using a wide range of experimental conditions,[141-144] allyl protecting groups were successfully removed following an adaptation of a procedure described by Ogawa.[144] Trisaccharide **105** was treated with zinc chloride and sodium acetate in a mixture of acetic acid and water as reported but tributyl tin hydride was added to the solution to avoid the formation of side products such as ketone in this case. The solution was filtered and the solvent was removed before the residue was taken up into ethyl acetate and washed with aqueous solution KF (1M) to eliminate the tin reagent. Purification by column chromatography on silica gel gave trisaccharide **106** in 77% yield. The next step was the removal of the acetate at the other end of trisaccharide **106**. Although this transformation is typically not extremely challenging, it proved problematic in this case. Conventional reagents, such as sodium methoxide and potassium carbonate, allowed the deprotection to be completed successfully. However, a difference of 16 in mass unit for the obtained compound was revealed by mass spectroscopy, which was explained by the opening of the phthalimido ring. After several unsuccessful attempts to reclose the ring, compound **106** was treated with potassium tert-butoxide in methanol for a short period of time, and diol **107** was afforded in 45% yield. This result was quite surprising because, the mechanism of this reaction is theoretically the same as previously tried reactions. This result was the first evidence that the presence of a methyl group at the C-6 position impacted the overall reactivity of the molecule.

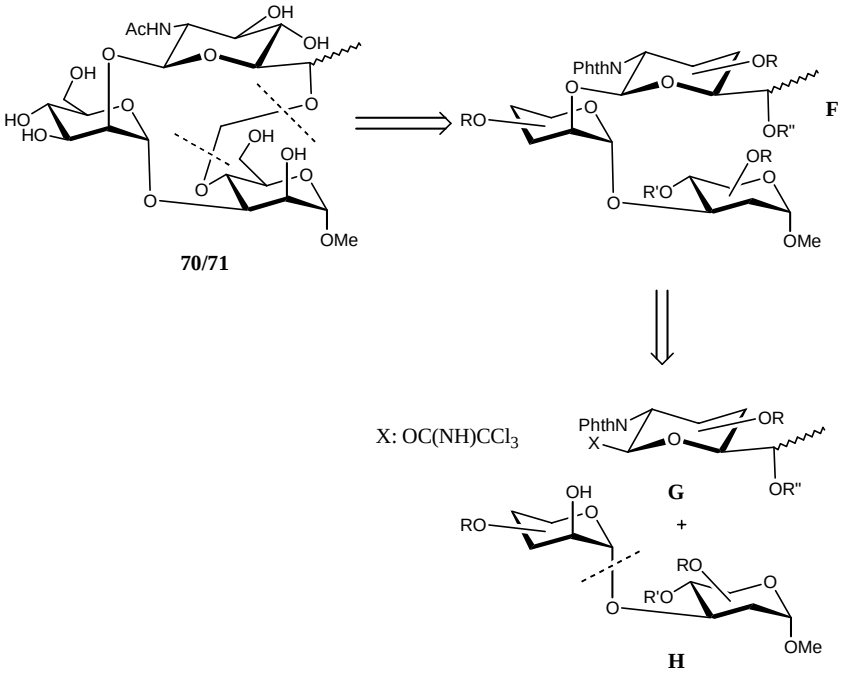
To achieve the final cyclization by intramolecular glycosylation, a leaving group had to be introduced selectively at the anomeric center of the glucosamine. It was

decided to follow the procedure described by Boons and co-workers,[45] which uses a trichloroacetimidate to perform the glycosylation. To that end, trisaccharide **107** was treated with trichloroacetonitrile in the presence of the base DBU.[145,146] However, the expected introduction of the trichloroacetimidate failed to occur and boosting the reaction conditions by adding more reagents only resulted in the decomposition of the trisaccharide. This failed approach prompted us to attempt the reaction with a thioglycoside instead of the previously used trichloroacetimidate. To do so, trisaccharide **107** was reacetylated to obtain compound **108** in a quantitative fashion. The introduction of a thioethyl moiety at the anomeric center was attempted using ethanethiol and a catalytic amount of TMSOTf.[111] After one hour, no conversion was noticed and more catalyst was added. Unfortunately, Maldi-Tof analyses conducted after one additional hour revealed the presence of two compounds in solution corresponding respectively to one monosaccharide and one disaccharide. The harshness of the experimental conditions caused the degradation of **108**. Furthermore, it is known that methylene acetal moieties are acid sensitive and using too much Lewis acid such as TMSOTf could only result in further degradation.

After several unsuccessful attempts to cyclize trisaccharide **107** via intramolecular glycosylation, a different approach was considered. The next section focuses on the use of an intramolecular methylene acetal bridge formation to synthesize the target cyclic trisaccharides.

2.3.2 Intramolecular Methylene Acetal Formation Approach

After failure to achieve the cyclization by intramolecular glycosylation, it was decided to use another synthetic route. A retrosynthesis of this new approach is depicted in scheme 2.7. It was decided to synthesize one of the targeted cyclic trisaccharides by intramolecular formation of the methylene acetal bridge from trisaccharide **F**.



Scheme 2.7

Linear trisaccharide **F** can be assembled by conventional glycosylation using monosaccharide building block donor **G** and disaccharide acceptor **H**.

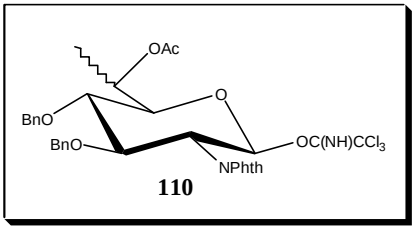
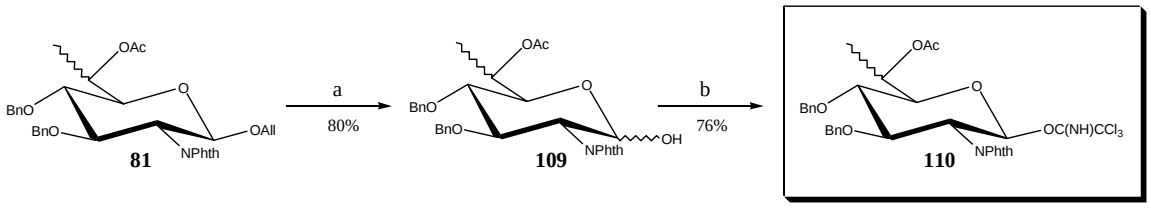


Figure 2.11

This approach requires only one new building block (Figure 2.11). Compound **110** can easily be synthesized from building block **81** (Scheme 2.8). Following Ogawa's adapted procedure (see deallylation of compound **105**), compound **81** was deallylled to afford **109** in 80% yield. Trichloroacetimidate was then introduced by treatment of compound **109** with trichloroacetonitrile in the presence of DBU, using methylene chloride as the solvent to afford building block **110** in 76% yield.

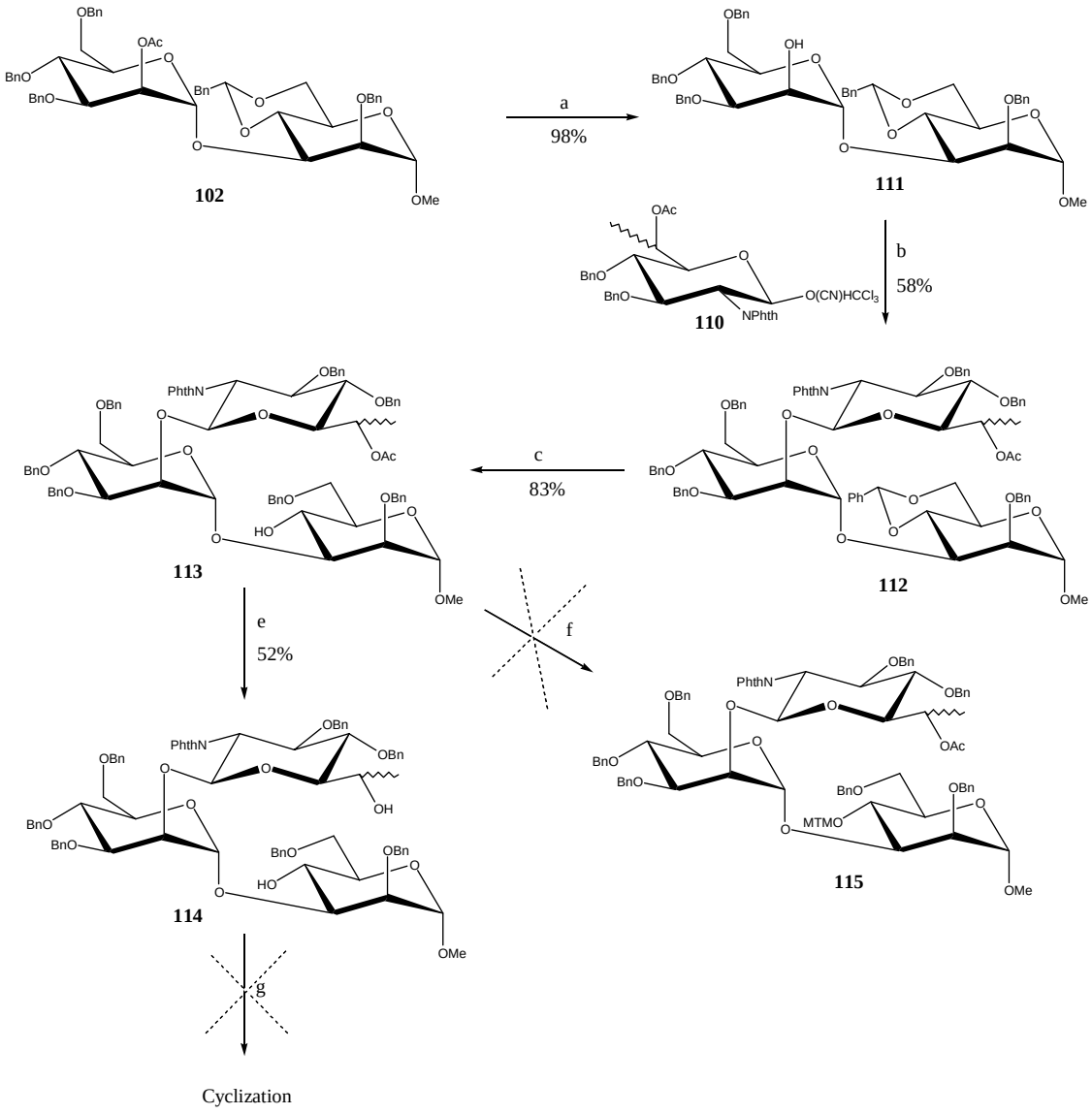


a) $ZnCl_2$, NaOAc, Bu_3SnH, AcOH, H_2O. b) trichloroacetonitrile, DBU, DCM.

Scheme 2.8

The new approach started with the deacetylation of previously synthesized disaccharide **102** to afford disaccharide **111** (Scheme 2.9). The glycosylation reaction was conducted by treating a mixture of glycosyl acceptor **111** and trichloroacetimidate **110** in dry methylene chloride at –20^{o}C with a catalytic amount of $BF_3.OEt_2$.[145,147-149]

Under these conditions, only the β-anomer of the trisaccharide **112** was formed.[150,151] This result was confirmed by ^{1}H NMR spectrum showing a large $J_{1",2"}$ coupling constant associated with trans diaxial hydrogen substituents ($J_{1",2"}$= 8.5 Hz).



a) NaOMe, MeOH. b) $BF_3.Et_2O$, DCM, -20 ^oC. c) $NaBH_3CN$, THF, HCl. e) NaOMe, MeOH. f) methyle sulfide, benzoyle peroxide, CH_3CN. g) NIS, formaldehyde diphenyl mercaptal, TfOH, CH_3CN, 4 Å MS.

Scheme 2.9

Since the R_f values of the disaccharide and trisaccharide were extremely close, regular silica gel column chromatography could not be used to purify compound **112**. The separation was therefore achieved by size exclusion chromatography (Sephadex LH-20, CH_2Cl_2/MeOH, 1/1, v/v) and pure trisaccharide **112** was obtained in an acceptable 58% yield.

The next step in the synthesis was the selective opening of the benzylidene acetal at the C-6 position of the reducing mannoside, using $NaBH_3CN$ and hydrogen chloride in THF.[138] Resulting trisaccharide **113** was then subjected to deacetylation by potassium tert-butoxide in methanol to afford diol **114** in 52% yield. Again, this low yield is the result of opening of one side of the phthalimido functionality.

Cyclization was attempted on trisaccharide **114** by intramolecular formation of a methylene acetal following a protocol described by Magnusson.[108] Compound **114** was treated with formaldehyde diphenylmercaptal activated with NIS and TfOH at –35 ^oC. Two hours after the reagents were added, no progression was observed. The reaction was carried on overnight with no success. Neither the addition of more triflic acid, nor raising the temperature to 0 ^oC yielded the desired cyclic compound. After two more hours of reaction, degradation of the starting material started to occur. The failure of that step led us to consider another approach to form the necessary methylene acetal.

As described in the first approach, reacting a free hydroxyl with a methyl thiomethyl moiety in the presence of triflic acid can form a methylene acetal. It was then decided to introduce a methyl thiomethyl moiety on the free hydroxyl at the C-4 position of the reducing mannoside of **113**. As previously described, this trisaccharide was treated with dimethyl sulfide with benzoyl peroxide added portionwise in acetonitrile.

Unfortunately, the reaction remained ineffective after 18 hours under the exclusion of light and any attempt to repeat the same reaction failed to give the desired product.

2.3.3 Conclusion

The synthesis of compounds **70** and **71** was attempted by two different approaches, an intramolecular glycosylation and an intramolecular methylene acetal formation. In both cases, the synthesis was successful up to the linear trisaccharide stage. However, unsuccessful synthetic steps occurring either during the preparation for cyclization or the cyclization itself resulted in the impossibility to obtain either targeted cyclic trisaccharides. After many challenges and lessons learned in spite of a disappointing outcome, our efforts focused on the synthesis of other conformationally constrained trisaccharides.

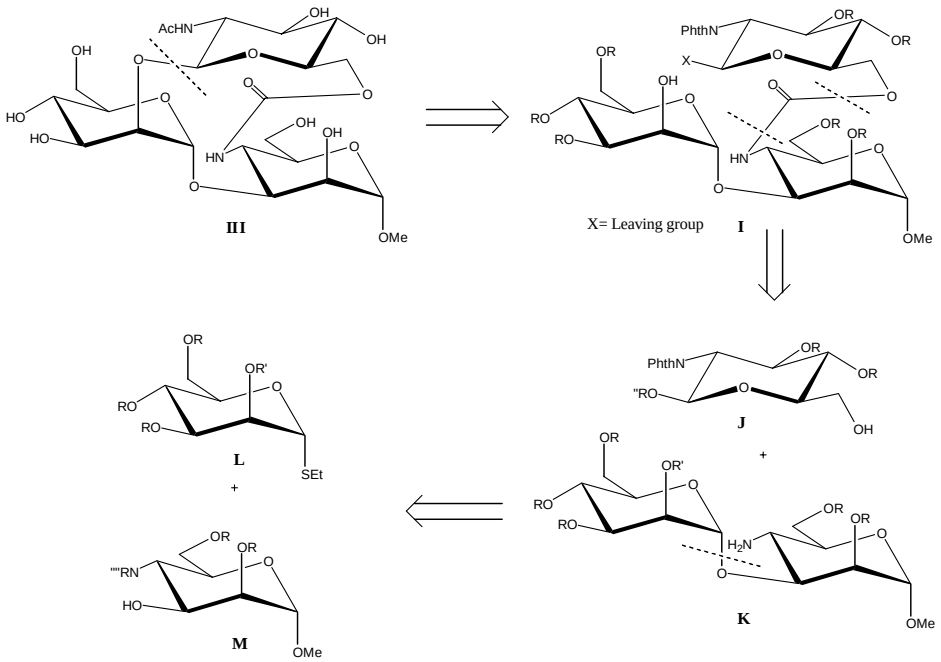
2.4. Synthesis of (2-N-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6”-carbamate (**72**)

2.4.1 Intramolecular Glycosylation Approach

Trisaccharide (2-N-acetyl-2- deoxy -β-D- glucopyranosyl)-(1→2)- (α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6”-carbamate (**72**) is a very unusual carbohydrate for two reasons. Its cyclic character is brought by a carbamate bond, which has rarely been used in the field of carbohydrate chemistry to link monosaccharides. The second interesting feature of this compound is the presence of a 4-amino sugar in its

structure. Since this type of amino sugar cannot be found in nature, it has to be synthesized starting from an existing natural monosaccharide.

The retrosynthesis describing the intramolecular glycosylation approach for the cyclization is depicted in Scheme 2.10. It appears that linear trisaccharide **I** could be used as a precursor in the cyclization leading to **72**. **X** represents a leaving group such as a trichloroacetimidate or an ethylthio moiety. Trisaccharide **I** could be assembled by formation of a carbamate bond between disaccharide **K**, where an amino group replaces an hydroxyl at the C-4 position, and monosaccharide building block **J,** which contains a free hydroxyl at the C-6 position. Disaccharide building block **K** could be obtained from the assembly of thioglycoside **L** and monosaccharide building block **M,** which also contains a free hydroxyl at the C-3 position.



Scheme 2.10

The synthesis of trisaccharide **72** then requires thioglycoside building block **79**, monosaccharide building block **116** and protected 4-amino sugar **117** (Figure 2.12)

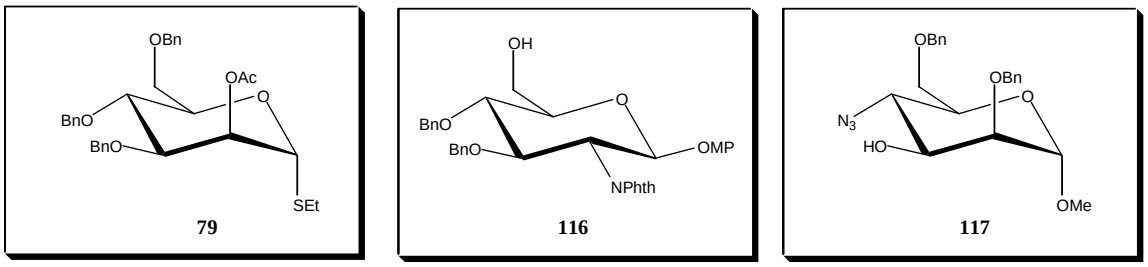
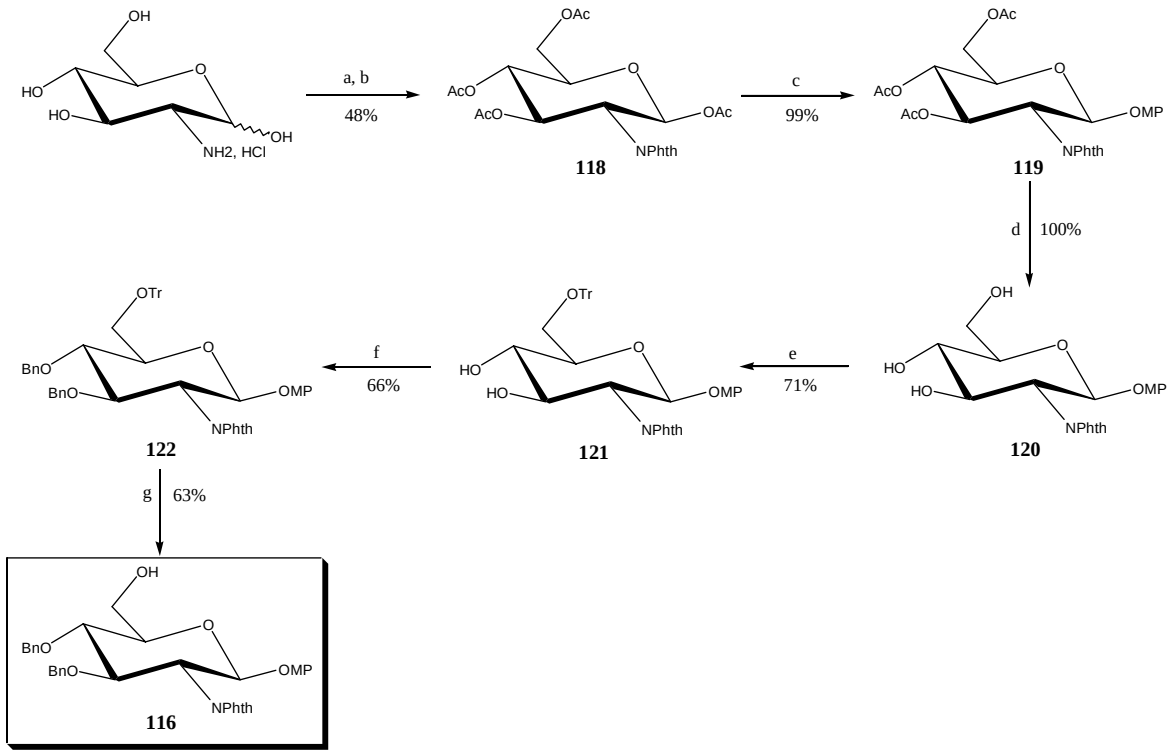


Figure 2.12

The synthesis of building block **79** was described in section 2.2.1. The synthesis of building block **116** started by the introduction of a phthalimido protecting group on the free amine of commercially available glucosamine. It was followed by the acetylation of all free hydroxyls (Scheme 2.11). Glucosamine hydrochloride was then treated with phthalic anhydride and sodium hydrogencarbonate as a base in water to afford 2-deoxy-2-N-phthalimido-β-D-glucopyranoside.[152] The latter intermediate was subsequently acetylated with acetic anhydride and sodium acetate to afford compound **118**, which was mainly formed as β-acetate (β/α > 20/1, 48% yield). It is interesting to note that the β-anomer can be selectively crystallized using 2-propanol and water.

The next step consisted in a glycosylation performed at the anomeric center, with *p*-methoxy phenol in the presence of TMSOTf as Lewis acid promoter to yield compound **119** in an excellent yield of 99%.[153-155] Deacetylation using freshly prepared methanolic sodium methoxide afforded triol **120** in a quantitative yield. This step was followed by the selective introduction of a trityl protecting group on the free hydroxyl at the C-6 position by treatment with trityl chloride in pyridine and a catalytic amount of

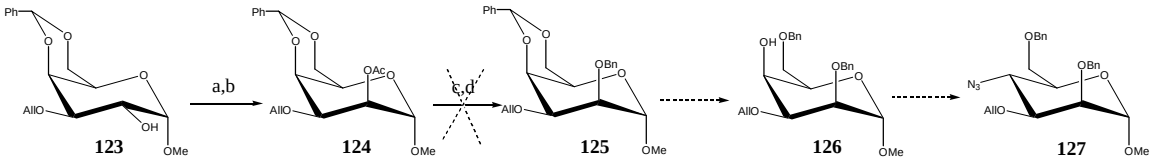
DMAP (80% yield). Resulting compound **121** was benzylated by refluxing benzyl bromide, sodium hydride and a catalytic amount of tetrabutyl ammonium iodide in dry THF. Special care had to be taken during the work up of this reaction, since the addition of methanol to quench the excess of NaH, could lead to the excessive formation of sodium methoxide, that would, in turn, harm the phthalimido protecting group. The reaction mixture was then poured into iced water, and the aqueous layer extracted with ethyl acetate, which gave benzylated compound **122** in 66% yield. The next step consisted in removing the trityl group at the C-6 position by treatment with tetrafluoroboric acid (48% in water) to afford building block **116** in 63% yield.[112,156]



a) Phtalic anhydride, $NaHCO_3$, H2O. b) NaOAc, Ac_2O. c) *p*-methoxyphenol, TMSOTf, DCM. d) NaOMe, MeOH. e) TrCl, pyridine, DMAP. f) BnBr, TBAI, THF, NaH, reflux. g) HBF_4, 48% in H_2O, acetonitrile.

Scheme 2.11

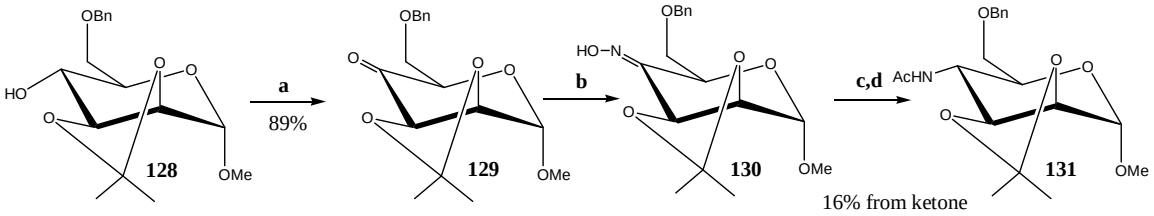
Building block **117** is the last of the three building blocks required to complete the synthesis of **72**. Compound **117** is an unnatural 4-amino sugar which synthesis had to be started from an existing sugar. It was first considered to start from a suitably protected galactoside, on which two inversions of configuration would be performed. The first inversion would be achieved at the C-2 position by introducing an ester such as acetate or benzoate.[134,135] A second inversion could be performed at the C-4 position by introducing an azido functionality,[157] which, when reduced to an amine, would yield the desired 4-amino sugar. Such a strategy is depicted in Scheme 2.12. Monosaccharide **123** was prepared using conventional protection-deprotection interconversions. A triflate leaving group was formed on the free hydroxyl at the C-4 position by treatment of compound **123** with triflic anhydride in pyridine.[134] The obtained triflate was displaced via a S_N2 mechanism using cesium acetate in DMF to afford taloside **124** in 47% yield. This low yield is not surprising since the S_N2 mechanism causes the nucleophile to approach by the top face, which is sterically disfavored by the presence of axial OH-4.[158,159] The regeneration of compound **123** can occur following two mechanisms: Either the triflate decomposes and the starting material is regenerated, or the triflate leaves, which will result in the formation of a carbocation leading to an S_N1 type reaction, where both isomer **123** and **124** can be produced. Because a permanent benzyl protecting group is needed at OH-2, the acetate must also be removed. Building block **124** was treated with freshly prepared methanolic sodium methoxide. Unfortunately this step resulted in the very surprising formation of a disaccharide, whose structure could not be determined. Any attempt to deprotect the acetyl was unsuccessful and another route had to be considered.



a) triflic anhydride, pyridine. b) CsOAc, DMF. c) NaOMe, MeOH.

Scheme 2.12

In a second attempt to synthesize building block **117**, a double inversion of configuration at the C-4 was considered. In view of the difficulties previously encountered to achieve a talose configuration using a S_N2 type reaction (steric interactions between a top face approaching nucleophile and an axial protected hydroxyl) it was decided adopt a different reaction sequence to inverse the configuration.



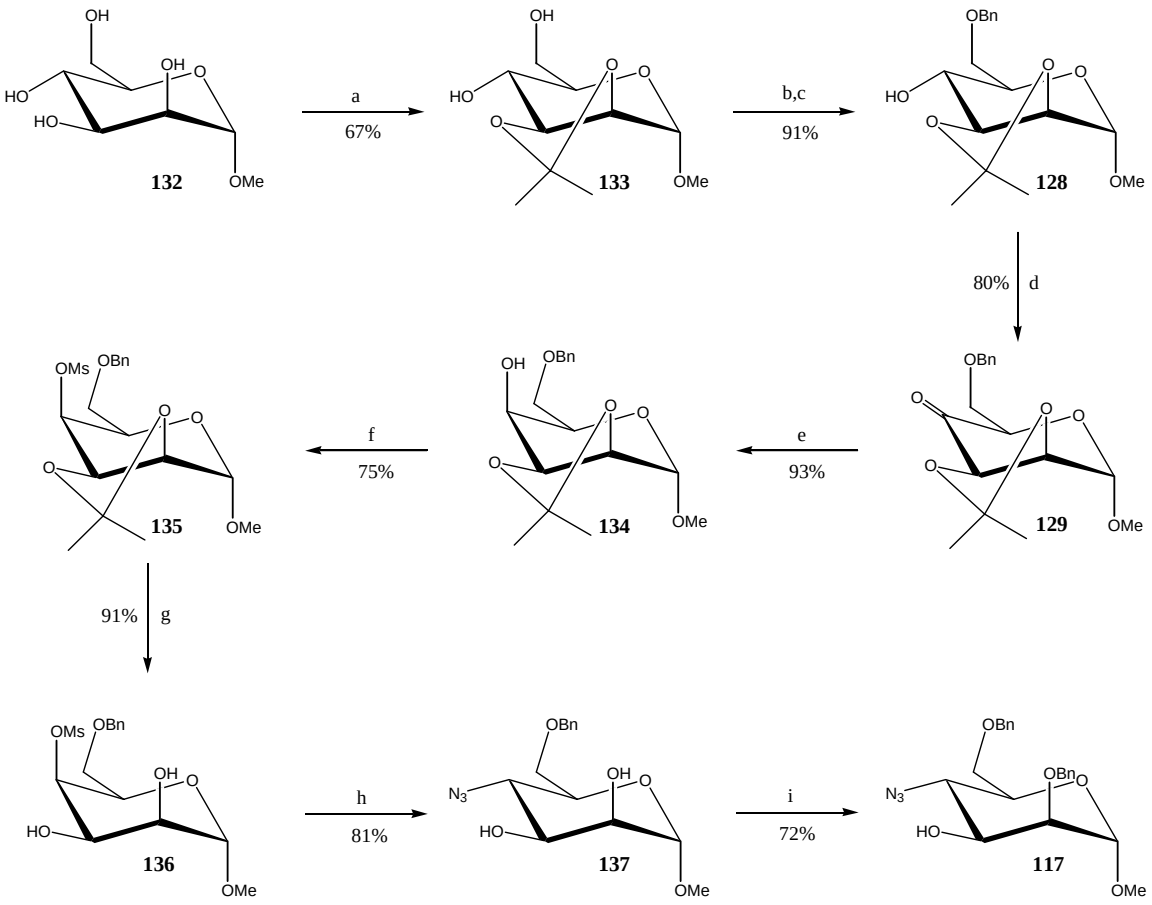
a) DMSO, TFAA, Et_3N,DCM, -65 ^oC. b) NaOH, NH_2OH, HCl. c) $LiAlH_4$, Et_2O. d) Ac_2O, pyridine.

Scheme 2.13

Starting from suitably protected mannoside **128**, Swern oxidation was achieved in 80% yield to give ketone **129**.[125] This monosaccharide was then treated with a mixture of NaOH and ammonium hydroxide in ethanol to yield unstable oxime **130** (Scheme 2.13).[131,160-163] Immediate reduction of the latter compound with lithium aluminium

hydride[164,165] in diethyl ether followed by an acetylation using conventional method afforded desired building block **131** in 16% yield over three steps. Unfortunately, this already extremely low yield was also associated with the 64% production of the other epimers. Furthermore, the removal of the acetate protecting group of compound **131**, which is necessary to form the amine required to synthesize the carbamate bond, proved impossible.

Since the oxime was reduced to an amine in axial configuration, it was hypothesized that the reduction of the ketone precursor would lead to the same result. This hypothesis was verified in a new route that was used to synthesize building block **117** (Scheme 2.14). Starting from commercially available methyl α-mannose **132**, the regioselective protection of 2,3-*cis* diol with isopropylidene group was achieved in two steps.[166] The treatment of compound **132** in dry acetone with 2,2-dimethoxypropane, using (±)-10-camphor sulfonic acid as the catalyst, gave the fully blocked 2,3:4,6-tetra-*O*-isopropyliene-α-D-mannopyranoside intermediate. Water was subsequently added to the solution to selectively hydrolyze the 4,6-acetal, which is more labile because of 1,3-diaxial interactions occurring in the 6-membered ring. This reaction had to be carefully monitored since 2,3-di-O-isopropylene can also be cleaved after prolonged reaction time, which in turn could cause its hydrolysis and a return to the starting material. However, the insolubility of the starting material indicated when to quench the reaction.



a) 2,2-dimethoxypropane, CSA, acetone. b)$Bu_3Sn(OMe)_2$, toluene, reflux. c) BnBr, TBAI, toluene, reflux. d) Oxalyl chloride, DMSO, DCM, Et_3N. e) $NaBH_4$, EtOH, H_2O. f) MsCl, DCM, Et_3N. g)20% TFA in DCM. h) NaN_3, DMF. i) BnBr, TBAI, DCM, H_2O, NaOH.

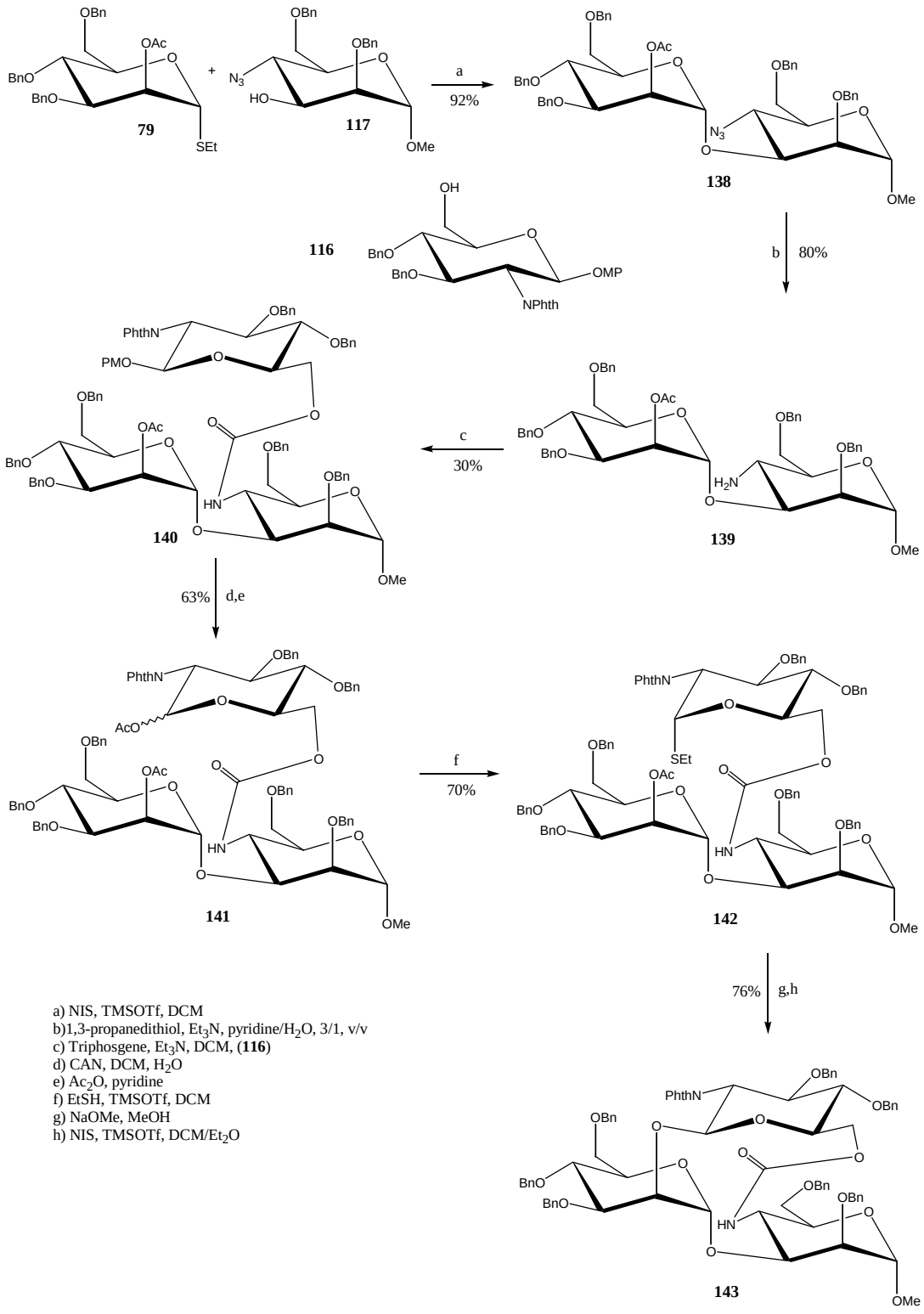
Scheme 2.14

In the next step, selective benzylation was achieved on the free hydroxyl at the C-6 position. Compound **133** was refluxed for 18 hours in the presence of tributlyltin diemthoxide in toluene. Benzyl bromide and tetrabutyl ammonium iodide were then added and the solution was refluxed 18 additional hours. After conventional work up and column chromatography, compound **128** was obtained as a single isomer in 91% yield. Monobenzylated building block **128** was subjected to Swern[125] oxidation to afford ketone **129** before reduction to compound **134** using sodium borohydride in a mixture of

ethanol and water.[167] A mesylate leaving group was then introduced at the free hydroxyl of taloside **134** to afford compound **135** in an acceptable yield of 75%.[167] The next step of the synthesis was to remove the isopropylidene to yield inversion of configuration precursor **136**. Monosaccharide **136** was then treated with sodium azide in hot DMF (100 ^{o}C) to afford mannoside **137** as a single isomer.[157] The final step was the selective benzylation of the free hydroxyl located at the C-2 position of **137** using phase transfer catalysis to afford target building block **117**.[168,169] The structure of the target compound was confirmed by acetylation of a small portion of the building block, on which it was verified that H-2 was chemically shifted downfield from 3.72 ppm to 5.12 ppm (compound **118**).

All three building blocks in hands, trisaccharide **72** was ready to be assembled. NIS/TMSOTf mediated glycosylation of donor **79** with acceptor **117** afforded the desired disaccharide **138** in a good yield of 92% (Scheme 2.15).

Treatment of disaccharide **138** with 1,3-propanedithiol in wet pyridine and in the presence of a catalytic amount of triethyl amine resulted in the reduction of the azido to an amine.[159,168-170] The complete conversion of the starting material was monitored by TLC analysis using ninhydrin as a revelator. Starting from the thus obtained amine **139**, the formation of the carbamate bond was attempted. Triphosgene was added to a solution of **139** in methylene chloride in the presence of triethyl amine, resulting in the formation of an isocyanate that was reacted with the free hydroxyl at the C-2 position of building block **116**.[171-173] Linear trisaccharide **140**, bearing a carbamate linker, was obtained in 30% yield.


79
117
a
92%
138
116
b
80%
c
30%
139
140
63%
d,e
141
f
70%
142
76%
g,h
143
a) NIS, TMSOTf, DCM
b)1,3-propanedithiol, Et3N, pyridine/H2O, 3/1, v/v
c) Triphosgene, Et3N, DCM, (116)
d) CAN, DCM, H2O
e) Ac2O, pyridine
f) EtSH, TMSOTf, DCM
g) NaOMe, MeOH
h) NIS, TMSOTf, DCM/Et2O

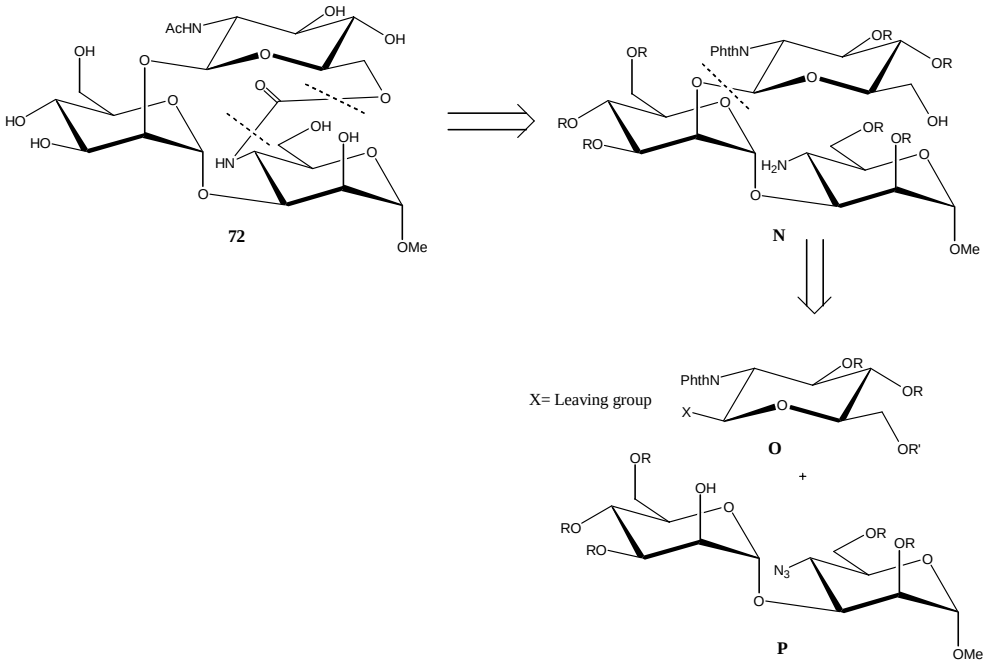
Scheme 2.15

Any attempt[174-176] to increase the previously obtained yield resulted in urethane formation when the amine of **139** reacted with the freshly formed isocyanate. In some cases, the excess triphosgene even caused two hydroxyls of two different molecules of **116** to form carbonate as a side product. The activation of **116** by formation of an alkoxide or a tin adduct before it was added to the reaction mixture also failed to improve the overall yield of the reaction.

The *p*-methoxy benzyl of **140** was removed using CAN[153,154] and the obtained trisaccharide was acetylated for purification purposes using acetic anhydride in pyridine to afford compound **141**. Ethanethiol in the presence of Lewis acid catalyst TMSOTf in methylene chloride was used to form thioglycoside **142** in 63% yield.[111] Deacetylation of the latter trisaccharide led to a cyclization precursor, which was treated with a diluted solution of NIS and TMSOTf to afford cyclic trisaccharide **143** in an excellent yield of 76%. However, NMR spectroscopy revealed the presence of two compounds in 1 to1 ratio, most probably isomers since only one mass was determined by mass spectroscopy. Both preparative TLC and HPLC failed to separate these two molecules. It was then hypothesized that during the glycosylation that led to cyclic trisaccharide **143**, an α/β mixture was obtained at the anomeric center of the glucosamine. To assess this theory and further attempt the cyclization of the desired trisaccharide, a second synthetic route was considered. The new approach consisted in the synthesis of a linear trisaccharide by conventional glycosylation, followed by a cyclization by carbamate bond formation.

2.4.2 Intramolecular Carbamate Bond Formation

A retrosynthetic analysis of cyclic trisaccharide **72** is shown in Scheme 2.16 below.



Scheme 2.16

Cyclization by carbamate bond formation could start from linear trisaccharide **N**, where a free amine at the C-4 position of the reducing mannose could react with the free hydroxyl at the C-6 position of the glucosamine using a reagent such as phosgene. It was proposed that compound **N** could be assembled from donor **O** and acceptor **P**.

To achieve the synthesis of **72** using this approach, **144** is the only required new building block (Figure 2.13).

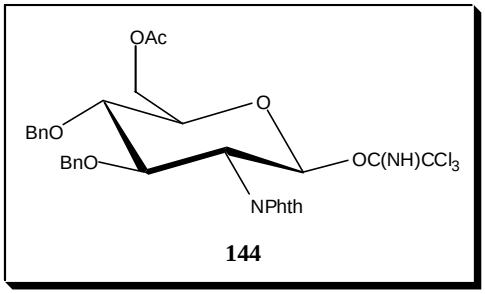
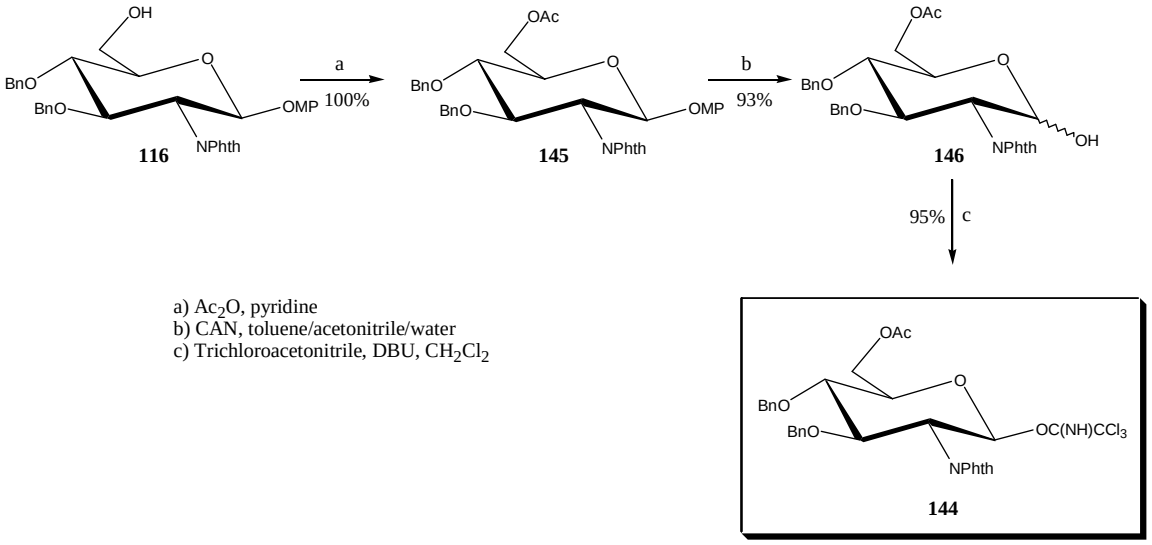


Figure 2.13

The synthesis of this building block can be achieved starting from previously described monosaccharide **116** (Scheme 2.17).

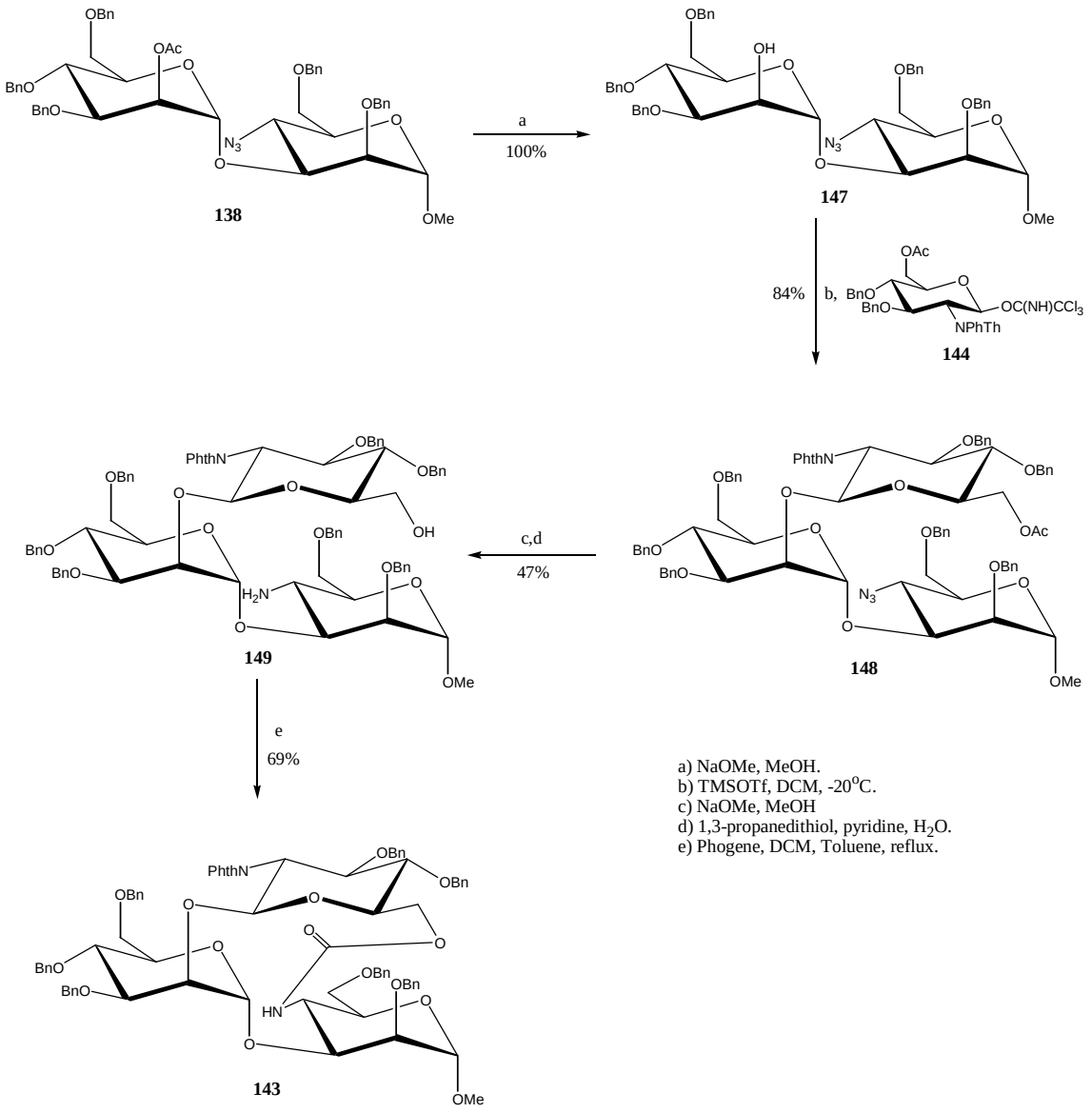


Scheme 2.17

Building block **116** was acetylated to afford compound **145** quantitatively. The removal of the *p*-methoxyphenyl protecting group of **145** was achieved using CAN[153,154] in a mixture of acetonitrile, toluene and water to afford compound **146** in 95% yield. In the last step of this synthesis, a trichloroacetimidate leaving group was introduced at the anomeric center of glucosamine **146** to yield building block **144**.

The synthesis of trisaccharide **72** can be achieved from disaccharide **138** and monomeric building block **144** (Scheme 2.18). The acetyl of disaccharide **138** was first removed by treatment with sodium methoxide in methanol to afford glycosyl donor **147**. Disaccharide **147** was then coupled with building block donor **144** in methylene chloride at low temperature, using TMSOTf Lewis acid as promoter.[147,148] Trisaccharide **148** was obtained as a single β-anomer in 84% yield. In the following steps, both ends of **148** were deblocked. First, the trisaccharide was deacetylated using freshly prepared methanolic sodium methoxide. In a second step, the azido group was reduced using 1,3-propanedithiol in wet pyridine to afford cyclization precursor **149**.[177] It is interesting to note that this step requires the use of wet pyridine to be successful. The cyclization step was first attempted using phosgene in methylene chloride in the presence of triethylamine.[178,179] After a few hours of reaction, TLC analysis showed complete conversion of the starting material into a new product. However, the starting amine was surprisingly recovered after an aqueous work up. The only explanation was that the obtained product was an isocyanate that reacted with water to regenerate the starting material. We assumed that an acyl chloride would be formed and react with the free hydroxyl. Instead, steric hindrance slowered the reaction, which, in turn, prompted the formation of an isocyanate from the rearrangement of the acyl chloride.

In a second attempt, the reaction was performed in toluene and after formation of the isocyanate, the reaction mixture was refluxed for two days. Trisaccharide **143** was thus obtained in a fairly good yield of 68%. However, to our surprise, the NMR spectra for the two approaches were identical. In the second approach, no anomeric mixture could possibly be obtained, since no stereoselectivity was involved.

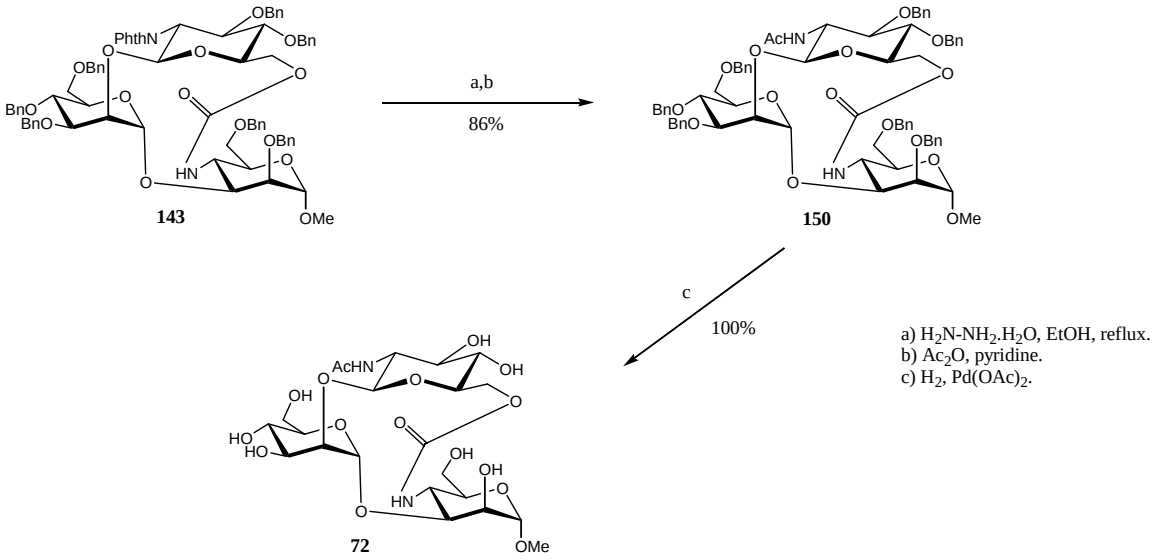


Scheme 2.18

In-depth NMR spectroscopy analyses revealed that the two obtained compounds that we initially thought were isomers, were in fact optic rotamers, which arise from the presence of the amide linkage of the carbamate functionality. ^{1}H NMR spectra recorded at different temperatures showed a shift from a mixture of two compounds to a single compound. Trisaccharide **143** being very rigid, the rotation between the two forms *cis*

and *trans* is probably extremely slow on then NMR timescale, resulting in an NMR spectrum showing two different molecules.

Trisaccharide **143** was then deprotected to yield target compound **72** (Scheme 2.19).



Scheme 2.19

The first deprotection step is the conversion of the phthalimido into an acetyl group. Compound **143** was then treated with hydrazine hydrate in refluxing ethanol.[180-183] The obtained free amine intermediate was acetylated using acetic anhydride and pyridine to yield the desired NHAc functionality. After column chromatography, trisaccharide **150** was obtained in 86% yield. Debenzylation by catalytic hydrogenation over $Pd(OAc)_2$ completed the deprotection and afforded conformationally constrained trisaccharide **72** in quantitative yield. This compound was obtained as a 1/1 mixture of *cis/trans* at the amide linkage.

The full assignment of the NMR spectrum for compound **72** was realized using a combination 2D experiments (COSY, HSQC, TOCSY, HSQC-TOCSY). HMBC and ROESY experiments allowed the assignment for each set of data to one of the *cis* or *trans* rotamer. These last two experiments and a measurements of $J_{C1,H1}$ coupling constants for both compounds confirmed the anomeric configurations for each monosaccharides constituting these trisaccharides and the *cis* and *trans* mixture at the carbamate amide bond. Exchanged cross peaks complicated further the ROESY spectrum where a lot of overlapping is occurring because of the presence of these two structures in solution. In this context, it became extremely difficult to conclude about the conformational properties of these trisaccharides and further NMR experiments are needed to realize this study.

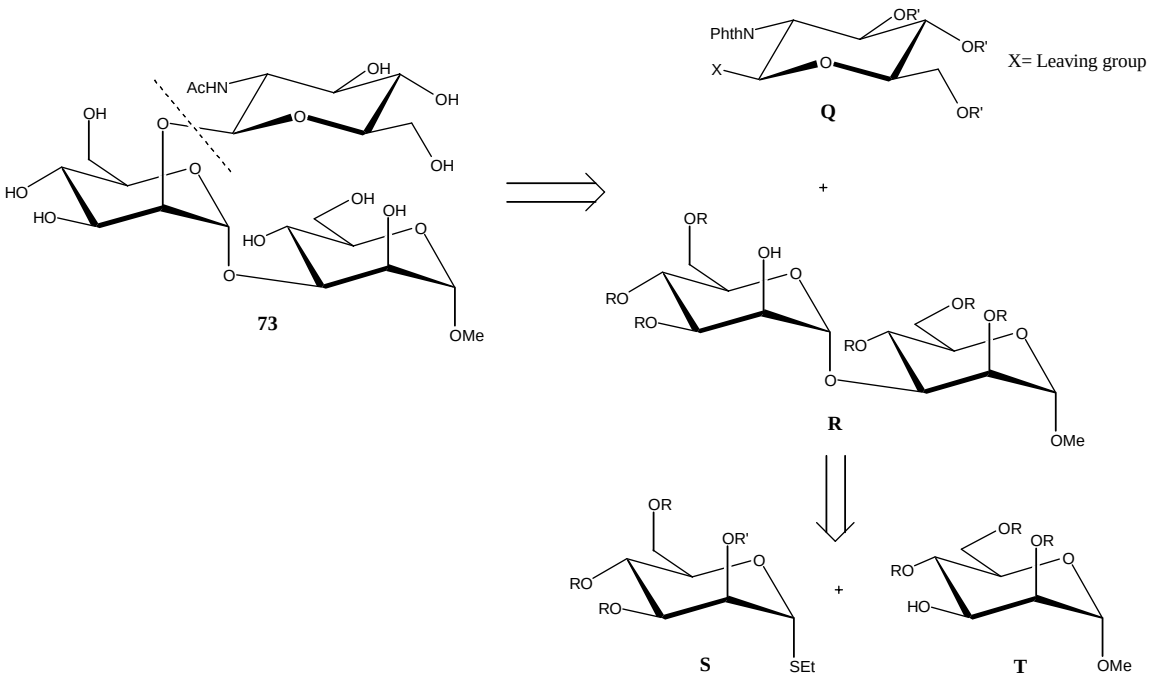
2.4.3 Conclusion

The synthesis of compound **72** was achieved using two different approaches. An intramolecular glycosylation route gave compound **72** but the low yielding intermolecular carbamate bond formation appeared to be a limiting step and doubts about the stereoselectivity in the glycosylation step prompted us to use a second approach. In the second route, an intramolecular carbamate bond formation was used for the macrocylization step that gave compound **143** in an excellent yield of 69%. Cyclic trisaccharide **72** was obtained as a mixture of *cis* and *trans* rotamers around the amide bond of the carbamate bond in rapid dynamic exchange. This compound is currently subject to an on-going thermodynamic study upon binding with lectin Con A.

2.5. Synthesis of Methyl (2-N-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (**73**)

2.5.1 Synthesis

Linear trisaccharide **73** will be used as a reference when testing conformationally constrained trisaccharides by ITC and therefore needs to be synthesized. As depicted in retrosynthetic analysis (Scheme 2.20), trisaccharide **73** could be assembled from monosaccharide donor **Q**, which contains a trichloroacetimidate at its reducing end, and disaccharide **R**, which has a free hydroxyl at the C-2 position of the non-reducing mannose. In a similar way, disaccharide building block **R** could be assembled from thioglycoside donor **S** and acceptor building block **T**.



Scheme 2.20

In order to achieve the synthesis of trisaccharide thioglycoside **79**, imidate **151** and glycosyl acceptor **152** (Figure 2.14) were synthesized using standard protection-deprotection interconversions.

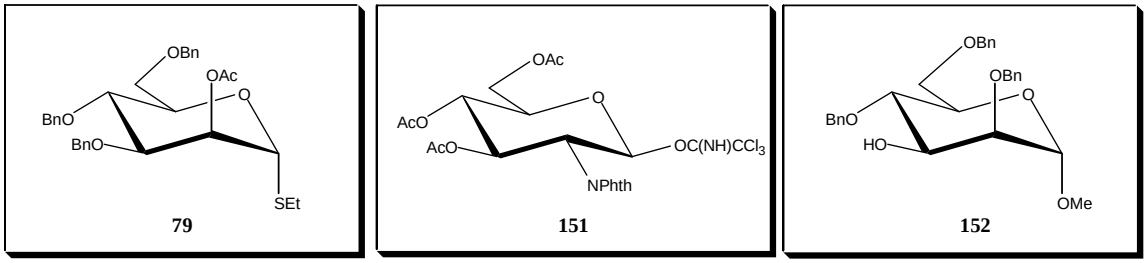
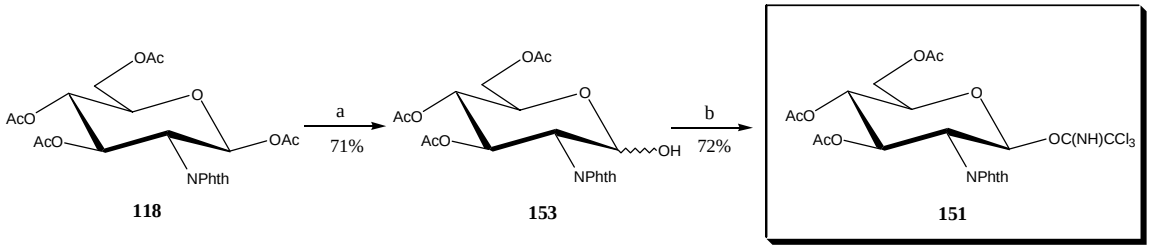


Figure 2.14

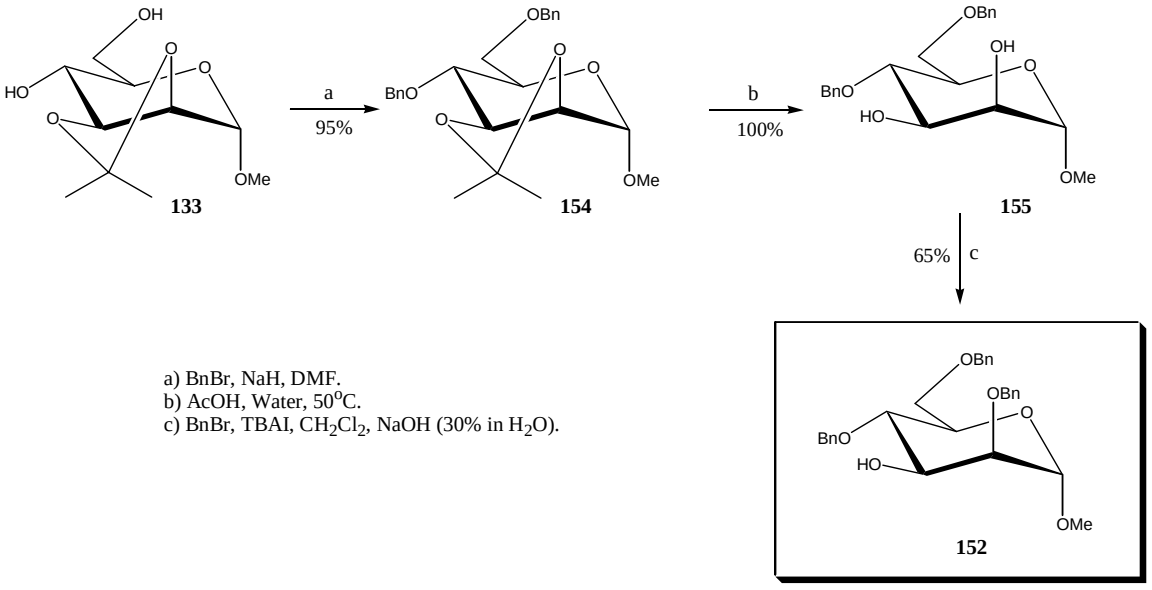
The synthesis of building block **79** is described in section **2.2.1**. Compound **151** was obtained in two steps, starting from previously described intermediate **118**. In a first step, the regioselective anomeric deacetylation was achieved by treatment with hydrazine acetate in DMF yielding compound **153** in 71% yield (Scheme 2.21).[180-182] The second step consisted in the introduction of trichloroacetimidate at the anomeric center of **153**, using trichloroacetonitrile in methylene chloride in the presence of a catalytic amount of DBU. After a rapid purification by column chromatography on silica gel, building block **151** was obtained in 72% yield and was immediately glycosydated.



a) NH_2NH_2. EtOAc, DMF. b) CCl_3CN, DBU, CH_2Cl_2.

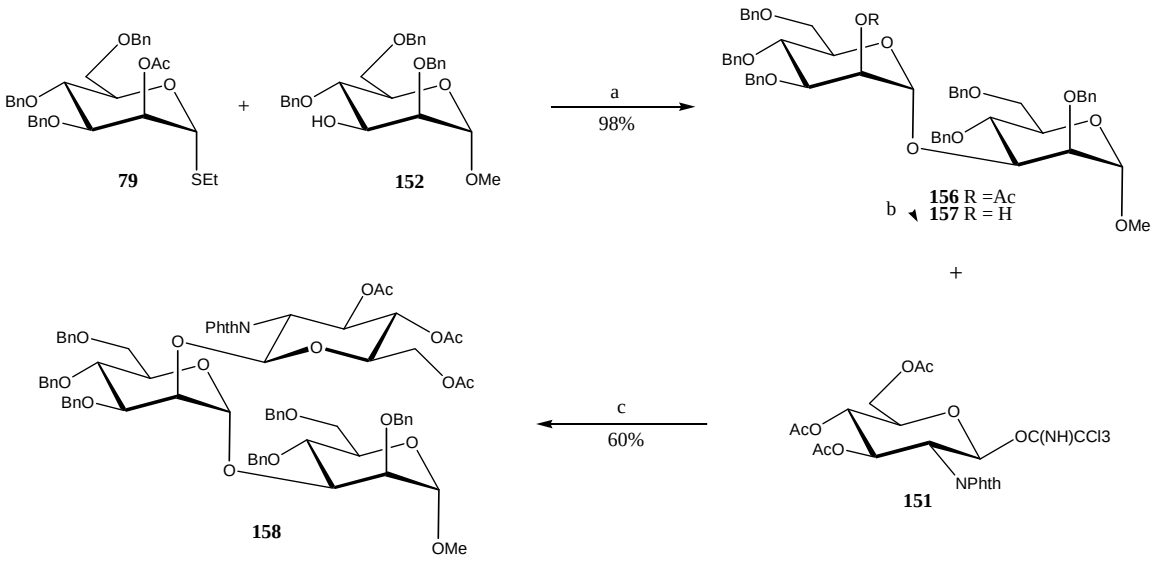
Scheme 2.21

Building block **152** was synthesized from a previously described intermediate (Scheme 2.22). Methyl 1,2-di-*O*-iospropylidene-α-D-mannopyranoside **133** was benzylated to afford compound **154** in 95% yield. This step was followed by the quantitative removal of the isopropylidene acetal by treatment of **154** with a mixture of glacial acetic acid and water (9/1, v/v) for 18 hours at 50°C to afford diol **155**. In the last step of this synthesis, the free hydroxyl at the C-2 position of **155** was regioselectively benzylated using phase transfer catalysis.[168,169] After treatment of **155** with BnBr in methylene chloride in the presence of aqueous sodium hydroxide and with TBAI as a catalyst, building block **152** was obtained in a good yield of 62%.



Scheme 2.22

Building blocks **79**, **151** and **152** being available, the synthesis of linear trisaccharide **73** was realized. The first step was the synthesis of disaccharide **156** (Scheme 2.23).



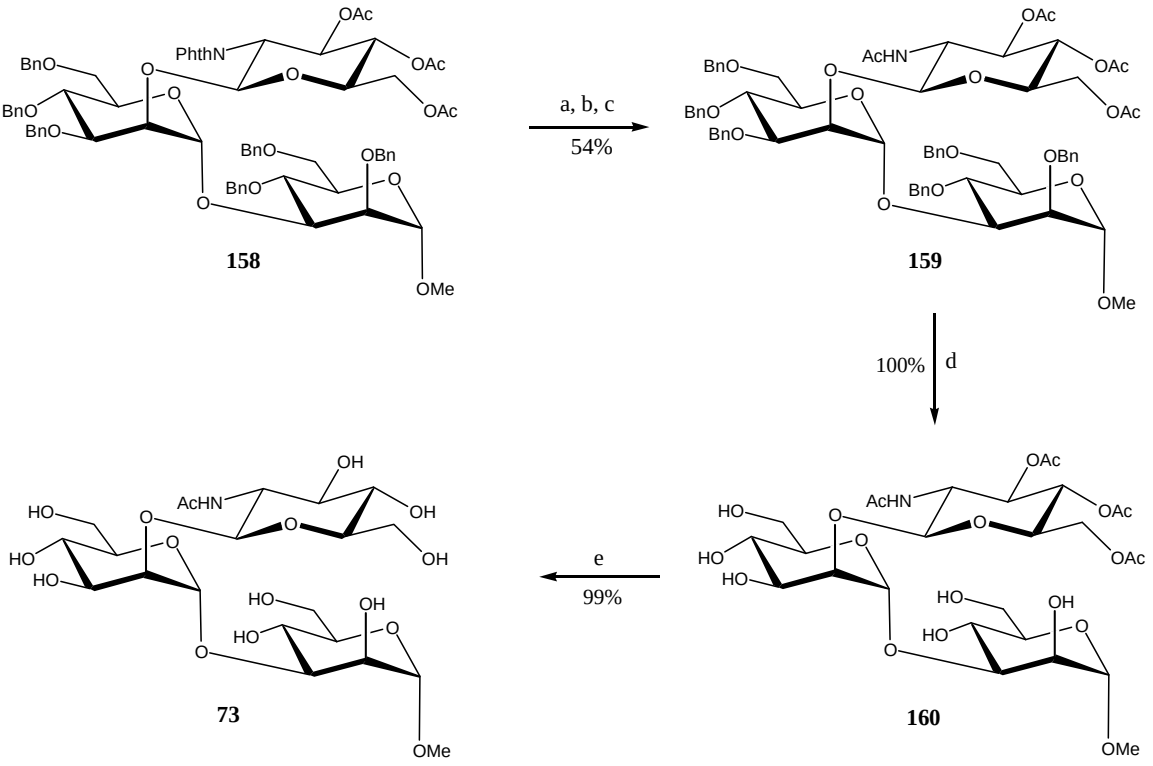
a) NIS, TMSOTF, CH_2Cl_2, Et_2O. b) NaOMe, MeOH. c) TMSOTf, CH_2Cl_2, -20°C.

Scheme 2.23

Thioglycoside **79** and glycosyl acceptor **152** were assembled by treatment with a solution containing NIS in methylene chloride and ether (4/6, v/v) in the presence of promoter TMSOTf.[137] Due to neighboring group participation of the C-2 acetyl group of **79**, **156** was produced as a single α-isomer. The acetyl group of disaccharide **156** was then removed by treatment with a solution of potassium *tert*-butoxide in methanol to afford **157** in quantitative yield after column chromatography purification. The final step consisted in the production of the protected linear trisaccharide. The glycosylation was realized by treating a mixture of glycosyl acceptor **157** and trichloroacetimidate **151** in dry methylene chloride with a catalytic amount of $BF_3.Et_2O$.[147,148] After one hour of reaction at –20 °C, the formation of trisaccharide **158** was complete, in spite of its contamination by disaccharide **157**. The newly formed trisaccharide was then purified by size exclusion column chromatography (Sephadex LH 20, methanol/CH_2Cl_2, 1/1, v/v) to

yield pure **158** in a relatively modest 60% yield. The β-linkage was selectively introduced because of neighboring group participation of the phthalimido protecting group at the C-2 position of **151.** That way, the free hydroxyl of **157** could only approach glycosyl acceptor **151** by its β-face.

The remaining of the synthesis consisted in the deprotection of trisaccharide **158** towards target product **73** (Scheme 2.24).



a) NaOMe, MeOH. b) $NH_2NH_2.H_2O$. c) Ac_2O, pyridine. d) H_2, $Pd(OAc)_2$. e) NaOMe, MeOH.

Scheme 2.24

In the first step trisaccharide **158** was quantitatively deacetylated by treatment with potassium *tert*-butoxide in methanol. The resulting intermediate was then treated with hydrazine monohydrate in refluxing ethanol to remove the phthalimido before

reacetylation, resulting in the introduction of the required NHAc functionality. Trisaccharide **159** was obtained in 53% yield over three steps. **159** was then hydrogenated using palladium (II) acetate as the catalyst to yield compound **160** in 100% yield. The last step of the synthesis was the deacetylation of the last three protected hydroxyls. Trisaccharide **160** was treated with methanolic potassium *tert*-butoxide and after three hours of reaction, target compound **73** was obtained in quantitative yield. NMR spectroscopy confirmed the removal of the three acetates, but also revealed the presence of impurities. Trisaccharide **73** was then reacetylated and purified by size exclusion chromatography (Sephadex LH20, MeOH/CH_2Cl_2, 1/1, v/v) before being subjected to a new deacetylation to afford the pure target compound.

NMR analysis and mass spectroscopy confirmed the structure of trisaccharide **73**. The obtained anomeric configurations were also verified by determining the coupling constant $J_{C,H}$ for each anomeric center. Results are as follows: $J_{C1,H1}$= 169.1 Hz, $J_{C1',H1'}$= 168.8 Hz, $J_{C1'',H1''}$= 158.8 Hz. Typically, coupling constants for β-linkages average $J_{C1,H1}$= 160 Hz and $J_{C1,H1}$= 170 Hz[184,185] for an α-linkage, which confirmed correct anomeric configurations for compound **73**.

2.5.2 Conclusion

For the most part, the synthesis of linear trisaccharide **73** was achieved without major challenges and in a high-yielding manner. Except from the transformation of the phthalimido into the acetyl functionality, the deprotection steps also provided good yields. Overall, the synthetic route proved effective and produced the final trisaccharide in a sufficient amount to conduct tests and analyses on its biological properties.

2.6. Synthesis of 3-amino-propyl (2-*N*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6" methylidene acetal (**74**) and (2-*N*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (**75**)

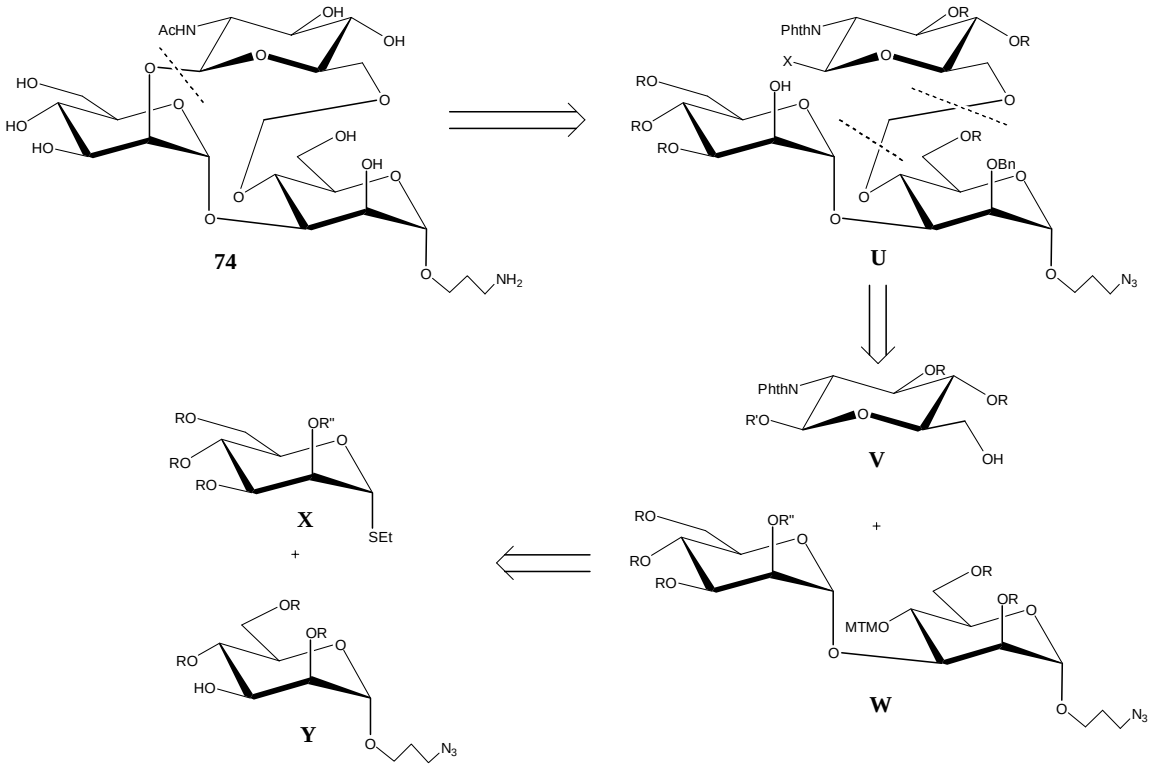
In general, carbohydrate protein-interactions are characterized by fast association and dissociation rates. And this characteristic has to be taken into account when designing a drug. A good inhibitor for a biological process will first be recognized by a receptor, in which it will stay long enough to successfully complete its inhibition task. Due to their high flexibility, natural carbohydrates are quickly accommodated in and released from the binding site of a receptor. Based on the above observation, they do not represent ideal inhibitors candidates. Conformationally constrained oligosaccharides however were thought to be better candidates. Their reduced flexibility would result in the slower accommodation of the ligand into the binding site, as well as a slower release from the binding pocket.

In order to study the effects of conformational constraints on the kinetics of binding between oligosaccharides and proteins, compounds **74** and **75** were designed and synthesized. An alkyl linker located at the reducing end of both molecules will allow their attachment to a microchip intended to conduct Biacore experiments.

*2.6.1 Synthesis of 3-amino-propyl (2-N-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6" methylidene acetal (**74**)*

Trisaccharide **74** being similar to compound **50**, the synthetic strategy reported by Boons and co-workers was adopted.[45,140] Trisaccharide intermediate **U** can be cyclized by intramolecular glycosylation, using a leaving group such as a thio ethyl or a

trichloroacetimidate (Scheme 2.25). The formation of a methylene acetal bridge between monosaccharide **V** and disaccharide **W** will lead to trisaccharide **U**. Disaccharide building block **W** can be synthesized from thioglycoside donor **X** and glycosyl acceptor **Y** using conventional glycosylation techniques.



Scheme 2.25

The three building blocks shown in Figure 2.15 are required for the assembly of trisaccharide **74**. Although the syntheses of thioglycoside **79** and building block **116** have already been described, a new synthetic route had to be designed for the last building block **161**.

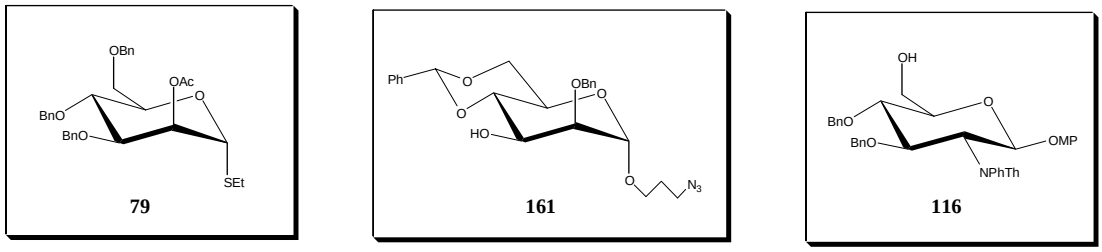
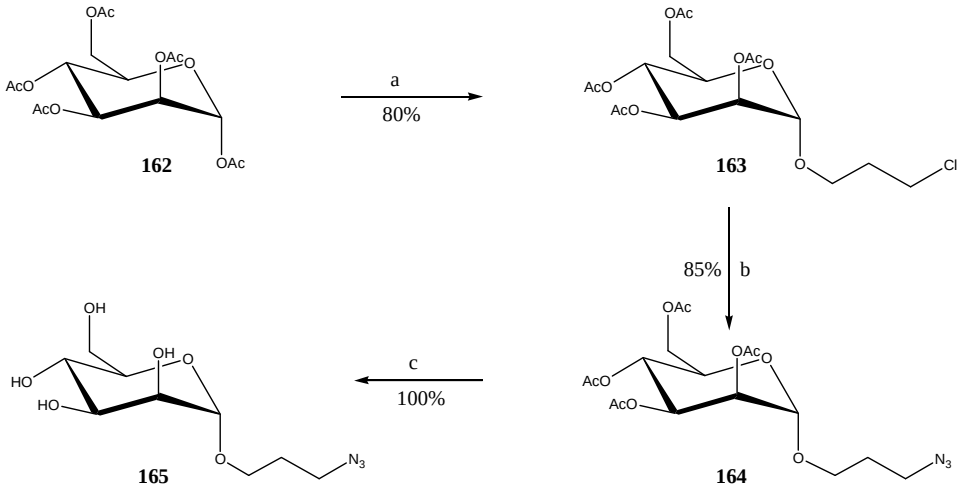


Figure 2.15

The first part of the synthesis of **161** is the introduction of the alkyl linker at the reducing end of the mannose (Scheme 2.26). Starting from *per*-acetylated α-D-mannose **162**, a three-step procedure led to 3-azido-propyl α-D-mannose **165**. A 3-chloropropyl group was first introduced at the anomeric position of mannoside **162** by treatment with 3-chloropropan-1-ol in the presence of Lewis acid $BF_3.Et_2O$.[186] Although the reaction was rather slow and required repeated additions of Lewis acid, compound **163** was nevertheless obtained. The chlorine atom was then displaced, using sodium azide in the presence of KI in DMF at 100°C for 18 hours to afford azido compound **164** in 85% yield. This reaction was very difficult to be monitored by TLC analysis, since both compounds have nearly identical *R*fs. After 18 hours, the reaction was stopped and the residue was purified by column chromatography. The displacement of the chlorine atom was confirmed by ^1H and ^{13}C NMR spectroscopy: compared to compound **163**, the signal corresponding to geminal protons in azido compound **164** was shifted to the right and was no longer overlapping the acetyl signals.

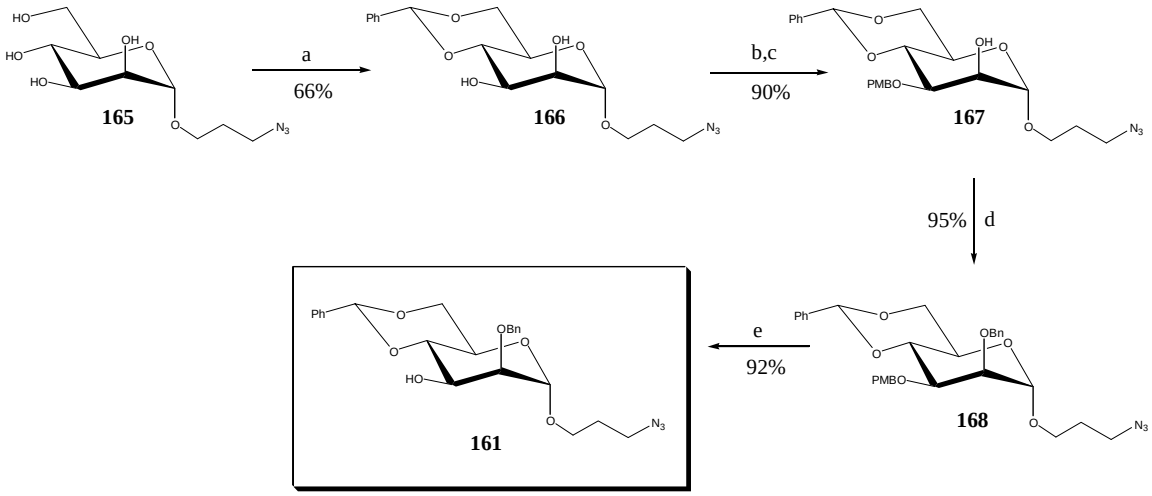


a) 3-chloropropan-1-ol, $BF_3.OEt_2$. b) KI, NaN_3, DMF. c) NaOMe, MeOH.

Scheme 2.26

In the last of the three steps, monosaccharide **164** was deacetylated using sodium methoxide in methanol to afford compound **165** in quantitative yield.

To achieve the synthesis of building block **161**, the selective introduction of a 4,6-di-O-benzylidene followed by the regioselective benzylation on the free hydroxyl at the C-2 position had to be performed. The low yielding procedure used to synthesize similar building block **80** (Section 2.2.1) was considered not suitable. Four synthetic steps are required to access starting material **165** and lose 75% of the compound at this stage would be unacceptable. It was decided to use a longer reaction sequence that was thought to give a better overall yield of 52% (instead of 15%). 3-azido-propyl α-D-mannoside **165** was treated with benzaldehyde dimethyl acetal in DMF, in the presence of tetrafluoroboric acid to afford 4,6-benzylidene compound **166** in 66% yield (Scheme 2.27).[112] ^{1}H NMR revealed the presence of a characteristic singlet at 5.55 ppm, which confirmed the successful introduction of the benzylidene.



a) Benzaldehyde dimethyl acetal, TFBA, DMF. b) $Bu_3Sn(OMe)_2$, benzene reflux. c) PMBCl, TBAI, benzene, reflux. d) BnBr, NaH, DMF. e) DDQ, DCM, H_2O.

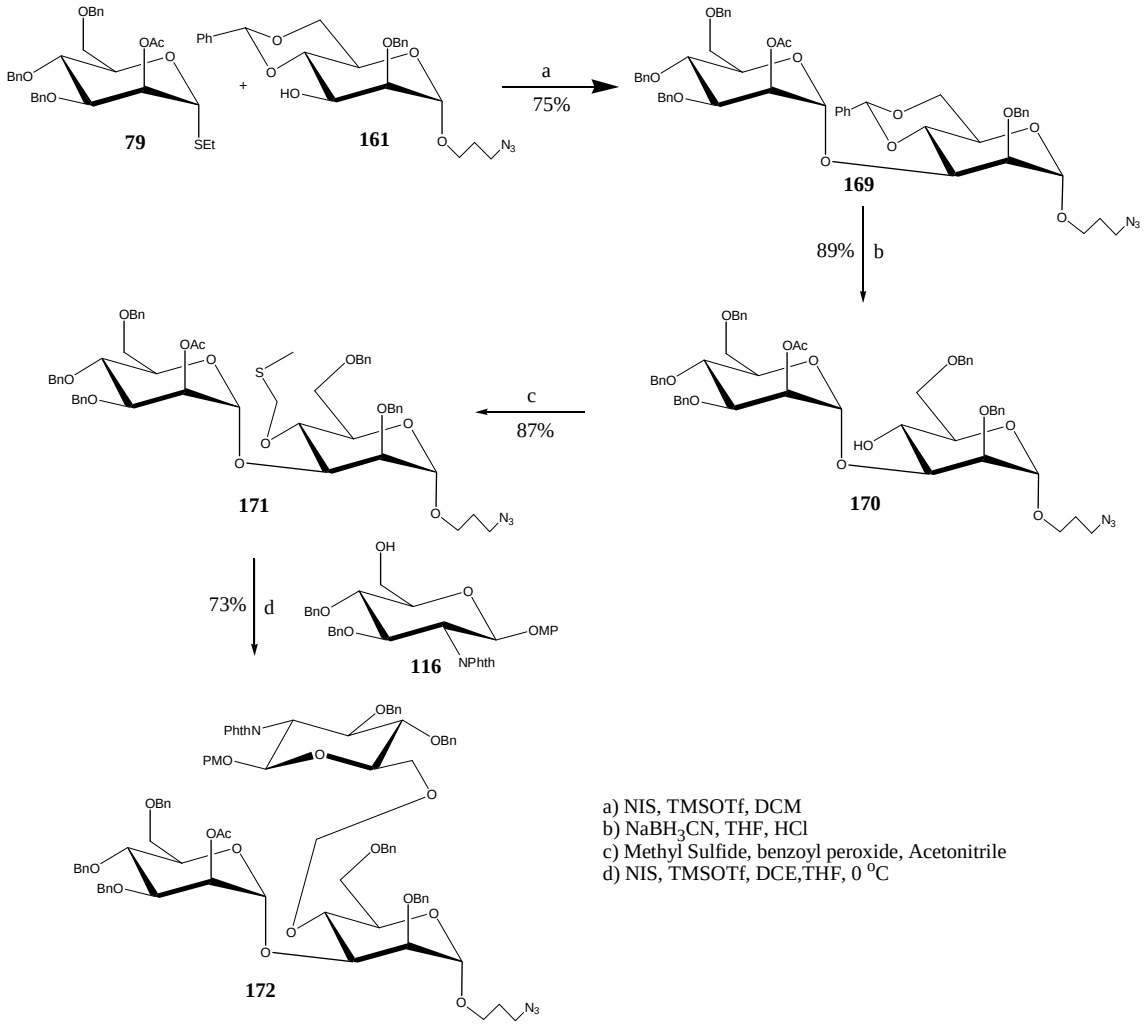
Scheme 2.27

p-methoxy benzyl was regioselectively introduced on the free hydroxyl at the C-3 position of **166** by treatment with tributyl tin dimethoxide in refluxing benzene, using a Dean Stark procedure.[187] After two hours, the Dean Stark equipment was removed, and *p*-methoxy benzyl chloride and TBAI were added to the reaction mixture. After a further two hours of reaction, an aqueous work up was performed with KF to get rid of the tin reagent. Purification by column chromatography on silica gel led to pure compound **167** in an excellent yield of 95%. The acetylation of **167** confirmed that the PMB group was introduced at the right position, and ^{1}H NMR revealed a downfield shift of the H-2 signal in the acetylated compound from 3.79 ppm to 5.18 ppm.

Monosaccharide **167** was then benzylated by treatment with sodium hydride and benzyl bromide in DMF to afford compound **168**. The PMB group was finally removed,

using phase transfer catalysis in water and a methylene chloride solvent system with DDQ to yield final building block **161** in 92% yield.[188]

In order to synthesize target trisaccharide **74**, the three necessary building blocks were assembled in a linear fashion before cyclization (Scheme 2.28).



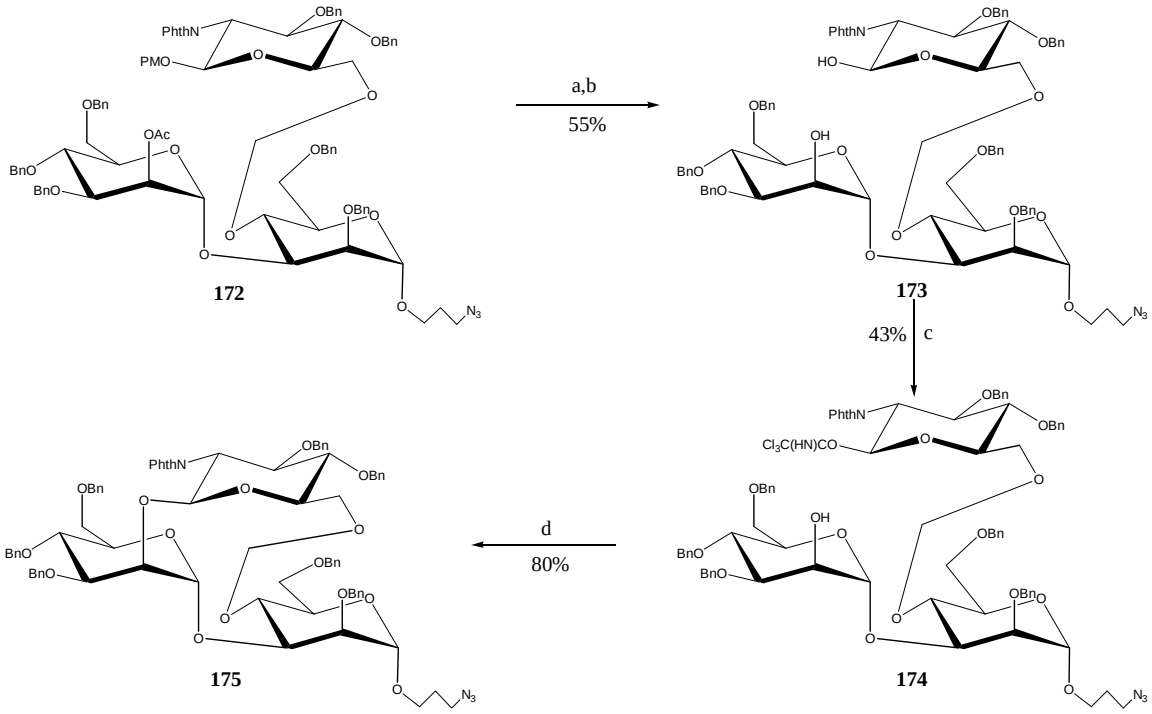
Scheme 2.28

In a first step, thioglycoside **79** and glycosyl acceptor **161** were assembled by treatment with NIS in methylene chloride in the presence of a catalytic amount of

TMSOTf to afford disaccharide **169** in a decent yield of 75%. As described before, neighboring participation of the acetate at the C-2 position of thioglycoside **79** resulted in the formation of single β-anomer. The opening of the benzylidene acetal at the C-6 position of the reducing mannose was then achieved.[138] Compound **169** was treated with sodium cyanoborohydride and hydrogen chloride in THF to afford disaccharide **170**, whose structure was verified by acetylation. In the next step, a methyl thiomethyl moiety was introduced on the free hydroxyl at the C-4 position of the reducing mannose by treatment of **170** with dimethyl sulfide and benzoyl peroxide added portionwise in acetonitrile under the exclusion of light. The obtained compound **171** and acceptor **116** were assembled by treatment with NIS and a catalytic amount of triflic acid in a solvent mixture of DCE and THF. Linear trisaccharide **172** was obtained in a good yield of 73%.

Methylene acetal linked compound **172** had next to be converted into a suitable glycosyl donor for intramolecular glycosylation (Scheme 2.29). To do so, the protecting group at the anomeric center of the glucosamine of trisaccharide **172** had to be replaced by an appropriate leaving group. A trichloroacetimidate moiety was identified as an ideal leaving group for several reasons. First, it can be introduced under mild conditions and activated by acid catalysis. It also offers the possibility to be converted into other types of leaving groups.

Deacetylation was achieved using standard conditions before *p*-methoxyl phenyl protecting group of trisaccharide **172** was removed by oxidation with CAN[154] to afford compound **173** in an overall yield of 55%.



a) NaOMe, MeOH. b) CAN, toluene, acetonitrile, water. c) Cl_3CN, DBU, methylene chloride. d) TMSOTf, methylene chloride, -20 ^{o}C.

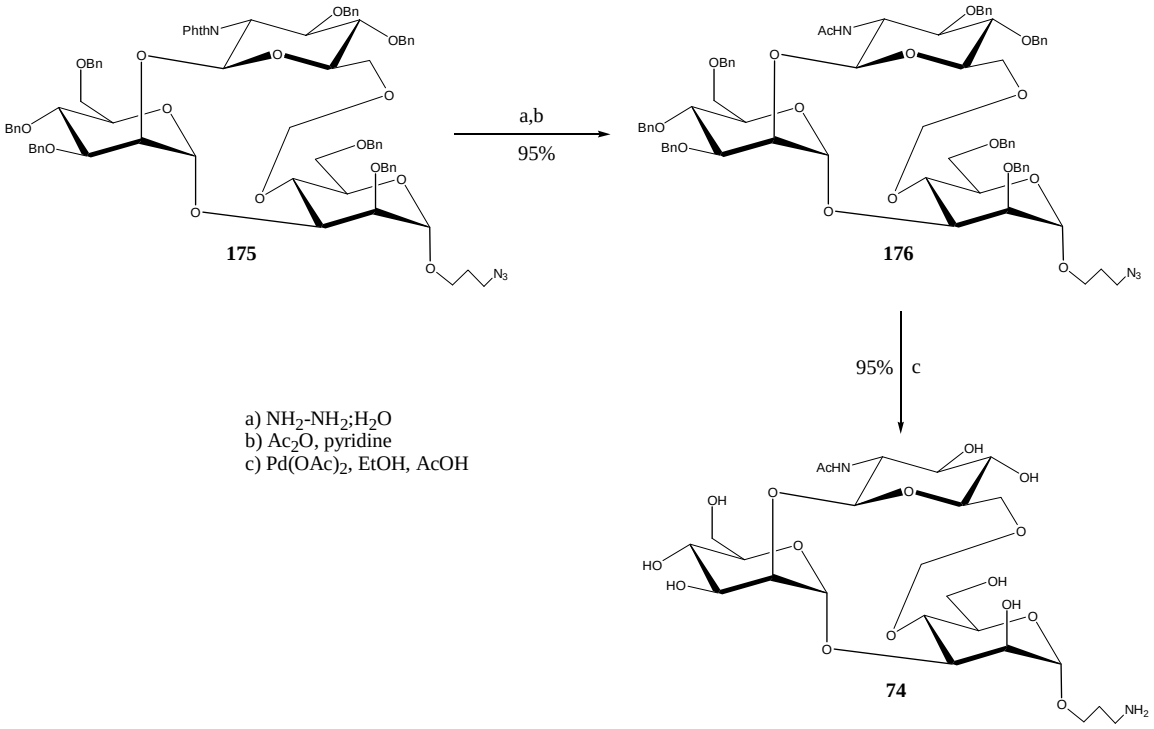
Scheme 2.29

Trisaccharide **173** was then treated with trichloroacetonitrile and DBU, resulting in the formation of trichloroacetimidate **174** in 43% yield. After 20 minutes of reaction, no more progress was observed and any attempt to boost the experimental conditions resulted in the formation of di-imidate. The reactivity of the anomeric hydroxyl is greater than a normal hydroxyl such as 2-OH, however, this reaction has to be performed cautiously to avoid the formation of di-trichloroacetimidate.

In the following step, cyclization was attempted and TMSOTf mediated glycosylation of compound **174** gave desired macrocyclic trisaccharide **175** in an

excellent yield of 80%. Its structure was then confirmed by mass spectrometry and NMR spectroscopy.

The remainder of the synthesis toward trisaccharide **74** consisted in the deprotection of cyclic intermediate **175**, which was achieved in two steps (Scheme 2.30).



Scheme 2.30

Trisaccharide **175** was treated with hydrazine hydrate in refluxing ethanol to convert the phthalimido group into the corresponding amine. The amine was immediately acetylated using acetic anhydride in pyridine to provide compound **176** bearing the desired NHAc functionality (95% yield). In a final deprotection step, debenzylation by catalytic hydrogenation over $Pd(OAc)_2$ was performed to give conformationally

constrained trisaccharide **74** in an almost quantitative yield. It is important to note that we used acetic acid to protonate the free amine and thus avoid catalyst poisoning.

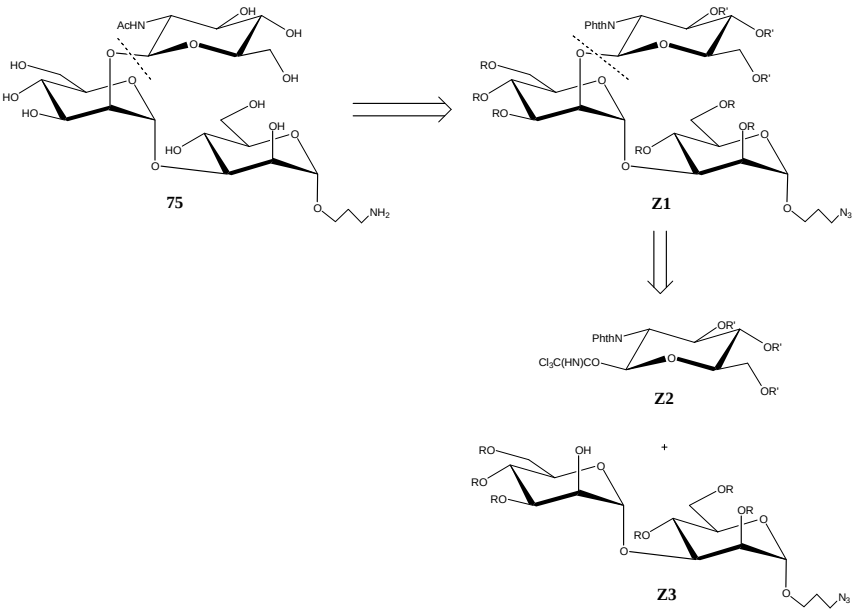
The structural integrity of compound **74** was confirmed by NMR spectroscopy. The ^{1}H and ^{13}C signals were unambiguously assigned by two-dimensional homo-nuclear correlation spectroscopy (COSY, TOCSY). Those results were backed up by hetero-nuclear proton-carbon chemical shift correlation (HSQC). The anomeric configurations were verified by measurement of $J_{C1,H1}$ (H-1, δ 4.69 ppm, $J_{C1,H1}$= 172.3 Hz; H-1', δ 5.48 ppm, $J_{C1',H1'}$= 177.6 Hz; H-1", δ 4.74 ppm, $J_{C1",H1"}$= 167.3 Hz)[184,185]. On the ^{1}H NMR spectrum, the methylene acetal function (O-CH_2-O) was displayed as two doublets, with chemical shifts at δ 4.96 and δ 4.83 ppm and a geminal coupling constant J_{gem} of 5.2 Hz, which are all characteristic of an AB system

*2.6.2 Synthesis of 3-amino-propyl (2-N-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (**75**)*

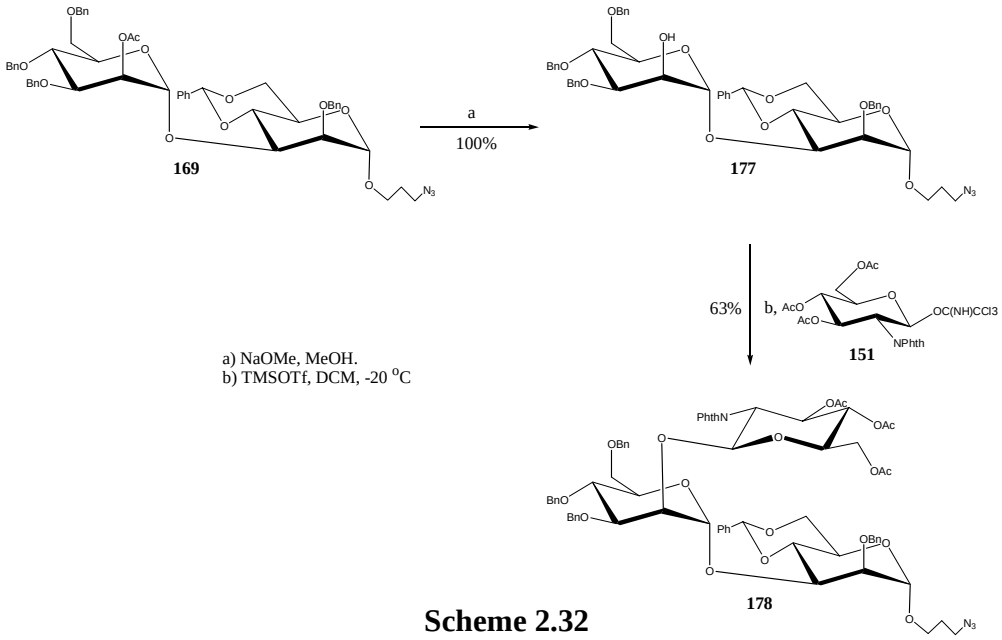
A retrosynthetic analysis of the synthesis of linear trisaccharide **75** is depicted in scheme 2.31. It was proposed that compound **75** could be assembled from glycosyl donor **Z1**, which contains a trichloroacetimidate group at its reducing end and disaccharide glycosyl acceptor **Z2** containing a free hydroxyl group at the C-3 position.

The synthesis of trisaccharide **75**, which was achieved using building blocks discussed in previous sections, is shown in Scheme 2.32. Disaccharide **169** was first deacetylated using conventional experimental conditions to yield glycosyl acceptor in quantitative yield. In a second step, a glycosylation was performed between **177** and trichloroacetimidate **151** in methylene chloride at –20 ^{o}C with a catalytic amount of

$BF_3.OEt_2$. Desired linear trisaccharide **178** was thus obtained as a single β-anomer. This structure was confirmed by ^1H NMR spectroscopy where a large $J_{1",2"}$ coupling constant associated with trans diaxial hydrogen substituents was observed ($J_{1",2"}$= 8.5 Hz).

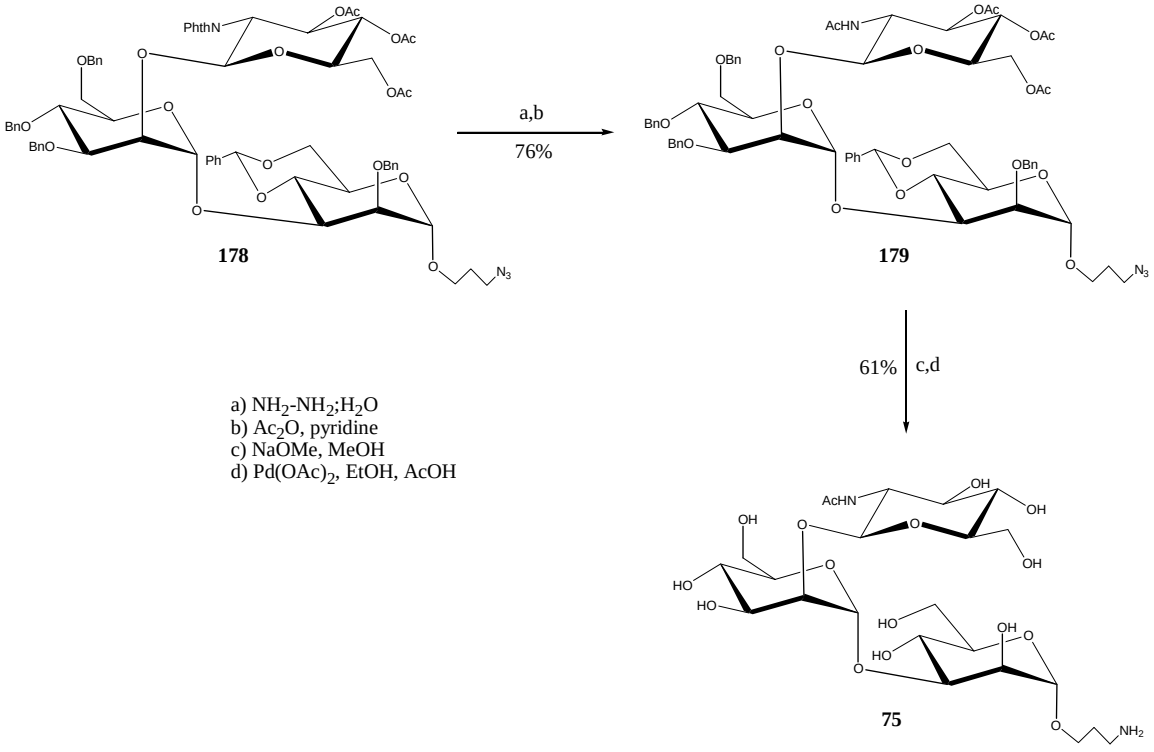


Scheme 2.31



Scheme 2.32

Linear trisaccharide **178** was then deprotected using a three-step procedure as depicted in Scheme 2.33. In the first step, the phthaloyl group of trisaccharide **178** was replaced by an acetyl group. Compound **178** was then treated with hydrazine hydrate in refluxing ethanol to provide a free amine. The amine, detected by TLC using ninhydrin, was acetylated with acetic anhydride in pyridine to trisaccharide **179** in 76% yield. **179** was first deacetylated with potassium carbonate and then debenzylated in ethanol and acetic acid by catalytic hydrogenation with $Pd(OAc)_2$,. Final linear trisaccharide **75** was obtained in 61% yield over two steps. The debenzylation of this compound proved more challenging than the previous trisaccharides and was achieved over four days. The deprotected product was finally purified by exclusion size chromatography (Sephadex G25, water).



Scheme 2.33

As before, NMR spectroscopy was used to confirm the structure of trisaccharide **75**. The ^{1}H and ^{13}C signals were unambiguously assigned by two-dimensional homo-nuclear correlation spectroscopy (COSY, TOCSY). Hetero-nuclear proton-carbon chemical shift correlation (HSQC) further confirmed those results. The anomeric configurations were verified by measurement of $J_{C1,H1}$ (H-1, δ 4.72 ppm, $J_{C1,H1}$= 172.4 Hz; H-1', δ 5.10 ppm, $J_{C1',H1'}$= 172.5 Hz; H-1", δ 4.43 ppm, $J_{C1",H1"}$= 162.1 Hz).

2.6.3 Conclusion

Efficient synthesis routes were developed for both conformationally constrained trisaccharide **74** and linear trisaccharide **75**. Protected cyclic trisaccharide **175** was assembled from building blocks **79**, **116** and **161**, and the formation of the methylene acetal bridge was achieved in good yield. The macrocyclisation by intramolecular glycosylation, which was clearly the most challenging step of the synthesis, also yielded excellent results. Linear protected trisaccharide **178** was assembled from disaccharide building block **169** and building block donor **151** in a reasonably good yield. However, the deprotection of the linear trisaccharide leading to final target compound **75** proved more difficult than anticipated, especially when compared to the deprotection step of **74**.

These two compounds are currently being prepared to undergo Biacore experiments. Two approaches are being considered to study their kinetics of binding. A first experiment would consist in attaching the sugars onto a gold activated matrix at the surface of a microchip in the Biacore measuring cell. A protein would be then dissolved in the buffer and run through the cell, allowing the kinetics of carbohydrate-protein interactions to be recorded. In a second experiment, the sugars would ideally be linked to

a protein and the mannose binding protein would be attached to the microchip. The approach would have the advantage of mimicking in vivo conditions. However, this experiment would require to be carefully designed: since proteins are rarely monomeric, there would be a risk that multivalency may influence the kinetics of binding.

CHAPTER THREE

CONCLUSIONS AND FURTHER WORK

This research project first consisted of a computer modeling study of cyclic trisaccharides **51** and **72**, in an attempt to understand the origin of enthalpy/entropy compensation reported to occur upon binding of cyclic trisaccharide **51** with the lectin Con A. Although no definite conclusion could be drawn from this study, it was found that both oligosaccharides bound with Con A in a similar fashion. It was then hypothesized that these compensative phenomenons could have different origins. A better water rearrangement around the cyclic compound would result in a higher enthaply of the dessolvation upon binding. A second hypothesis was that an oligosaccharide needed to adopt a certain conformation upon binding, which could be more energy demanding for cyclic trisaccharide **51** than its natural counterpart **72**.

Cyclic trisaccharides **70**, **71** and **72** were also designed by computer modeling. It was anticipated that compounds **70** and **71** would be pre-organized toward specific conformations, while **72**, as a very rigid molecule, would be locked in a given conformation. Based on these observations, further studies on these three compounds could prove interesting to study the role of conformational properties of oligosaccharides in protein-carbohydrate interactions.

A second part of this project started with the synthesis of cyclic trisaccharides **70** and **71**. The first challenge was the introduction of a methyl moiety at the C-6 position of the glucosamine. This difficult step was achieved using a Grignard reaction with methyl

magnesium bromide in diethyl ether, but afforded only one of the two required diastereoisomers. This building block was used toward the synthesis of **70** and **71**, which was successful up to the linear stage of trisaccharides **107** and **114**. Two different approaches were used to obtain the linear compounds. Unfortunately, all attempts to cyclize these precursors were unsuccessful.

It was then decided to focus on the synthesis of **72** where a carbamate bond was introduced between the C-4 of the reducing mannose and the C-6 position of the *N*-acetyl-glucosamine. The synthesis of this compound was achieved using two different routes. The first approach, for which the cyclization was completed by intramolecular glycosylation, afforded low yields when the carbamate bond was introduced, which prompted the need for a more productive route. In the new approach, starting from linear trisaccharide **149**, the cyclization step was performed by intramolecular carbamate bond formation, which gave an excellent yield of 69%. Linear trisaccharide **73** was also synthesized to serve as a reference when testing cyclic compound **72** for its biological properties.

Independently from the rest of the project, trisaccharides **74** and **75** were designed and synthesized to study the kinetics of protein-carbohydrate interactions. The only challenge was the synthesis of building block **161** with an alkyl linker at the anomeric center of a mannoside. An effective synthetic route was found and the rest of the synthesis towards **74** and **75** was achieved in a rather high yielding fashion.

In the future, it might be of interest to attempt the synthesis of **70** and **71** using a different approach. First, the synthesis of building blocks **81** and **82** could be started from 6-*R*-C-methyl mannoside and 6-*S*-C-methyl mannoside, on which an inversion of

configuration could lead to the required glucosamine intermediates. Linear precursors could then be synthesized using the intramolecular methylene acetal bridge formation approach. Although at this stage, the introduction of a methyl thiomethyl failed, it might be possible to use another method to introduce this moiety, which would result in a precursor that could be cyclized more easily. In view of the problems presented by the syntheses described in this project, it might also be pertinent to further assess the protecting group strategy. As an example, the phthalimido group could be replaced by a dibenzyl protecting group that could also be used as neighboring participating group during glycosylation steps.

It could also be interesting to synthesize a modified version of trisaccharide **72**, where the position of the atom in the carbamate bond would be changed. An amino group at the C-6 position of the glucosamine and a hydroxyl group at the C-4 position of the reducing mannose could be reacted with phosgene to give a new carbamate bond. This modification could well be useful to modulate the conformational properties of this compound while conserving its rigidity.

CHAPTER FOUR

EXPERIMENTAL

4.1 General methods and materials

All chemical were purchased from Aldrich and VWR. 4 Å molecular sieves were purchased from Avocado, activated at 300 ^{o}C for 5 h and stored at 180 ^{o}C.

All reaction solvents were distilled prior to use: methylene chloride, 1,2-dichloroethane, diethyl ether, acetonitrile, toluene, *N*,*N*-dimethylformamide (DMF) and pyridine from CaH_2, tetrahydrofuran (THF) from sodium and benzophenone.

All NMR spectra (1D- ^{1}H and ^{13}C and 2D- COSY, HSQC, TOCSY, HMBC) were recorded on Varian 300 MHz and 500 MHz spectrometers equipped with Sun off-line editing workstations. Chemical shifts (δ) were recorded in parts per millions (ppm) using tetramethylsilane as internal standard. Liquid Secondary Ion (LSI) mass spectra were recorded using VG ZabSpec Spectrometer with m-nitrobenzyl alcohol as matrix. Maldi-Tof mass spectra were recorded with HP Maldi-Tof Spectrometer with Gentisic Acid as matrix. Masses are reported in Dalton (Da).

Chromatography was performed using Merck 7734 silica and flash chromatography using Crosfield ES70X microspheroidal silica. TLC analyses were performed on silica gel plates (Merck 1.05554 Kieselgel 60 F254). Compounds on TLC were revealed by UV light and/or dipping in H_2SO_4/MeOH (1/10, v/v) followed by subsequent charring at 140 ^{o}C.

4.2 Computer Modeling

4.2.1 Starting models for the oligosaccharides and for Con A

The trisaccharide βGlcNac(1→2)αMan(1→3)Man was constructed with monosaccharides obtained from a database of three-dimensional structures.[189] All subsequent calculations were performed by SYBYL software. The coordinates of Concanavalin A were taken from the 2.0 Å resolution crystal structure of the Con A-mannose complex from the Protein Data Bank. Four conserved water molecules, which are involved in the coordination of the structural cations, were incorporated into the model. All hydrogen atoms were added and their positions optimized with the Tripos force field.[190]

4.2.2 Systematic Conformational Search For The Trisaccharides

For the linear trisaccharide, a four-dimensional systematic search was performed by rotating the ϕ and ψ of the two glycosidic linkages in 10° increments. The SEARCH procedure of the SYBYL software was used for this purpose, together with energy parameters appropriate for carbohydrates.[27] In order to avoid limitation of conformational space due to steric conflicts in the rigid-residue approach, the hydroxyl hydrogens were omitted and the hydroxymethyl group at C-6 were replaced by methyl groups. To limit the computation time needed, all conformations with penetration of van der Waals spheres larger than 40% were rejected prior to any energy calculations.

A systematic search of the possible conformations of the 13-membered ring has been performed on the cyclic trisaccharide. In the 13-membered ring of this cyclic

trisaccharide, nine torsion angles could be rotated, one of which was used as a ring-closure bond. An eight-dimensional systematic conformational search was performed by rotating the ϕ and ψ torsion angles of the two glycosidic linkages as well as the torsion angles of the methylene acetal bridge in 10° increments, except for ω_3, which corresponds to the ring-closure bond.

To establish the correct stereochemistry at the ring-closure point and to relieve minor steric conflicts, each of the resulting conformations of the cyclic trisaccharide was submitted to several steps of energy minimization. The hydroxyl atoms were restored, and the charges and atom types specifically defined for carbohydrate parameterization were used. A dielectric constant of 4 was chosen for the calculations. Energy minimization has been conducted with the conjugate gradient method, until a rms gradient of 0.05 is attained. The structures obtained after the systematic search were checked for the puckering of the pyranose rings and chirality of carbon atoms.

A family analysis method was used to identify groups of conformations. In this approach, a family algorithm was used, which concludes that an object belongs to a group if there is only a single step change to at least one of the objects of the group. For this purpose, the torsional angles of the βGlcNac(1→2)Man and the αMan(1→3)Man glycosidic linkages were used as selection criteria and the step limit was fixed at 30^o.

4.2.3 Docking in the Binding Site of Con A

The lowest energy conformation of compound **73** and each of the low energy conformations of compound **72**, in an energy window of 10 kcal/mol, were used as starting structures to be docked in the binding site of Con A. Lectins three-dimensional

structures are taken from the Protein Data Bank were used. Hydrogen atoms were added in the structure and partial charges calculated using the Pullman procedure. Oligosaccharides were docked in the lectin binding site by fitting on the known orientation of mannose or glucose in the corresponding crystal structure of legume lectin/carbohydrate complexes.[62] Hydroxyl groups are manually reoriented in order to optimise the hydrogen bond network. Optimisation is performed in three steps:

- Optimisation of the hydrogen atoms of the oligosaccharide and the amino acids in a 5 Å sphere around.
- Optimisation of the whole oligosaccharide and the hydrogen atoms around.
- Optimisation of the oligosaccharide and the side chain of the amino acids around.

A distance dependant dielectric constant is used. Minimisation procedure uses the Powell method and the rms gradient is chosen at a value of 0.05.

4.3 Synthesis of (2-*N*-acetyl-2-deoxy-6-R-*C*-methyl-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6”-methylene acetal (**70**) and (2-N-acetyl-2-deoxy-6S-*C*-methyl-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6”-methylene acetal (**71**)

4.3.1 Intramolecular Glycosylation Approach

Acetyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside (83) - A solution of D-mannose (11.4g, 63 mmol) in a mixture of pyridine/acetic anhydride (1/1, v/v, 120 mL) was stirred at room temperature for 17 h. The solvent was removed under reduced pressure and the residue thus obtained was co-evaporated with, respectively, toluene (5 × 100 mL), ethanol (100 mL) and methylene chloride (100 mL) to give **83** (α/β~4/1) as a yellow oil which was used without any further purification (24.6 g, 100%). R_f (acetone/methylene

chloride, 5/95, v/v)= 0.83; δ_H ppm (300 MHz, $CDCl_3$): 6.09 (1H, d, $J_{1\alpha,2\alpha}$=1.8 Hz, H-1α), 5.85 (1H, d, $J_{1\beta,2\beta}$, 1.1 Hz, H-1β), 5.48 (1H, dd, $J_{2\beta,3\beta}$= 3.2 Hz, H-2β), 5.35-5.25 (4H, m, H-2α, H-3α, H-4α, H-4β), 5.12 (1H, dd, $J_{3\beta,4\beta}$= 9.9 Hz, H-3β), 4.33-4.00 (5H, m, H-5α, H-6aα, H-6bα, H-6aβ, H-6bβ), 3.80 (1H, m, H-5β); δ_C ppm (75 MHz, $CDCl_3$): 170.6, 170,0, 169.7, 169.5, 168.0 (CH_3-C=O), 90.5 (C-1α), 90.4 (C-1β), 70.5, 68.7, 68.3, 65.5 (C-2, C-3, C-4, C-5), 62.0 (C-6), 20.8, 20.8, 20.5 (CH_3-C=O); m/z: calc= 390, LSI= 413 (100%, $[M+Na]^+$).

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl bromide (84) – To a cooled (0 ^oC) solution of Acetyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside (**83**) (23.8 g, 60.9 mmol) in dry methylene chloride (100 mL) was added a saturated solution of hydrobromic acid in acetic acid (70 mL). The reaction mixture was stirred under argon for 18 h and diluted with methylene chloride (100 mL) before being poured into an ice-water bath (300 mL). The organic layer was collected and washed with, successively, aqueous $NaHCO_3$ (saturated, 3 × 100 mL) and water (300 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated under reduced pressure to afford **84** as a yellow oil, which was used without any further purification (24.0 g, 95%). R_f (acetone/methylene chloride, 3/97, v/v)= 0.64; δ_H ppm (300 MHz,$CDCl_3$): 6.28 (1H, d, $J_{1,2}$= 1.1 Hz, H-1), 5.71 (1H, dd, $J_{3,2}$= 3.5 Hz, $J_{3,4}$= 10.1 Hz, H-3), 5.44 (1H, dd, H-2), 5.36 (1H, dd, $J_{4,5}$=$J_{4,3}$= 10.1 Hz, H-4), 4.33 (1H, dd, $J_{6a,5}$= 4.8 Hz, $J_{6a,6b}$= 12.5 Hz, H-6a), 4.22 (1H, m, H-5), 4.12 (1H, dd, $J_{6b,5}$=2.2 Hz, H-6b), 2.17, 2.10, 2.07, 2.00 (12H, 4s, CH_3-C=O). δ_C ppm (75 MHz, $CDCl_3$): 170.5, 169.7, 169.6 (CH_3-C=O, acetyls), 83.1 (C-1), 72.8, 72.10, 67.9, 65.3 (C-2, C-3, C-4, C-5), 61.4 (C-6), 20.8, 20.7 (CH_3-C=O).

3,4,6-tri-*O*-acetyl-1,2-di-*O*-(1'-methoxyethylidene)-α-D-mannopyranoside (85) - To a solution of 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**84**) (23.7 g, 58 mmol) in a mixture of methanol/methylene chloride (1/1, v/v, 250 mL) was added collidine (12.1 mL, 92 mmol). The solution was stirred under argon for 18 h, after which time it was concentrated to dryness. The residue was taken up in methylene chloride (150 mL) and resulting solution was washed with, respectively, water (150 mL), aqueous $NaHCO_3$ (saturated, 150 mL) and brine (150 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. Recrystallization of the yellow residue from methanol-water gave **85** as a white solid. The mother liquid was evaporated under reduced pressure and the residue was purified by silica column chromatography (methylene chloride/acetone, 99/1, v/v) to afford compound **85** as a white solid (16.8 g, 80%). R_f (acetone/methylene chloride, 3/97, v/v)= 0.49; m.p.= 108-109 °C; $[\alpha]_d$= -37.3 (methylene chloride, c= 2.0 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 5.48 (1H, d, $J_{1,2}$= 2.6 Hz, H-1), 5.27 (1H, dd, $J_{4,3}$=$J_{4,5}$= 9.8 Hz, H-4), 5.13 (1H, dd, $J_{3,2}$= 4.0 Hz, H-3), 4.60 (1H, dd, H-2), 4.23 (1H, dd, $J_{6a,5}$= 5.1 Hz, $J_{6a,6b}$= 12.1 Hz, H-6a), 3.68 (1H, m, H-5), 3.27 (3H, s, C$\underline{H}_3$ orthoester), 2.11, 2.06, 2.04 (9H, 3s, C$\underline{H}_3$-C=O), 1,73 (3H,s, O-C$\underline{H}_3$ orthoester); δ_C ppm (75 MHz, $CDCl_3$): 170.6, 170.4, 169.4 (CH_3-$\underline{C}$=O), 124.5 (Cq orthoester), 97.3 (C-1), 76.6, 71.2, 70.6, 65.4 (C-2, C-3, C-4, C-5), 66.3 (C-6), 49.9 (O-C$\underline{H}_3$ orthoester), 24.4 (C$\underline{H}_3$ orthoester), 20.8 ($\underline{C}H_3$-C=O); m/z: calc= 362, LSI= 385 (25%,[M+Na]+).

3,4,6-Tri-*O*-benzyl-1,2-*O*-(1'-methoxyethylidene)-α-D-mannopyranoside (86) - To a solution of 3,4,6-Tri-*O*-acetyl-1,2-*O*-(1'-methoxyethylidene)-α-D-mannopyranoside (**85**) (16.3 g, 45.1 mmol) in methanol (100 mL) was added potassium *tert*-butoxide (5.6 g, 45.8 mmol) and the mixture was stirred for 4 h under argon. The solution was then neutralized using Dowex 50WH+ resin, filtered and the solvent was removed under reduced pressure. The white solid thus obtained was used without further purification and was dissolved in dimethyl formamide. The solution was cooled to 0 °C and NaH, 60% in mineral suspension (7g, 175 mmol) was added carefully. The reaction mixture was stirred at 0°C for 15 min, after which time the ice bath was removed. Benzyl bromide (17.6 mL, 148.9 mmol) was added dropwise and the solution was stirred under argon for 18 h. The excess of NaH was destroyed with methanol and the solvent was removed under reduced pressure. The residue was dissolved in diethyl ether (150 mL), and the solution was washed with water. The aqueous layer was then extracted with ether (3 × 100 mL) and the combined layers were dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The resulting pale yellow oil was purified by silica gel column chromatography (acetone/methylene chloride, 2/98, v/v) to afford **86** as a white solid (10.03 g, 49%). R_f (acetone/methylene chloride, 3/97, v/v)= 0.72; $[\alpha]_d$= +8.5 (methylene chloride, c= 10.3 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.31-7.26 (15H, m, arom H), 5.35 (1H, d, $J_{1,2}$= 2.2 Hz, H-1), 4.91-4.52 (6H, m, C<u>H</u>$_2$ benzyls), 4.39 (1H, dd, $J_{2,3}$= 3.9 Hz, H-2), 3.92 (1H, dd, $J_{4,3}$=$J_{4,5}$= 9.4 Hz, H-4), 3.73 (3H, m, H-3, H-6a, H-6b), 3.40 (1H, m, H-5), 3.28 (3H, s, C<u>H</u>$_3$ orthoester), 1.74 (3H, s, O-C<u>H</u>$_3$ orthoester); δ_C ppm (75 MHz, $CDCl_3$): 138.3 (Cq), 128.6-127.6 (arom C), 97.6 (C-1), 79.1, 77.2, 76.2, 74.3 (C-2,

C-3, C-4, C-5), 75.3, 73.4, 72.4 (C<u>H</u>$_2$ benzyls), 69.0 ppm (C-6), 49.8 (O-C<u>H</u>$_3$ orthoester), 24.5 (C<u>H</u>$_3$ orthoester); m/z: calc= 506, LSI= 529 (100%, $[M+Na]^+$).

Acetyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (87) - A solution of 3,4,6-Tri-*O*-benzyl-1,2-di-*O*-(1'-methoxyethylidene)-α-D-mannopyranoside (**86**) (9.79 g, 19.3 mmol) in a mixture of glacial acetic acid (100 mL) and acetic anhydride (20 mL) was stirred at 50 °C for 1h and then at room temperature for 18 h. The solvent was co-evaporated with, successively, toluene (4×100 mL), then ethanol (100 mL) and methylene chloride (100 mL) to give **87** as a pale yellow oil which was used without further purification (9.79 g, 93%). R_f (acetone/methylene chloride, 3/97, v/v)= 0.85; $[\alpha]_d$= +22.4 (methylene chloride, c= 8.4 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.31-7.16 (15H, m, arom H), 6.13 (1H, d, $J_{1,2}$= 2.2 Hz, H-1), 5.37 (1H, m, H-2), 4.88-4.48 ppm (6H, m, C<u>H</u>$_2$ benzyls), 4.09-3.64 ppm (5H, m, H-3, H-4, H-5, H-6a, H-6b), 2.17, 2.07 ppm (6H, 2s, C<u>H</u>$_3$ acetyls). δ_C ppm (75 MHz, $CDCl_3$): 170.2, 168.5 (CH$_3$-<u>C</u>=O), 128.5-127.7 (arom C), 91.3 (C-1), 77.7, 74.6, 73.9, 73.7 (C-2, C-3, C-4, C-5), 77.6, 77.2, 76.7 (C<u>H</u>$_2$ benzyls), 75.5 (C-6), 21.2, 21.1 (C<u>H</u>$_3$-C=O); m/z: calc= 534, LSI= 557 (50%, $[M+Na]^+$).

Ethyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (88) - To a cooled solution (0 °C) of Acetyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**87**) (9.51 g, 17.8 mmol) in methylene chloride (50 mL) were added 4 Å molecular sieves (5 g), ethanethiol (2.63 mL, 35.5 mmol) and TMSOTf (1.94 mL, 7.12 mmol). The ice bath was removed and the solution was stirred overnight. The mixture was then filtered

through celite and the solution was washed with aqueous potassium fluoride (1M, 3 × 200 mL) and aqueous $NaHCO_3$ (saturated, 200 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (acetone/methylene chloride, 1/99, v/v) to afford building block **88** as a pale yellow oil (8.02 g, 84%). R_f (acetone/methylene chloride, 2/98, v/v)= 0.90; $[\alpha]_d$= +92.7 (methylene chloride, c= 11.7 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.29-7.17 (15H, m, arom H), 5.43 (1H,dd, $J_{2,1}$= 1.8 Hz, $J_{2,3}$= 2.9 Hz, H-2), 5.32 (1H, d, H-1), 4.85-4.43 (6H, m, C<u>H</u>$_2$ benzyls), 4.17 (1H, m, H-5), 3.93 (1H,m, H-3), 3.83 (1H, dd, $J_{6a,5}$= 4.2 Hz, $J_{6a,6b}$= 10.8 Hz, H-6a), 3.76 (1H, m, H-4), 3.69 (1H, dd, $J_{6b,5}$=1.8 Hz, H-6a), 2.65 (2H, m, C<u>H</u>$_2$-CH_3 SEt), 2.17 (3H, s, C<u>H</u>$_3$ acetyl), 1.27 (3H, t, $J_{vicinal}$= 7.35 Hz, CH_2-C<u>H</u>$_3$ SEt). δ_C ppm (75 MHz, $CDCl_3$): 170.4 (CH_3-<u>C</u>=O), 138.4, 138.8, 137.7 (Cq), 128.5-127.7 (arom C), 82.5, 82.3, 81.6, 80.0, 78.6 (C-1, C-2, C-3, C-4, C-5), 75.2, 73.5, 71.9 (C<u>H</u>$_2$ benzyls), 68.8 (C-6), 25.5 (<u>C</u>H_2-CH_3 SEt), 21.2 (<u>C</u>H_3-C=O), 14.9 (CH_2-<u>C</u>H_3 SEt); m/z: calc= 536, LSI= 559 (25%, $[M+Na]^+$).

Methyl 4,6-*O*-Bensylidene-α-D-mannopyranoside (90) - To a solution of Methyl α-D-mannopyranoside (**89**), (20.0 g, 103 mmol) in DMF (500 mL, 5% w/v) were added benzaldehyde dimethyl acetal (16.3 mL, 108 mmol) and tetrafluoroboric acid (52% in diethyl ether, 17.4 mL). The reaction mixture was stirred under argon for 18 h and TLC analysis revealed the conversion of the starting material to a major product. The solution was then neutralized with Et_3N and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (methylene chloride/acetone, 95/5, v/v, then 90/10, v/v) to give **90** as a colorless oil (16.76 g, 58%).

R_f (methylene chloride/methanol, 95/5, v/v)= 0.57; $[\alpha]_d$= +27.4 (methylene chloride, c= 5.2 mg/mL, 25 ^oC) ; δ_H ppm ($CDCl_3$, 300MHz): 7.58-7.33 (5H, m, arom H), 5.57 (1H, s, C<u>H</u> benzylidene), 4.77 (1H, s, $J_{1,2}$= 1.5 Hz, H-1), 4.29 (1H, dd, $J_{3,2}$= 5.0 Hz, $J_{3,4}$= 8.0 Hz, H-3), 4.10-4.03 (2H, m, H-2, H-5), 3.96-3.76 (3H, m, H-4, H-6), 3.40 (3H, s, OC<u>H</u>$_3$); δ_C ppm ($CDCl_3$, 75 MHz): 137.4 (Cq benzylidene), 129.4, 128.3, 128.1, 126.4, 126.2 (arom C), 102.2 (<u>C</u>H benzylidene), 101.4 (C-1), 78.9, 70.9, 68.4, 63.1 (C-2, C-3, C-4, C-5), 68.7 (C-6), 54.9 (OC<u>H</u>$_3$); m/z: calc= 282, Maldi-Tof= 305 $[M+Na]^+$.

Methyl 2-*O*-Benzyl-4,6-*O*-bensylidene-α-D-mannopyranoside (80) - Benzyl bromide (6.78 mL, 57.0 mmol) was added to a solution of methyl 4,6-*O*-bensylidene-α-D-mannopyranoside (**90**) (16.56 g, 54.3 mmol) in DMF (300 mL) containing silver oxide (15.25 g, 109 mmol). The mixture was stirred under argon in the dark for 48 h. The reaction mixture was filtered and the solid residue obtained was rinsed with methylene chloride (200 mL). The solvent of the mother liquid was then removed and the residue was taken up into diethyl ether (250 mL). The resulting solution was washed with, successively, aqueous $NaHCO_3$ (saturated, 2 × 250 mL) and water (250 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. Purification of the crude product by silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v then 90/10, 80/20 and 70/30) afforded building block **80** as a colorless oil (5.05 g, 25%); R_f (hexane/ethyl acetate, 70/30, v/v)= 0.52; $[\alpha]_d$= +12.7 (methylene chloride, c= 7.3 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 300MHz): 7.52-7.46 (2H, m, arom H), 7.41-7.30 (8H, m, arom H), 5.58 (1H, s, C<u>H</u> benzylidene), 4.76, 4.69 (2H, 2d, J_{gem}= 11.5 Hz, C<u>H</u>$_2$ benzyl), 4.75 (1H, d, $J_{1,2}$= 1.5 Hz, H-1), 4.26 (1H, dd, $J_{3,2}$= 4.0

Hz, $J_{3,4}$= 9.0 Hz, J3,OH= 7.5 Hz, H-3), 4.14-4.03 (1H, m, H-5), 3.91 (1H, dd, $J_{4,5}$= 9.0 Hz, H-4), 3.87-3.72 (3H, m, H-6, H-2), 3.36 (3H, s, OC<u>H</u>$_3$), 2.36 (1H, d, O<u>H</u>); δ_C ppm ($CDCl_3$, 75 MHz): 137.7, 137.4 (Cq benzylidene, Cq benzyl), 129.1-126.3 (arom C), 102.3 (<u>C</u>H benzylidene), 99.5 (C-1), 79.5, 78.5, 68.7, 63.4 (C-2, C-3, C-4, C-5), 73.8 (<u>C</u>H$_2$ benzyl), 68.8 (C-6), 55.0 (O<u>C</u>H$_3$); m/z: calc=372, Maldi-Tof= 395 $[M+Na]^+$.

Allyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-*N*-Acetyl-β-D-glucopyranoside (92) - To a solution of Acetyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-*N*-Acetyl-β-D-glucopyranoside (**91**) (30g, 77.1 mmol) in 1,2-dichloroethane (300 mL), was added TMSOTf (15.7 mL, 84.8 mmol) and the reaction mixture was stirred under argon at 50 ^oC for 18 h. 4 Å molecular sieves (5 g) and allyl alcohol (15.7 mL, 231.3 mmol) were added and the reaction mixture was stirred for 18 h. The solution was filtered through celite, the solid residue was rinsed with a mixture methanol/methylene chloride (1/10, v/v, 250 mL) and the resulting solution was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (methylene chloride/methanol, 95/5, v/v) to afford compound **92** as a colorless oil (21.4 g, 72%). R_f (methylene chloride/methanol, 95/5, v/v)= 0.63; $[\alpha]_d$= +31.4 (methylene chloride, c= 11.0 mg/mL, 25 ^oC), δ_H ppm (300 MHz, $CDCl_3$): 5.92-5.72 (1H, m, CH_2-C<u>H</u>=CH_2), 5.47 (1H, d, NHAc), 5.35-5.12 (3H, m, H-2, CH_2-CH=C<u>H</u>$_2$), 5.04 (1H, d, $J_{3,2}$=$J_{3,4}$= 9.5 Hz, H-3), 4.71 (1H, d, $J_{1,2}$= 8.4 Hz, H-1), 4.40-4.01 (4H, m, H-6, C<u>H</u>$_2$-CH=CH_2), 3.86 (1H, d, $J_{4,3}$= 10.6 Hz, $J_{4,5}$= 8.8 Hz, H-4), 3.72-3.59 (1H, m, H-5), 2.11-1.88 (12H, 4s, C<u>H</u>$_3$-C=O); δ_C ppm (75 MHz, $CDCl_3$): 170.2, 169.8 (CH_3-<u>C</u>=O), 133.8 (CH_2-<u>C</u>H=CH_2), 118.2 (CH_2-CH=<u>C</u>H$_2$), 99.8 (C-1), 73.1 (C-6), 71.6,

69.8, 68.7 (C-3, C-4, C-5), 62.4 ($\underline{C}H_2$-CH=CH_2), 55.6 (C-2) 23.8, 20.6 ($\underline{C}H_3$-C=O); m/z: calc= 387, Maldi-Tof= 409 $[M+Na]^+$.

Allyl 2-Deoxy-2-*N*-acetyl-β-D-glucopyranoside (93) - To a solution of Allyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-Acetyl-β-D-glucopyranoside (**92**) (21.30 g, 55.0 mmol) in water (300 mL) was added barium hydroxide *octa*-hydrate (41.88 g, 330 mmol) and the reaction mixture was stirred for 18 hours at 90 ^oC. NMR analysis of a small aliquot of the reaction mixture showed complete deacetylation. Bubbling of carbon dioxide in the solution resulted in the precipitation of barium carbonate that was filtered through celite several times. The solvent was evaporated and fully deacetylated compound **93** was obtained as a slightly yellow foam (12.05 g, 100%). R_f (methylene chloride/methanol, 90/10, v/v)= 0; δ_H ppm (300 MHz, CD_3OD): 6.06-5.90 (1H, m, CH_2-C$\underline{H}$=CH_2), 5.38-5.16 (2H, m, CH_2-CH=C$\underline{H}_2$), 4.47-4.34 (2H, m, $J_{1,2}$= 7.7 Hz, H-1, C$\underline{H}_2$-CH=CH_2), 4.19-4.11 (1H, m, C$\underline{H}_2$-CH=CH_2), 3.78 (1H, dd, $J_{6a,6b}$= 12.5 Hz, $J_{6a,5}$= 2.1 Hz, H-6a), 3.69 (1H, dd, $J_{6b,5}$= 5.2 Hz, H-6b), 3.41-3.23 (4H, m, H-2, H-3, H-4, H-5); δ_C ppm (300 MHz, CD_3OD): 133.6 (CH_2-$\underline{C}$H=CH_2), 116.2 (CH_2-CH=$\underline{C}H_2$), 98.4 (C-1), 75.7, 74.8, 74.2, 71.3 (C-2, C-3, C-4, C-5), 69.7 ($\underline{C}H_2$-CH=CH_2), 61.0 (C-6); m/z: calc= 219, Maldi-Tof= 241 $[M+Na]^+$.

Allyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-azido-β-D-glucopyranoside (94) - To a solution of Allyl 2-Deoxy-2-*N*-Acetyl-β-D-glucopyranoside (**93**) (5.48 g, 25 mmol) in methanol (150 mL) were added DMAP (3.36 g, 26.5 mmol) and freshly prepared triflic azide solution (0.4 M in methylene chloride, 2.6 eq, 163 mL). After stirring under argon for 96 h, TLC

analysis indicated almost complete conversion of the starting material to a new product. Pyridine (30 mL) was added to the solution and the solvent was evaporated to a minimum volume. The residue was then dissolved in a mixture of acetic anhydride/pyridine (1/1, v/v, 150 mL) and the resulting solution was stirred under argon for 18 hours. The reaction mixture was poured into an ice/water solution (500 mL) and the aqueous layer was extracted with methylene chloride (4 × 200 mL). The combined organic layer was then washed with aqueous HCl (1M, 2×100 mL) and aqueous $NaHCO_3$ (Saturated, 100 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed. The oily residue was purified by silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then 90/10, 80/20 and 70/30) to afford compound **94** as a colorless oil (6.87 g, 74%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.85; $[\alpha]_d$= +23.2 (methylene chloride, c= 6.3 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 6.01-5.85 (1H, m, CH_2-C<u>H</u>=CH_2), 5.39-5.20 (2H, m, CH_2-CH=C<u>H</u>$_2$), 5.05-4.92 (2H, m, H-6), 4.47-4.34 (2H, m, $J_{1,2}$= 8.4 Hz, H-1, C<u>H</u>$_2$-CH=CH_2), 4.31-4.07 (3H, m, H-3, H-4, C<u>H</u>$_2$-CH=CH_2), 3.69-3.60 (1H, m, H-2), 3.56-3.48 (1H, m, H-5), 2.11-1.98 (9H, 3s, C<u>H</u>$_3$-C=O); δ_C ppm (75 MHz, $CDCl_3$): 133.2 (CH_2-CH=<u>C</u>H$_2$), 118.5 (CH_2-<u>C</u>H=CH_2), 101.0 (C-1), 72.8, 72.1, 68.8, 64.0 (C-2, C-3, C-4, C-5), 70.9 (<u>C</u>H$_2$-CH=CH_2), 62.2 (C-6), 21.0, 20.9 (<u>C</u>H$_3$-C=O); m/z: calc= 371, Maldi-Tof= 392 $[M+Na]^+$.

Allyl 2-Deoxy-2-azido-β-D-glucopyranoside (95) - To a solution of Allyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-azido-β-D-glucopyranoside (**94**) (6.77 g, 18.2 mmol) in methanol (150 mL) was added sodium methoxide (catalytic amount). The solution was stirred under argon for 1h. The solution was then neutralized using Dowex 200WH+ resin, filtered and

the solvent was removed to afford compound **95** as a white foam that was used without any further purification (4.43 g, 99%). Rf (methylene chloride/methanol, 95/5, v/v)= 0.12; δ_H ppm (300 MHz, CD_3OD): 6.02-5.88 (1H, m, CH_2-C<u>H</u>=CH_2), 5.49-5.14 (2H, m, CH_2-CH=C<u>H</u>$_2$), 4.45-4.14 (2H, m, C<u>H</u>$_2$-CH=CH_2, $J_{1,2}$= 7.6 Hz, H-1), 4.21-4.11 (1H, m, C<u>H</u>$_2$-CH=CH_2), 3.86 (1H, dd, $J_{6a,6b}$= 12.4 Hz, $J_{6a,5}$= 2.1 Hz, H-6a), 3.67 (1H, dd, $J_{6b,5}$= 5.6 Hz, H-6b), 3.38-3.11 (4H, m, H-2, H-3, H-4, H-5); δ_C ppm (300 MHz, CD_3OD): 133.5 (CH_2-<u>C</u>H=CH_2), 116.2 (CH_2-CH=<u>C</u>H$_2$), 100.8 (C-1), 76.3, 70.8, 67.9 (C-2, C-3, C-4, C-5), 70.9 (<u>C</u>H$_2$-CH=CH_2), 61.9 (C-6); m/z: calc= 245, Maldi-Tof= 268 $[M+Na]^+$.

Allyl 2-Deoxy-2-azido-4,6-di-*O*-benzylidene-β-D-glucopyranoside (96) - To a solution of Allyl 2-Deoxy-2-azido-β-D-glucopyranoside (**95**) (4.38 g, 17.9 mmol) in dry acetonitrile (100 mL) were added benzaldehyde dimethyl acetal (5.37 mL, 35.8 mmol) and CSA until *p*H= 3. The reaction mixture was stirred under argon for 18 h. The reaction mixture was then washed with water (100 mL) and the aqueous layer was extracted with methylene chloride (3×100 mL). The combined organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (methylene chloride/acetone, 98/2, v/v) to afford compound **96** as white solid (5.95 g, 100%). Rf (methylene chloride/acetone, 97/3, v/v)= 0.62; m.p.= 72-75 ^oC; $[\alpha]_d$= +11.7 (methylene chloride, c= 3.7 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.56-7.32 (5H, m, arom H), 6.02-5.89 (1H, m, CH_2-C<u>H</u>=CH_2), 5.54 (1H, s, C<u>H</u> benzylidene), 4.57-4.28 (3H, m, $J_{1,2}$= 8.2 Hz, H-1, CH_2-CH=C<u>H</u>$_2$), 4.17 (1H, dd, $J_{6a,6b}$= 12.3 Hz, $J_{6a,5}$= 5.5 Hz, H-6a), 3.88 (1H, dd, $J_{4,3}$=$J_{4,5}$=7.6 Hz, H-4), 3.69 (1H, dd, $J_{3,4}$=$J_{3,2}$=7.6 Hz, H-3), 3.54 (1H, dd, $J_{6b,5}$=8.4 Hz), 3.51-3.46 (2H, m, H-2, H-5);

δ_C ppm (75 MHz, $CDCl_3$): 137.1 (arom Cq), 133.7 (CH_2-$\underline{C}H$=CH_2), 129.8, 128.6, 126.2 (arom C), 118.5 (CH_2-CH=$\underline{C}H_2$), 102.4 ($\underline{C}$H benzylidene), 101.6 (C-1), 81.4, 72.3, 66.4, 66.1 (C-2, C-3, C-4, C-5), 71.2 (C-6), 68.7 ($\underline{C}H_2$-CH=CH_2); m/z: calc= 333, Maldi-Tof= 355 $[M+Na]^+$.

Allyl 2-Deoxy-2-azido-3-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-glucopyranoside (97) - To a solution of Allyl 2-Deoxy-2-azido-4,6-di-*O*-benzylidene-β-D-glucopyranoside (**96**) (5.85 g, 17.6 mmol) in DMF (80 mL) was added NaH (0.64 g, 26.4 mmol) at 0 ^oC. The reaction mixture was stirred for 15 min at this temperature after which time the ice bath was removed. TBAI (catalytic amount) and Benzyl bromide (2.51 mL, 21.1mmol) were added and the reaction mixture was stirred under argon for 5h. The excess of NaH was then destroyed with methanol and the solvent was removed under reduced pressure. The residue was taken up into diethyl ether (100 mL) and washed with water (100 mL). The aqueous layer was extracted with diethyl ether (3×50 mL) and the combined organic layer was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo*. The yellow residue was purified by silica gel column chromatography (methylene chloride/acetone, 99/1, v/v) to give **97** a colorless oil (7.13 g, 96%). R_f (methylene chloride/acetone, 99/1, v/v)= 0.90; $[\alpha]_d$= +26.3 (methylene chloride, c= 8.7 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.52-7.24 (10H, m, arom H), 6.02-5.86 (CH_2-$\underline{C}H$=CH_2), 5.56 (1H, s, $\underline{C}H$ benzylidene), 5.39-5.18 (2H, m, CH_2-CH=$C\underline{H}_2$), 4.91, 4.77 (2H, J_{gem}= 11.3 Hz, J_{gem}= 11.6 Hz, $C\underline{H}_2$ benzylidene), 4.43-4.29 (3H, m, $J_{1,2}$= 7.7Hz, H-1, H-6a, $C\underline{H}_2$-CH=CH_2), 4.19-4.09 (1H, m, $C\underline{H}_2$-CH=CH_2), 3.84-3.65 (2H, m, $J_{6b,6a}$= 10.2 Hz, $J_{6b,5}$= 5.2 Hz, H-6b, $J_{3,4}$=$J_{3,2}$= 9.1 Hz, H-3), 3.59-3.31 (3H, m, H-4, H-2, H-5); δ_C

ppm (75 MHz, $CDCl_3$): 138.2 (arom Cq), 133.6 (CH_2-$\underline{C}H$=CH_2), 118.5 (CH_2-CH=$\underline{C}H_2$), 100.7 C$\underline{H}$ benzylidene), 100.5 (C-1), 83.6, 77.3, 70.6, 68.5 (C-2, C-3, C-4, C-5), 77.2 (C$\underline{H}_2$ benzyls), 69.7 (C-6), 67.6 ($\underline{C}H_2$-CH=CH_2); m/z: calc= 423, Maldi-Tof= 447 $[M+Na]^+$.

Allyl 2-Deoxy-2-azido-3,4-di-*O*-benzyl-β-D-glucopyranoside (98) - To a cooled solution (0^oC) of Allyl 2-Deoxy-2-azido-3-*O*-benzyl-4,6-di-*O*-benzylidene-β-D-glucopyranoside (**97**) (7.03 g, 16.6 mmol) in a mixture of methylene chloride/diethyl ether (5/1, v/v, 80 mL) were added borane-trimethylamine complex (18.19 g, 250 mmol) and 4 Å molecular sieves (5 g). Aluminium chloride (4 eq, 8.78 g, 66.4 mmol) in diethyl ether (80 mL) was then added and the reaction mixture was stirred for 30 min. The solution was filtered trough a pad of celite and the solid was washed with methylene chloride (2 × 100 mL). The combined organic layer was washed with aqueous sulfuric acid (1M, 300 mL) using vigorous stirring for 30 min. The organic phase was then collected and washed with, successively, aqueous $NaHCO_3$ (saturated, 2 × 300 mL) and water (300 mL). The organic phase was dried ($MgSO_4$) and the solvent was removed. The residue was co-evaporated with toluene (5 × 100 mL) before being purified by silica gel column chromatography (methylene chloride/acetone, 99/1, v/v) to afford building block **98** as a colorless oil (4.94 g, 70%). R_f (methylene chloride/acetone, 99/1, v/v)= 0.46; $[\alpha]_d$= +17.6 (methylene chloride, c= 8.3 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.41-7.24 (10H, m, arom H), 6.02-5.86 (CH_2-C$\underline{H}$=CH_2), 5.39-5.18 (2H, m, CH_2-CH=C$\underline{H}_2$), 4.92-4.74 (3H, d, J_{gem}= 10.9 Hz, J_{gem}= 11.4 Hz, C$\underline{H}_2$ benzyls), 4.67 (1H, d, J_{gem}= 11.0 Hz, C$\underline{H}_2$ benzyl), 4.43-4.35 (2H, m, $J_{1,2}$= 7.4 Hz, $\underline{C}H_2$-CH=CH_2), 4.20-4.09

(1H, m, C<u>H</u>$_2$-CH=CH$_2$), 3.84 (1H, dd, $J_{6a,6b}$= 12.0 Hz, $J_{6a,5}$= 2.6 Hz, H-6a), 3.64 (1H, dd, $J_{6b,5}$= 4.2 Hz, H-6b), 3.52 (1H, dd, $J_{3,4}$=$_{3,2}$= 9.1 Hz, H-3), 3.43-3.22 (3H, m, H-2, H-4, H-5); δ_C ppm (75 MHz, $CDCl_3$): 137.9 (arom Cq), 133.9 (CH$_2$-<u>C</u>H=CH$_2$), 129.5-128.7 (arom C), 118.7 (CH$_2$-CH=<u>C</u>H$_2$), 100.8 (C-1), 83.2, 77.6, 75.7, 66.9 (C-2, C-3, C-4, C-5), 75.8, 75.4 (C<u>H</u>$_2$ benzyls), 70.9 (<u>C</u>H$_2$-CH=CH$_2$), 61.9 (C-6); m/z: calc= 425, Maldi-Tof= 448 $[M+Na]^+$.

Allyl 6-*O*-Acetyl-2-deoxy-2-azido-3,4-di-*O*-benzyl-β-D-glucopyranoside (98Ac)– A solution of Allyl 2-Deoxy-2-azido-3,4-di-*O*-benzyl-β-D-glucopyranoside (**98**) (20 mg, 0.05 mmol) in a mixture pyridine/acetic anhydride (1/1, v/v, 2 mL) was stirred under argon for 4 h. The solvents were co-evaporated with, successively toluene (5 × 20 mL), ethanol (20 mL) and methylene chloride (20 mL) to afford **98Ac** as a slightly yellow oil (22 mg, 100%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.78; δ_H ppm (300 MHz, $CDCl_3$): 7.35-7.12 (10H, m, arom H), 5.98-5.79 (1H, m, CH$_2$-C<u>H</u>=CH$_2$), 5.37-5.15 (2H, m, CH$_2$-CH=C<u>H</u>$_2$), 4.90-4.67 (3H, m, J_{gem}= 11.0 Hz, J_{gem}= 12.3 Hz, C<u>H</u>$_2$ benzyls), 4.51 (1H, d, J_{gem}= 11.0 Hz, C<u>H</u>$_2$ benzyls), 4.36-4.19 (3H, m, H-1, H-6a, C<u>H</u>$_2$-CH=CH$_2$), 4.16-4.00 (2H, m, H-6b, C<u>H</u>$_2$-CH=CH$_2$), 3.49-3.28 (4H, m, H-2, H-3, H-4, H-5), 1.98 (3H, s, C<u>H</u>$_3$-C=O); m/z: calc= 467, Maldi-Tof= 490 $[M+Na]^+$.

Allyl 2-deoxy-2-azido-3,4-di-*O*-benzyl-6-*C*-methyl-β-D-glucopyranoside (99) - To a cooled solution (–78 ^oC) of DMSO (80.6 µl, 1.13 mmol) in methylene chloride (4 mL) was added dropwise a solution of oxalyl chloride (50.2 µl, 0.75 mmol) in methylene chloride (2 mL) at and the reaction mixture was stirred for 15 min under argon. Allyl 2-

Deoxy-2-azido-3,4-di-*O*-benzyl-β-D-glucopyranoside (**98**) (200 mg, 0.24 mmol) in methylene chloride (4 mL) was added dropwise to the reaction mixture over a period of 15 min keeping the temperature in the flask below –65 ^{o}C. The solution was then stirred for 45 more min at –78 ^{o}C before adding Et_3N (0.4 mL) dropwise. After 5 min, the cooling bath was removed and the solution was allowed to warm up to room temperature was stirred for 1 h before adding water (2 mL). The organic phase was separated, dried ($MgSO_4$), filtered and the solvent was removed. The thus obtained unstable aldehyde was used immediately and dissolved in diethyl ether (2 mL). This solution was then added dropwise to a solution of MeMgBr (3M in diethyl ether, 0.31 mL, 0.48 mmol) and the reaction mixture was stirred for 2 h. Aqueous NH_4Cl (15%, 5 mL) previously cooled to 0 ^{o}C was added and the solution was vigorously stirred for 15 min. TLC analysis indicated the formation of a major compound as well as a few side products. The organic layer was then separated, dried ($MgSO_4$) and the solvent was removed. The yellow residue was purified by silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then 90/10, 80/20 and 70/30) to afford compound **99** as a pale yellow oil (61.2 mg, 30%, only one isomer). R_f (hexane/ethyl acetate, 70/30, v/v)= 0.62; $[\alpha]_d$= +3.2 (methylene chloride, c= 10.5 mg/mL, 25 ^{o}C); δH ppm (300 MHz, $CDCl_3$): 7.43-7.24 (10H, m, arom C), 5.92-5.78 (1H, m, CH_2-C<u>H</u>=CH_2), 5.42-5.21 (2H, m, CH_2-CH=C<u>H</u>$_2$), 4.94-4.67 (4H, J_{gem}= 11.0 Hz, C<u>H</u>$_2$ benzyls), 4.46-4.30 (2H, H-1, C<u>H</u>$_2$-CH=CH_2), 4.23-4.14 (1H, m, C<u>H</u>$_2$-CH=CH_2), 4.11-3.99 (1H, m, H-6), 3.72 (1H, dd, $J_{2,1}$=$J_{2,3}$= 8.0 Hz, H-2), 3.51-3.38 (2H, m, H-3, H-4), 3.11 (1H, dd, $J_{5,6}$=1.2 Hz, $J_{5,4}$= 8.7 Hz, H-5), 1.22 (3H, d, $J_{Me,6}$= 6.8 Hz, C<u>H</u>$_3$/C-6); δ_C ppm (75 MHz, $CDCl_3$): 145.8 (arom Cq), 133.6 (CH_2-<u>C</u>H=CH_2), 128.7-128.0 (arom C), 118.2 (CH_2-CH=<u>C</u>H$_2$), 101.4 (C-1), 83.4, 78.0, 77.8, 66.6, 65.3 (C-2, C-

3, C-4, C-5, C-6), 75.8, 75.5 ($\underline{C}H_2$ benzyls), 70.8 ($\underline{C}H_2$-CH=CH_2), 20.6 ($\underline{C}H_3$/C-6); m/z: 439, Maldi-Tof= 462 $[M+Na]^+$. Elemental analysis: Calc. C: 65.59, H: 6.65, Found: C: 65.71, H: 6.81.

Allyl 2-Deoxy-2-amino-3,4-di-*O*-benzyl-6-*C*-methyl-β-D-glucopyranoside (100) - To a cooled (–78 ^oC) solution of DMSO (8.84 mL, 24 mmol) in methylene chloride (40 mL) was added dropwise a solution of oxalyl chloride (0.52 mL, 11.5 mmol) in methylene chloride (20 mL) and the reaction mixture was stirred under argon for 15 min. Allyl 2-Deoxy-2-azido-3,4-di-*O*-benzyl-β-D-glucopyranoside (**98**) (2.03 g, 4.78 mmol) in methylene chloride (40 mL) was added dropwise to the reaction mixture over a period of 45 min keeping the temperature in the flask below –65 ^oC. The solution was then stirred for 45 more min at –78 ^oC before adding dropewise Et_3N (4.2 mL). After 5 min, the cooling bath was removed and the solution was allowed to warm up to room temperature and the solution was stirred for 1 h before adding water (50 mL). The organic phase was separated, dried ($MgSO_4$), filtered and the solvent was removed. The thus obtained unstable aldehyde was used immediately and dissolved in diethyl ether (30 mL). This solution was then added dropwise to a solution of MeMgBr (3M in diethyl ether, 6.34 mL, 19.1 mmol) and the reaction mixture was stirred for 2 h. Aqueous NH_4Cl (15%, 30 mL) previously cooled to 0 ^oC was added and the solution was vigorously stirred for 15 min. TLC analysis indicated the formation of a complex mixture of polar compounds. The organic layer was then separated, dried ($MgSO_4$) and the solvent was removed. The yellow residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then 90/10, 80/20 and 70/30) to afford **100** as a yellow oil (888 mg,

45%, only one isomer). R_f (hexane/ethyl acetate, 70/30, v/v)= 0.35; $[\alpha]_d$= +9.9 (methylene chloride, c= 5.7 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.39-7.24 (10H, m, arom C), 6.00-5.82 (1H, m, CH_2-C<u>H</u>=CH_2), 5.35-5.18 (2H, m, CH_2-CH=C<u>H_2</u>), 5.03-4.70 (4H, *J*gem= 11.3 Hz, C<u>H_2</u> benzyls), 4.41-4.01 (4H, H-1, C<u>H_2</u>-CH=CH_2, H-6), 3.76 (1H, dd, $J_{4,3}$=$J_{4,5}$= 6.3 Hz, H-4), 3.55 (1H, m, H-3), 3.17 (1H, dd, $J_{5,6}$= 9.6 Hz, H-5), 2.87 (1H, dd, $J_{2,1}$= 8.0 Hz, $J_{2,3}$= 7.5 Hz, H-2), 1.22 (3H, d, $J_{Me,6}$= 6.0 Hz, C<u>H_3</u>/C-6); δ_C ppm (75 MHz, $CDCl_3$): 134.1 (CH_2-CH=<u>C</u>H_2), 128.7-128.0 (arom C), 118.0 (CH_2-<u>C</u>H=CH_2), 103.4 (C-1), 85.2, 78.9, 78.1, 65.4, 57.4, 57.3 (C-2, C-3, C-4, C-5, C-6), 75.6, 75.2, 70.8 (<u>C</u>H_2 benzyls, <u>C</u>H_2-CH=CH_2), 20.7 (<u>C</u>H_3/C-6); m/z: calc= 413, found (Maldi-Tof)= 432 $[M+Na]^+$; Elemental analysis: Calc. C: 69.71, H: 7.56, Found: C: 69.89, H: 7.75.

Allyl 6-*O*-Acetyl-2-deoxy-3,4-di-*O*-benzyl-6-*C*-methyl-2-*N*-phthalimido-β-D-gluco-pyranoside (101) - To a solution of Allyl 2-Deoxy-2-amino-3,4-di-*O*-benzyl-6-*C*-methyl-β-D-glucopyranoside (**100**) (878 mg, 2.1 mmol) in toluene (30 mL) were added phthalic anhydride (0.38 g, 2.5 mmol) and Et_3N (0.28 mL, 1.7 mmol). The reaction mixture was refluxed for 2 h after which time the solvent was evaporated. The residue was dissolved in a mixture pyridine/acetic anhydride (1/1, v/v, 20 mL) and the resulting solution was stirred under argon for 18 h. The solvent was then co-evaporated with, successively, toluene (5 × 40 mL), ethanol (40 mL) and methylene chloride (40 mL). The obtained brown residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v then 90/10, 80/20 and 70/30) to afford compound **101** as a colorless oil (858 mg, 69%). R_f (hexane/ethyl acetate, 70/30, v/v)= 0.48; $[\alpha]_d$= +16.1 (methylene chloride, c= 8.9 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.60 (4H, br-s,

phthalimido arom H), 7.50-6.78 (10H, m, arom H), 5.71-5.54 (1H, m, CH_2-C<u>H</u>=CH_2), 5.21 (1H, m, H-6), 5.16-5.04 (2H, m, $J_{1,2}$= 8.4 Hz, CH_2-CH=C<u>H</u>$_2$), 5.01-4.90 (1H, m, CH_2-CH=C<u>H</u>$_2$), 4.78-4.69 (2H, m, C<u>H</u>$_2$ benzyls), 4.44-4.23 (3H, m, H-3, C<u>H</u>$_2$ benzyls), 4.20-4.09 (2H, m, H-2, C<u>H</u>$_2$-CH=CH_2), 4.01-3.91 (1H, m, C<u>H</u>$_2$-CH=CH_2), 3.61 (1H, dd, $J_{4,3}$=$J_{4,5}$= 9.1 Hz, H-4), 3.36 (1H, d, $J_{5,6}$= 10.9 Hz, H-5), 2.04 (3H, s, C<u>H</u>$_3$-C=O), 1.32 (3H, d, $J_{Me,6}$= 6.7 Hz, C<u>H</u>$_3$/C-6); δ_C ppm (300 MHz, $CDCl_3$): 134.2-123.8 (arom C), 133.8 (CH_2-<u>C</u>H=CH_2), 98.9 (C-1), 80.1, 78.2, 75.1, 67.8, 55.9 (C-2, C-3, C-4, C-5, C-6), 75.6, 75.4 (<u>C</u>H_2 benzyls), 69.8 (<u>C</u>H_2-CH=CH_2), 21.2 (<u>C</u>H_3-C=O), 17.9 (<u>C</u>H_3/C-6); m/z: calc= 585, found (Maldi-Tof)= 607 $[M+Na]^+$.

Allyl 2-Deoxy-3,4-di-*O*-benzyl-6-*C*-methyl-2-*N*-phthalimido-β-D-glucopyranoside (81) - To a solution of Allyl 6-*O*-Acetyl-2-deoxy-3,4-di-*O*-benzyl-6-*C*-methyl-2-*N*-phthalimido-β-D-glucopyranoside (**101**) (808 mg, 1.4 mmol) in methanol (20 mL) was added sodium methoxide (catalytic amount) and the reaction mixture was stirred under argon for 18 h. The solution was then neutralized using Dowex 200WH^+ resin, filtered and the solvent was removed under reduced pressure to give building block **81** as a colorless oil that was used without any further purification (752 mg, 100%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.32; $[\alpha]_d$= +20.2 (methylene chloride, c= 6.3 mg/mL, 25 ^oC);δ_H ppm (300 MHz, $CDCl_3$): 7.65 (4H, br-s, H phthalimido), 7.39-6.79 (10H, m, arom H), 5.78-5.61 (1H, m, CH_2-C<u>H</u>=CH_2), 5.24-4.74 (7H, m, CH_2-CH=C<u>H</u>$_2$, H-1, J_{gem}=11.0 Hz, CH_2 benzyls), 4.49-4.26 (2H, m, C<u>H</u>$_2$-CH=CH_2), 4.21-3.97 (4H, m, H-2, H-3, H-4, H-5), 3.84 (1H, dd, $J_{6,5}$= 8.6 Hz, H-6), 3.28 (1H, d, $J_{OH,6}$= 9.9 Hz, OH), 1.38 (3H, d, $J_{vicinal}$= 6.5 Hz, C<u>H</u>$_3$/C-6,); δ_C ppm (75 MHz, $CDCl_3$): 138.3, 138.2 (Cq

benzyls), 133.8 (CH_2-CH=$\underline{C}H_2$), 128.7-127.5 (arom C), 117.7 (CH_2-$\underline{C}$H=CH_2), 97.8 (C-1), 79.9, 79.4, 78.0, 65.5, 56.2, (C-2, C-3, C-4, C-5, C-6), 75.3, 75.0, 70.4 ($\underline{C}H_2$ benzyls, $C\underline{H}_2$-CH=CH_2), 20.7 ($\underline{C}H_3$/C-6); m/z: calc= 543, found (Maldi-Tof)= 566 $[M+Na]^+$; Elemental analysis: Calc. C: 70.70, H: 6.12, Found: C: 70.86, H: 6.32.

Methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→2)-2-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranoside (102) - To a solution of acetyl 3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (**79**) (865 mg, 1.61 mmol) and methyl 2-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranoside (**80**) (500 mg, 1.34 mmol) in methylene chloride (30 mL) were added 4 Å molecular sieves (1 g) and the solution was stirred for 1 h under argon. NIS (373 mg, 1.61 mmol) was added quickly to the solution as well as TMSOTf (29 µl, 0.16 mmol). The reaction mixture was stirred for 1 h at room temperature, after which time it was neutralized using Et_3N. The solution was then filtered through celite, diluted with methylene chloride (40 mL) and washed with, successively, aqueous $Na_2S_2O_3$ (15%, 2 × 80 mL) and water (80 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated to dryness. Purification of the crude product by silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then 90/10 and 80/20) afforded **102** as a colorless oil (727 mg, 64%). R_f (hexane/ethyl acetate, 70/30, v/v)= 0.62; $[\alpha]_d$= +12.1 (CH_2Cl_2, c= 13.13 mg.mL^{-1}); δ_H ppm (300 MHz, $CDCl_3$): 7.52-7.15 (25H, m, arom H), 5.63 (1H, s, C$\underline{H}$ benzylidene), 5.64-5.60 (1H, m, H-2'), 5.32 (1H, d, $J_{1',2'}$= 2.0 Hz, H-1'), 4.89 (1H, d, J_{gem}= 11.0 Hz, C$\underline{H}_2$ benzyl), 4.67 (1H, d, $J_{1,2}$= 1.5 Hz, H-1), 4.77-4.60 (4H, m, C$\underline{H}_2$ benzyls), 4.48, 4.47, 4.41 (3H, 3d, J_{gem}= 12.0 Hz, C$\underline{H}_2$ benzyls), 4.30-4.20 (2H, m, H-3, H-5'), 3.98 (1H, dd, $J_{3',2'}$= 3.5 Hz, $J_{3',4'}$= 9.0 Hz,

H-3'), 3.92-3.65 (8H, m, H-4, H-4', H-5, H-2, H-6, H-6'), 3.30 (3H, s, O-C$\underline{H}_3$), 2.10 (3H, s, C$\underline{H}_3$ acetyl); δ_C ppm (75 MHz, $CDCl_3$): 170.0 (CH_3-$\underline{C}$=O), 138.7, 138.4, 137.9, 137.4 (Cq benzyl, Cq benzylidene), 128.6-126.1 (arom C), 101.1 ($\underline{C}$H benzylidene), 100.6, 98.9 (C-1, C-1'), 79.1, 78.0, 77.4, 74.3, 73.2, 72.2, 68.2, 63.9 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 75.1, 73.7, 73.5, 71.6 ($\underline{C}H_2$ benzyls), 69.1, 68.8 (C-6, C-6'), 54.9 (O-$\underline{C}H_3$), 21.1 ($\underline{C}H_3$-C=O); m/z: calc= 846, found (LSI)= 869 (100%, $[M+Na]^+$).

Methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→2)-2,6-di-*O*-benzyl-α-D-mannopyranoside (103) - To a solution of methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→2)-2-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-manno-pyranoside (**102**) (677 mg, 0.80 mmol) in THF (15 mL) were added 4 Å molecular sieves (1 g) and sodium cyanoborohydride (503 mg, 8 mmol). Hydrogen chloride (2M in diethyl ether) was added until no more gas was released and the reaction mixture was stirred for under argon 2 h. The reaction mixture was neutralized with Et_3N, filtered through celite and the resulting solution was washed with, successively aqueous $NaHCO_3$ (saturated, 2 × 40 mL) and water (40 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated to dryness. The crude product was purified by silica gel column chromatography (methylene chloride/acetone, 97/3, v/v) to afford desired disaccharide **103** as a colorless oil (590 mg, 87%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.10; $[\alpha]_d$= +24.5 (CH_2Cl_2, c=10.3 mg/mL); δ_H ppm (300 MHz, $CDCl_3$): 7.40-7.12 (25H, m, arom H), 5.53-5.47 (1H, m, H-2'), 5.37 (1H, s, H-1'), 4.89 (1H, d, J_{gem}= 11.0 Hz, C$\underline{H}_2$ benzyl), 4.75-4.45 (11H, J_{gem}= 11.4 Hz, C$\underline{H}_2$ benzyls, H-1), 4.11 (1H, dd, $J_{4,3}$= 9.2 Hz, H-4), 4.05-3.91 (3H, m, H-3', H-5, H-5'), 3.84-3.63 (7H, m, H-2, H-3, H-6, H-4', H-6'),

3.33 (3H, s, O-C<u>H</u>$_3$), 2.10 (3H, s, C<u>H</u>$_3$-C=O); δC ppm (75 MHz, $CDCl_3$): 170.4 (CH_3-<u>C</u>=O), 138.6, 138.0, 137.9 (Cq benzyl), 128.4-127.6 (arom C), 98.8, 98.7 (C-1, C-1'), 77.9, 77.5, 74.5, 71.9, 71.0, 68.8, 68.6 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 74.9, 73.7, 72.6, 71.8, 70.8, 69.4 (<u>C</u>H$_2$ benzyls, C-6, C-6'), 54.9 (O-<u>C</u>H$_3$), 21.1 (<u>C</u>H$_3$-C=O); m/z: calc= 848, (LSI)= 871 (100%, $[M+Na]^+$).

Methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→2)-2,6-di-*O*-benzyl-4-*O*-methyl thiomethyl-α-D-mannopyranoside (104) - Dimethyl sulfide (0.48 mL, 6.6 mmol) was added to a cooled solution (0 ^oC) of methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→2)-2,6-di-*O*-benzyl-α-D-mannopyranoside (**103**) (560 mg, 0.66 mmol) in acetonitrile (10 mL). Benzoyl peroxide (639 mg, 2.64 mmol) was added in 4 portions over 45 min and the reaction mixture was stirred in the dark for 18 h, gradually reaching room temperature. The solution was diluted with ethyl acetate (25 mL) and washed with, respectively, aqueous NaOH (1M, 2 × 40 mL) and brine (40 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated to dryness. The crude product was purified by silica gel column chromatography (methylene chloride/acetone, 97/3, v/v) to afford **104** as a colorless oil (564 mg, 94%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.72; $[\alpha]_d$= + 37.9 (CH_2Cl_2, c= 15.47 mg/mL); δH ppm (300 MHz, $CDCl_3$): 7.39-7.15 (25H, m, arom H), 5.35 (1H, dd, $J_{2',1'}$= 1.8 Hz, $J_{2',3'}$= 3.3 Hz, H-2'), 5.10 (1H, s, H-1'), 4.89 (1H, d, J_{gem}= 11.0 Hz, C<u>H</u>$_2$ benzyl), 4.78 (1H, d, J_{gem}= 11.4 Hz, C<u>H</u>$_2$ benzyl), 4.72 (1H, d, $J_{1,2}$=1.8 Hz, H-1), 4.67 (1H, d, J_{gem}= 12.1 Hz, C<u>H</u>$_2$ benzyl), 4.64-4.43 (9H, m, C<u>H</u>$_2$-S-CH_3, C<u>H</u>$_2$ benzyls), 4.06 (1H, dd, $J_{3,2}$= 3.3 Hz, $J_{3,4}$= 9.2 Hz, H-3), 3.97 (1H, dd, $J_{3',4'}$= 9.2 Hz, H-3'), 3.93-3.84 (2H, m, H-4, H-5'), 3.82-3.72

(4H, m, H-2, H-4, H-6a, H-6a'), 3.71-3.64 (3H, m, H-5, H-6b, H-6b'), 3.30 (3H, s, O-C$\underline{H}_3$), 2.22 (C$\underline{H}_3$-C=O acetyl), 2.02 (3H, s, S-C$\underline{H}_3$); δC ppm (75 MHz, $CDCl_3$): 170.3 (CH_3-$\underline{C}$=O), 138.7, 138.4, 138.1, 137.9 (Cq benzyls), 128.5-127.6 (arom C), 99.8, 99.3 (C-1, C-1'), 77.9, 77.3, 74.5, 72.2, 71.4, 69.1 (C2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 75.0, 73.5, 73.4, 73.0, 72.1, 72.0, 69.5, 69.3 ($\underline{C}H_2$ benzyls, $\underline{C}H_2$-S-CH_3, C-6, C-6'), 54.9 (O-$\underline{C}H_3$), 21.2 ($\underline{C}H_3$-C=O), 14.8 (S-$\underline{C}H_3$); m/z: calc= 808, found (LSI)= 932 (100%, $[M+Na]^+$).

Methyl 4,6-di-*O*-benzilydene-3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside-allyl 3",4"-di-*O*-benzyl-2"-deoxy-2"-6"-*C*-methyl-*N*-phthalimido-β-D-glucopyranoside-2,6"-methylene acetal (105) - To a solution of methyl (2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranoside)-(1→2)-2,6-di-O-benzyl-4-O-methyl thiomethyl-α-D-mannopyranoside (**104**) (435 mg, 0.50 mmol) allyl 2-deoxy-3,4-di-*O*-benzyl-6-*C*-methyl-2-*N*-phthalimido-β-D-glucopyranoside (**81**) (242 mg, 0.41 mmol) in a mixture THF/1,2-dichloroethane (1/1, v/v, 20 mL) were added 4 Å molecular sieves and the reaction mixture was stirred under argon for 1h. The solution was cooled to 0 ^oC. In a separate vessel, N-iodosuccinimide (113 mg, 0.50 mmol) was dissolved in a mixture THF/1,2-dichloroethane (1/1, v/v, 5 mL). Trifluoromethanesulfonic acid (5 µl, 0.05 mmol) was added and the solution was stirred for 30 seconds. The resulting mixture (0.1M, 5 mL) was added quickly to the initial solution, which was stirred for 20 min at 0 ^oC. The reaction mixture was neutralized by the addition of Et_3N and filtered through celite. The resulting solution was diluted with methylene chloride and washed with aqueous $Na_2S_2O_3$ (15%, 2 × 40 mL), aqueous $NaHCO_3$ (40 mL) and brine (40 mL). The

organic layer was dried ($MgSO_4$), filtered and the solvent was removed. Purification of the crude product by silica gel column chromatography (methylene chloride/acetone, 97/3, v/v) afforded desired trisaccharide **105** as a colorless oil (371 mg, 67%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.73; $[\alpha]_d$= +24.7 (methylene chloride, c= 5.1 mg/mL, 25 ^oC);δ_H ppm (500 MHz, $CDCl_3$): 7.65 (4H, br-s, phthalimido H), 7.60-6.84 (35H, m, arom H), 5.73-5.62 (1H, m, CH_2-C<u>H</u>=CH_2), 5.37 (1H, m, H-2'), 5.14 (1H, d, $J_{1",2"}$= 8.2 Hz, H=1"), 5.08-4.97 (4H, m, CH_2-CH=C<u>H</u>$_2$, H-1', O-C<u>H</u>$_2$-O), 4.91-4.42 (18H, m, O-C<u>H</u>$_2$-O, H-1, C<u>H</u>$_2$-CH=CH_2, C<u>H</u>$_2$ benzyls), 4.37 (1H, d, $J_{3",4"}$=$J_{3",2"}$= 9.1 Hz, H-3"), 4.28-4.19 (2H, m, H-2", H-4), 4.01-3.79 (10H, m, H-3, H-5, H-3', H-4", H-4', H-2, H-6', H-6), 3.69 (1H, m, H-5'), 3.35 (1H, m, H-6"), 3.22 (3H, s, OC<u>H</u>$_3$), 2.14 (3H, s, C<u>H</u>$_3$-C=O), 1.31 (3H, d, $J_{vicinal}$= 7.6 Hz, C<u>H</u>$_3$/C-6). δ_C ppm (500 MHz, $CDCl_3$): 133.9-124.7 (arom C), 133.4 (CH_2-<u>C</u>H=CH_2), 117.8 (CH_2-CH=<u>C</u>H$_2$), 100.1 (C-1'), 98.3 (C-1), 97.9 (C-1"), 95.7 (O-C<u>H</u>$_2$-O), 80.2, 79.6, 78.3, 78.2, 78.0, 77.8, 75.8, 72.4, 72.1, 69.9, 69.1, 56.3 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2", C-3", C-4", C-5", C-6"), 75.9, 72.0 (C<u>H</u>$_2$ benzyls), 73.8 (<u>C</u>H$_2$-CH=CH_2), 71.2, 70.0 (C-6, C-6'), 55.2 (O-<u>C</u>H$_3$), 20.3 (<u>C</u>H$_3$-C=O), 17.9 (<u>C</u>H$_3$/C-6); m/z: calc= 1403, found (Maldi-Tof)= 1427 $[M+Na]^+$; Elemental analysis: Calc. C: 70.97, H: 6.39, Found: C: 71.08, H: 6.53.

Methyl 4,6-di-*O*-benzilydene-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside 3",4"-di-*O*-benzyl-2"-deoxy-2"-6"-*C*-methyl-*N*-phthalimido-β-D-glucopyranoside-2,6"-methylene acetal (107) - To a solution of methyl 4,6-di-*O*-benzilydene-3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside-allyl 3",4"-di-*O*-benzyl-2"-deoxy-2"-6"-*C*-methyl-*N*-phthalimido-β-D-glucopyra-

noside-2,6”-methylene acetal (**105**) (321 mg, 0.23 mmol) in acetic acid/water (9/1, v/v, 5 mL) were added palladium (II) chloride (285 mg, 1.61 mmol), sodium acetate (285 mg, 3.45 mmol) and *tri*-butyltin hydride (0.25 mL,0.92 mmol). The reaction mixture was stirred for 18 h. The solution was then filtered through celite and the solvent was removed. The residue was taken up into ethyl acetate (5 mL) and washed with, successively, aqueous KF (1M, 2 × 10 mL) and water (10 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure to afford **106** as a white foam (244 mg, 77%) that was used without further purification. It was dissolved in methanol (5 mL) and sodium methoxide (catalytic amount) was added. The reaction mixture was allowed to stir under argon for 4 h. The solution was neutralized with dowex 200WH+ resin, filtered and the solvent was evaporated. The residue was purified by silica gel preparative TLC (methylene chloride/methanol, 98/2, v/v) to afford trisaccharide **107** as a white foam (115 mg, 49%). R_f (methylene chloride/methanol, 98/2, v/v)= 0.57; $[\alpha]_d$= +20.8 (methylene chloride, c= 7.7 mg/mL, 25 ^oC); δ_H ppm (500 MHz, $CDCl_3$): 7.64 (4H, br-s, phthalimido H), 7.55-6.65 (35H, m, arom H), 5.19 (1H, br-s, H-1”), 5.08 (1H, s, H-1’), 4.94-4.34 (20H, m, H-1, H-3, H-3”, H-4, O-C$\underline{H}_2$-O, C$\underline{H}_2$ benzyls), 4.16 (1H, s, H-2’), 4.12-3.84 (3H, m, H-2”, H-6”, H-3”), 3.82-3.53 (10H, m, H-2, H-4’, H-5”, H-4”, H-5, H-6, H-5’, H-6’), 3.34 (3H, s, O-C$\underline{H}_3$), 1.32 (3H, br s, C$\underline{H}_3$/C-6); δ_C ppm (500 MHz, $CDCl_3$): 134.1-123.6 (arom C), 100.7 (C-1’), 98.6 (C-1), 93.1 (C-1”), 79.1, 77.2, 74.8, 73.5, 72.1, 72.0, 69.6, 69.1, 68.5, 57.7, 50.4 (C-2, C-3, C-4, C-5, C-2’, C-3’, C-4’, C-5’, C-2”, C-3”, C-4”, C-5”, C-6”), 71.9, 69.5 (C-6, C-6’), 61.5 (O-$\underline{C}H_2$-O), 55.0 (O-$\underline{C}H_3$), 29.5 ($\underline{C}H_3$/C-6); m/z: calc= 1321, found (Maldi-Tof)= 1344 $[M+Na]^+$; Elemental analysis: Calc. C: 70.68, H: 6.24, Found: C: 70.78, H: 6.37.

Methyl 4,6-di-*O*-benzilydene-3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside-acetyl 3",4"-di-*O*-benzyl-2"-deoxy-2"-6"-*C*-methyl-*N*-phthalimido-β-D-glucopyranoside-2,6"-methylene acetal (108) - Methyl 4,6-di-*O*-benzilydene-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside 3",4"-di-*O*-benzyl-2"-deoxy-2"-6"-*C*-methyl-*N*-phthalimido-β-D-glucopyranoside-2,6"-methylene acetal (**107**) (50 mg, 0.04 mmol) was dissolved in a mixture pyridine/acetic anhydride (1/1, v/v, 2 mL) and the reaction mixture was stirred under argon for 4 h. The solvent was co-evaporated with, successively, toluene (5 × 10 mL), ethanol (10 mL) and methylene chloride (10 mL) to give **108** as a colorless oil (52 mg, 100%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.72; $[\alpha]_d$= +30.4 (methylene chloride, c= 10.9 mg/mL, 25 ^oC); δ_H ppm (500 MHz, $CDCl_3$): 7.75 (4H, br-s, arom H phthalimido), 7.72-6.82 (35H, m, arom H), 6.33 (1H, d, $J_{1",2"}$= 8.8 Hz, H-1"), 5.34 (1H, s, H-2'), 5.07 (1H, s, H-1'), 4.90-4.22 (22H, m, H-1, H-3", H-4, H-3, $J_{2",1"}$=$J_{2",3"}$= 9.8 Hz, H-2", H-6", O-C$\underline{H}_2$-O, J_{gem}= 11.2 Hz, C$\underline{H}_2$ benzyls), 4.06-3.62 (11H, m, H-3', H-5', H-5", H-2, H-4', H-4", H-5, H-6, H-6'), 3.36 (3H, s, O-C$\underline{H}_3$), 2.08, 1.89 (6H, 2s, C$\underline{H}_3$-C=O), 1.31 (3H, br-s, C$\underline{H}_3$/C-6); δ_C ppm (500 MHz, $CDCl_3$): 134.0-123.6 (arom C), 99.6 (C-1'), 98.7 (C-1), 90.0 (C-1"), 79.4, 78.8, 77.8, 76.5, 76.1, 74.2, 71.8, 69.4, 68.9, 54.6, 50.4 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2", C-3", C-4", C-5", C-6"), 76.7, 70.3 ($\underline{C}H_2$ benzyls), 63.0 (O-$\underline{C}H_2$-O), 54.9 (O-$\underline{C}H_3$), 29.5 ($\underline{C}H_3$/C-6), 20.7 ($\underline{C}H_3$-C=O) acetyl); m/z: calc= 1405, found (Maldi-Tof)= 1430 $[M+Na]^+$.

4.3.1 Intramolecular Methylene Acetal Formation Approach

6-*O*-acetyl-2-acetimido-3,4-di-*O*-benzyl-6-*C*-methyl-D-glucopyranoside (109) - To a solution of Allyl 6-*O*-acetyl-2-deoxy-3,4-di-*O*-benzyl-6-*C*-methyl-2-*N*-phthalimido-β-D-glucopyranoside (**81**) (300 mg, 0.51 mmol) in a mixture acetic acid/water (9/1, v/v, 3 mL) were added palladium (II) chloride (636 mg, 3.59 mmol), sodium acetate (636 mg, 7.65 mmol) and *tri*-butyltin hydride (0.55 mL, 2.04 mmol). The reaction mixture was stirred for 18 h after which time it was filtered through celite. The solvent was evaporated and the residue taken up into ethyl acetate (5 mL). The resulting solution was washed with, successively, aqueous KF (1M, 2 × 10 mL) and water (10 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed. The residue was purified by flash silica gel column chromatography (methylene chloride/acetone, 99/1, v/v) to afford compound **109** as a colorless oil (224 mg, 80%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.19; $[\alpha]_d$= +2.3 (methylene chloride, c= 7.2 mg/mL, 25 ^oC) δ_H ppm (300 MHz, $CDCl_3$): 7.75 (4H, br-s, phthalimido H), 7.71-6.83 (10H, m, arom H), 5.42-5.28 (2H, m, H-1, H-6), 4.87-4.76 (2H, m, C$\underline{H}_2$ benzyls), 4.54-4.37 (3H, m, H-3, J_{gem}= 10.2 Hz, C$\underline{H}_2$ benzyls), 4.11 (1H, dd, $J_{2,3}$=$J_{2,1}$= 9.5 Hz, H-2), 3.67 (1H, dd, $J_{4,3}$=$J_{4,5}$= 9.3 Hz, H-4), 3.50 (1H, m, H-5), 2.91 (1H, d, $J_{1,OH}$= 7.9 Hz, O$\underline{H}$), 2.09 (3H, s, C$\underline{H}_3$-C=O), 1.33 (3H, d, $J_{vicinal}$= 6.7 Hz, C$\underline{H}_3$/C-6); δ_C ppm (300 MHz, $CDCl_3$): 134.0-122.4 (arom C), 93.5 (C-1), 79.9, 79.8, 76.9, 68.7, 59.2 (C-2, C-3, C-4, C-5, C-6), 75.0 (C$\underline{H}_2$ benzyls), 21.7 (C$\underline{H}_3$-C=O), 18.3 (C$\underline{H}_3$/C-6); m/z: calc= 543, found (Maldi-Tof)= 567 $[M+Na]^+$; Elemental analysis: Calc. C: 68.25, H: 5.73, Found: C: 68.48, H: 5.98.

Trichloroacetimidate 6-*O*-acetyl-2-acetimido-3,4-di-*O*-benzyl-6-*C*-methyl-β-D-gluco-pyranoside (110) - To a solution of 6-*O*-acetyl-2-acetimido-3,4-di-*O*-benzyl-6-*C*-methyl-D-glucopyranoside (**109**) in methylene chloride (190 mg, 0.35 mmol) were added trichloroacetonitrile (13 eq, 0.46 mL, 0.66 mmol) and DBU (0.25 eq, 13 µl, 0.09 mmol). The solution was stirred under argon for 18 h. The solvent was removed under reduced pressure and the residue was purified by short flash silica gel column chromatography (methylene chloride/acetone, 97/3, v/v, then 95/5) to afford building block **110** as a white foam (182 mg, 76%). R_f (methylene chloride/acetone, 95/5, v/v)= 0.78; $[\alpha]_d$= +10.4 (methylene chloride, c= 5.6 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.72 (4H, br-s, phthalimido), 7.70-6.82 (10H, m, arom H), 6.33 (1H, d, $J_{1,2}$= 7.9 Hz, H-1), 5.37 (1H, m, H-6), 4.89-4.76 (2H, m, C$\underline{H}_2$ benzyls), 4.58-4.39 (4H, m, H-3, H-2, C$\underline{H}_2$ benzyls), 3.81-3.69 (1H, m, H-4), 3.58 (1H, m, H-5), 2.13 (3H, s, C$\underline{H}_3$-C=O), 1.34 (3H, br-s, C$\underline{H}_3$/C-6). δ_C ppm (300 MHz, $CDCl_3$): 135.8-123.7 (arom C), 94.9 (C-1), 79.8, 78.6, 77.7, 67.9, 57.8 (C-2, C-3, C-4, C-5, C-6), 75.9 (C$\underline{H}_2$ benzyls), 21.8 ($\underline{C}H_3$-C=O), 16.5 ($\underline{C}H_3$/C-6); m/z: calc= 688, found (Maldi-Tof)= 567 ($[M+Na]^+$-trichloroacetimidate).

Methyl 3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzyl-4,6-di-*O*-benzyl-idene-α-D-mannopyranoside (111) - To a solution of Methyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→3)-2-*O*-benzyl-4,6-di-*O*-benzilydene-α-D-mannopyra-noside (**102**) (183 mg, 0.22 mmol) in methanol (5 mL) was added sodium methoxide (catalytic amount) and the reaction mixture was stirred under argon for 4 h. The reaction mixture was then neutralized using Dowex 200WH+ resin, filtered and the solvent was removed to give compound **111** as a white foam that was used without any further

purification (170 mg, 98%). R_f (hexane/ethyl acetate, 70/30, v/v)= 0.52; $[\alpha]_d$= +22.1 (methylene chloride, c= 13.2 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.53-7.35 (25H, m, arom H), 5.70 (1H, s, C<u>H</u> benzylidene), 5.34 (1H, s, H-1'), 4.80-4.48 (11H, m, J_{gem}=11.3 Hz, C<u>H</u>$_2$ benzyls, H-1), 4.38-4.12 (4H, m, H-3, H-3', H-2', H-6a), 3.97-3.68 (8H, m, H-2, H-4, H-5, H-6b, H-4', H-5', H-6'), 3.30 (O-C<u>H</u>$_3$); δ_C ppm (75 MHz, $CDCl_3$): 136.2, 135.5, 135.1 (arom Cq), 128.5-123.7 (arom C), 93 (C<u>H</u> benzylidene), 98.2, 97.8 (C-1, C-1'), 77.6, 76.8, 75.3, 72.1, 69.7, 65.7, 61.7 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 72.6, 71.2, 69.5, 67.0, 66.6 (<u>C</u>H$_2$ benzyls, C-6, C-6'), 52.6 (O-<u>C</u>H$_3$); m/z: calc= 805, found (Maldi-Tof)= 827 $[M+Na]^+$.

Methyl (6"-*O*-acetyl-3",4"-di-*O*-benzyl-2"-deoxy-2"-6"-*C*-methyl-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4,6-di-*O*-benzilydene-α-D-mannopyranoside (112) - To a solution of Methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→2)-2,6-di-*O*-benzyl-4-*O*-methyl thiomethyl-α-D-mannopyranoside (**111**) (170 mg, 0.21 mmol) and trichloroacetimidate 6-*O*-acetyl-2-acetimido-3,4-di-*O*-benzyl-6-*C*-methyl-β-D-glucopyranoside (**110**) (175 mg, 0.25 mmol) in methylene chloride (5 mL) were added 4 Å molecular sieves (0.5 g) and the reaction mixture was stirred under argon for 1 h. The solution was then cooled to –20 ^oC. TMSOTf (5 µl, 0.025 mmol) was then added and the reaction mixture was stirred under argon at –20 ^oC for 1 h. The solution was then neutralized by addition of Et_3N, filtered trough celite and the solvent was removed *in vacuo*. The residue was purified by flash silica gel column chromatography (methylene chloride/acetone, 99/1, v/v) to afford the desired trisaccharide **112** as a white foam (248

mg, 88%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.63; $[\alpha]_d$= +21.5 (methylene chloride, c= 6.8 mg/mL, 25 ^oC); δ_H ppm (500 MHz, $CDCl_3$): 7.85-6.82 (39 H, m, arom H), 5.57 (1H, s, C<u>H</u> benzylidene), 5.03 (1H, s, H-1'), 5.02-4.98 (1H, m, H-6"), 4.93-4.34 (14H, m, H-1", H-1, J_{gem}= 11.7 Hz, C<u>H</u>$_2$ benzyls), 4.26-3.95 (6H, m, H-2", H-2', $J_{4',3'}$=$J_{4',5'}$= 8.4 Hz, H-4', H-3, H-2, H-6a), 3.90-3.35 (10H, m, H-3', H-4, H-5', H-3", H-5", H-6b', H-4", H-5, H-6), 3.31 (3H, s, O-C<u>H</u>$_3$), 2.04 (3H, s, C<u>H</u>$_3$-C=O), 1.10 (3H, d, $J_{vicinal}$= 6.6 Hz, C<u>H</u>$_3$/C-6); δ_C ppm (500 MHz, $CDCl_3$): 133.9-123.3 (arom C), 102.8 (<u>C</u>H benzylidene), 100.6 (C-1), 98.0 (C-1'), 96.0 (C-1"), 79.1, 77.8, 77.2, 74.7, 74.2, 72.7, 70.4, 67.2, 63.9, 55.3 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2", C-3", C-4", C-5", C-6"), 74.7, 73.5, 70.2 (C-6, C-6'), 54.8 (O-CH3), 21.0 (<u>C</u>H$_3$-C=O), 15.9 (<u>C</u>H$_3$/C-6); m/z: calc= 1332, found (Maldi-Tof)= 1355 $[M+Na]^+$; Elemental analysis: Calc. C: 71.21, H: 6.13, Found: C: 71.37, H: 6.32.

Methyl (6"-*O*-acetyl-3",4"-di-*O*-benzyl-2"-deoxy-2"-6"-*C*-methyl-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside (113) - To a solution of methyl (6"-*O*-acetyl-3",4"-di-*O*-benzyl-2"-deoxy-2"-6"-*C*-methyl-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4,6-di-*O*-benzilydene-α-D-manno-pyranoside (**112**) (228 mg, 0.17 mmol) in THF (5 mL) were added 4 Å molecular sieves (0.5 g) and sodium cyanoborohydride (108 mg, 1.71 mmol). Hydrogen chloride (2M in diethyl ether) was added until no more gas was released. The reaction mixture was then allowed to stir under argon for 1 h. The reaction mixture was then filtered through celite and the resulting solution was washed with, successively, aqueous $NaHCO_3$ (saturated, 2

× 30 mL) and water (30 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The yellow crude product was purified by flash silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then 90/10, 80/20, and 70/30) to afford trisaccharide **113** as a colorless oil (190 mg, 83%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.57; $[\alpha]_d$= +29.9 (methylene chloride, c= 10.7 mg/mL, 25 ^oC); δ_H ppm (500 MHz, $CDCl_3$): 7.70 (4H, br-s, phthalimido), 7.68-6.82 (35H, m, arom H), 5.40-5.34 (1H, m, H-6"), 5.27 (1H, m, H-1"), 5.17 (1H, s H-1'), 4.96-4.41 (17H, m, H-3", H-1, H-2, C$\underline{H}_2$ benzyls), 4.32 (1H, m, H-2"), 4.16 (1H, br-s, H-2'), 4.06-3.57 (11H, m, H-6a, H-3', H-4, H-6b, H-4', H-3", H-3, H-5", H-5', H-6'), 3.46-3.38 (2H, m, H-5, H-4"), 3.27 (3H, s, O-C$\underline{H}_3$), 2.14 (3H, s, C$\underline{H}_3$-C=O), 1.37 (3H, d, $J_{vicinal}$= 6.9 Hz, C$\underline{H}_3$/C-6); δ_C ppm (500 MHz, $CDCl_3$): 133.1-123.3 (arom C), 98.7 (C-1), 97.8 (C-1'), 96.8 (C-1"), 79.3, 79.1, 77.9, 77.8, 75.9, 74.9, 74.5, 73.8, 70.9, 68.0, 67.4, 55.9 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2", C-3", C-4", C-5", C-6"), 75.1, 70.8 (C$\underline{H}_2$ benzyls), 72.9, 70.8 (C-6, C-6'), 54.7 (O-C$\underline{H}_3$), 21.0 (C$\underline{H}_3$-C=O), 16.3 (C$\underline{H}_3$/C-6); m/z: calc= 1334, found (Maldi-Tof)= 1359 $[M+Na]^+$.

4.4 Synthesis of (2-*N*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6"-carbamate (**72**)

4.4.1 Intramolecular Glycosylation Approach

Acetyl 3,4,6-Tri-*O*-acetyl-2-deoxy-*N*-phtalimido-β-D-glucopyranoside (118) - To a solution of D-glucosamine hydrochloride (30 g, 139 mmol) in distilled water (150 mL) were added $NaHCO_3$ (11.7 g, 0.139 mmol) and phtalic anhydride (20.6 g, 0.139 mmol) and the solution was stirred at 40 ^oC for 1 h. More $NaHCO_3$ (11.7 g, 0.139 mmol) was

added portionwise over a period of 4 h and the mixture was stirred for 18 h at 40 ^{o}C. The solution was acidified with aqueous HCl (2M, 120 mL) and the resulting precipitate was filtered off, washed with cold water, dried over P_2O_5 and used without further purification. Sodium acetate (6 g, 73 mmol) was dissolved in acetic anhydride (150 mL) and heated to reflux. The compound previously obtained was added portionwise to the solution through the condenser and the mixture was kept under reflux for ½ h. After cooling to room temperature, it was poured into ice-water (3 L) and stirred with an overhead stirrer for 24 h. The crystals formed were filtered off and dissolved in methylene chloride (500 mL). The resulting solution was washed with water (2 × 500 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. Recrystallisation of the crude product from propan-2-ol afforded the desired compound **118** in the β-anomer form as a white foam (34.5 g, 52% yield); R_f (ethyl acetate/toluene, 40/60, v/v)= 0.68; $[\alpha]_d$ = +57.0 (methylene chloride, c=11.4 mg/mL); δ_H ppm (300 MHz, $CDCl_3$): 7.91-7.84 (2H, m, arom H), 7.81-7.73 (2H, m, arom H), 6.52 (1H, d, $J_{1,2}$= 9.0 Hz, H-1), 5.89 (1H, dd, $J_{3,2}$= 11.0 Hz, $J_{3,4}$= 9.0 Hz, H-3), 5.22 (1H, dd, $J_{4,5}$=9.0 Hz, H-4), 4.48 (1H, dd, H-2), 4.38 (1H, dd, $J_{6a,5}$= 4.5 Hz, $J_{6a,6b}$= 12.5 Hz, H-6a), 4.15 (1H, dd, $J_{6b,5}$= 2.5 Hz, H-6b), 4.04 (1H, m, H-5), 2.12, 2.04, 2.01, 1.87 (12H, 4s, C$\underline{H}_3$-C=O); δ_C ppm (75 MHz, $CDCl_3$): 170.6, 169.9, 164.4, 168.6, 167.3 (C=O), 134.5-123.8 (arom C), 131.2 (Cq-Phth), 89.7 (C-1), 72.6, 70.5, 68.3 (C-3, C-4, C-5), 61.5 (C-6), 53.5 (C-2), 20.7, 20.6, 20.4 ($\underline{C}H_3$-C=O); m/z: calc= 477, found (LSI)= 500 Da (100%, $[M+Na]^+$).

***p*-Methoxyphenyl 3,4,6-Tri-*O*-acetyl-2-deoxy-*N*-phthalimido-β-D-glucopyranoside (119)** - To a cooled solution (0 ^{o}C) of Acetyl 3,4,6-Tri-*O*-acetyl-2-deoxy-*N*-phthalimido-β-D-glucopyranoside (15) (10.0 g, 21 mmol) in methylene chloride (150 mL) were added *p*-methoxyphenol (4.0 g, 33 mmol) and TMSOTf (0.41 mL, 2.1 mmol). The reaction mixture was stirred under argon for 3h. Ethyl acetate was then added and the solution was washed with, successively, aqueous $NaHCO_3$ (saturated, 2×100 mL) and brine (100 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (methylene chloride/acetone, 99/1, v/v) to afford **118** as a pale yellow oil (11.2 g, 99%). *R*f (methylene chloride/acetone, 96/4, v/v)= 0.56; $[\alpha]_d$ = +47.1 (methylene chloride, c= 9.7 mg/mL, 25 ^{o}C)δ_H ppm (300 MHz, $CDCl_3$): 7.90-7.81 (2H, m, arom H phthalimido), 7.76-7.70 (2H, m, arom H phthalimido), 6.89-6.82 (2H, m, arom H MP), 6.77-6.71 (2H, m, arom H MP), 5.89 (2H, d and dd, $J_{1,2}$= 8.5 Hz, $J_{3,4}$= 8.8 Hz, $J_{3,2}$= 10.7 Hz, H-1, H-3), 5.26 (1H, dd, $J_{4,5}$= 9.9 Hz, H-4), 4.59 (1H, dd, H-2), 4.35 (1H, dd, H-2), 4.35 (1H, dd, $J_{6a,5}$=5.1 Hz, $J_{6a,6b}$= 12.5 Hz, H-6a), 4.19 (1H, dd, $J_{6b,5}$= 2.2 Hz, H-6b), 3.98 (1H, m, H-5), 3.72 (3H, s, O-C$\underline{H}_3$), 2.12, 2.06, 1.90 (9H, 3s, C$\underline{H}_3$-C=O); δ_C ppm (75 MHz, $CDCl_3$): 170.6, 170.2, 169.5 (C=O), 155.8, 150.5 (Cq MP), 134.4, 123.7 (arom C phthalimido), 134.4, 123.7 (arom C phthalimido), 131.3 (Cq phthalimido), 118.9, 114.5 (arom C MP), 97.5 (C-1), 72.0, 70.7, 68.9 (C-3, C-4, C-5), 62 .0 (C-6), 55.6, 54.6 (C-2, O-$\underline{C}H_3$), 20.8, 20.7 ($\underline{C}H_3$-C=O); m/z: calc= 541, (LSI)= 564 (100%, $[M+Na]^+$).

***p*-Methoxyphenyl 2-deoxy-2-phtalimido-β-D-glucopyranoside (120)** - Methanolic sodium methoxide (0.2 M, 11 mL) was added to a solution of *p*-methoxyphenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside **(119)** (11.11 g, 20.5 mmol) in methanol (100 mL) and the reaction mixture was stirred under argon at room temperature for 2 h. The mixture was neutralized with Dowex-50WX8-[H^+], filtered and concentrated under reduced pressure which yielded compound **120** as a white foam (8.54 g, 100% yield). R_f (methanol/methylene chloride, 20/80, v/v) = 0.62; δ_H (300 MHz, CD_3OD): 7.93-7.78 (4H, m, arom H phthalimido), 6.88-6.80 (2H, m, arom H MP), 5.66 (1H, d, $J_{1,2}$ = 8.1 Hz, H-1), 4.31 (1H, dd, $J_{3,2}$ = 10.7 Hz, $J_{3,4}$ = 7.7 Hz, H-3) 4.21 (1H, dd, H-2), 3.95 (1H, dd, $J_{6b,5}$ = 2.0Hz, $J_{6b,6}$, 12.0 Hz, H-6b), 3.78 (1H, dd, $J_{6a,5}$ = 4.8 Hz, H-6a), 3.68 (3H, s, O-C$\underline{H}_3$), 3.60-3.45 (2H, m, H-4, H-5); δ_C (75 MHz, CD_3OD), 157.5, 153.2 (Cq MP), 136.3, 124.9 (arom C phthalimido), 133.6 (Cq phthalimido), 119.8, 116.1 (arom C MP), 99.9 (C-1), 79.2, 73.3, 72.9 (C-3, C-4, C-5), 63.1 (C-6), 59.1, 56.6 (C-2, O-$\underline{C}H_3$); m/z: calc= 415, found (LSI)= 438 (100%, $[M+Na]^+$).

***p*-Methoxyphenyl 2-deoxy-2-phtalimido-6-O-trityl-β-D-glucopyranoside (121)** - To a solution of *p*-Methoxyphenyl 2-deoxy-2-phtalimido-β-D-glucopyranoside **(120)** (2.8 g, 6.74 mmol) in pyridine (80 mL) were added trityl chloride (4.8 g, 17.2 mmol) and DMAP (catalytic amount). The solution was stirred for five hours after which time the solvent was evaporated *in vacuo* and the obtained oil was co-evaporated with toluene (3 x 80 mL). The residue was taken up in methylene chloride and the solution was washed with, successively, aqueous $NaHCO_3$ (saturated, 2 x 100 mL) and brine (100 mL). The organic layer was dried (MgSO4), filtered and concentrated to dryness under reduced

pressure. The oily residue was purified by silica gel column chromatography (methanol/ methylene chloride, 2/98, v/v) to afford **121** as a yellow foam (3.54 g, 80% yield); R_f (methanol/CH_2Cl_2, 3/47, v/v) = 0.56; $[\alpha]_D$ = +9.8 (methylene chloride, c = 11.3 mg/mL); δ_H (300 MHz, $CDCl_3$) 7.88-7.79 (2H, m, arom H phthalimido), 7.50-7.15 (15H, m, arom H), 6.98-6.89 (2H, m, arom H MP), 6.78-6.69 (2H, m, arom H MP), 5.72 (1H, d, $J_{1,2}$ = 8.1 Hz, H-1), 4.48-4.35 (2H, m, H-2, H-3), 3.72 (3H, s, O-C$\underline{H}_3$), 3.69-3.61 (2H, m, H-6), 3.53-3.51 (2H, m, H-4, H-5); δ_C (75 MHz, $CDCl_3$), 168.6 ($\underline{C}$ = 0), 155.3, 151.0 (Cq MP), 143.9 (Cq trityl), 134.2, 123.5 (arom C, phtalimido), 131.5 (Cq phthalimido), 128.8-124.2 (arom C trityl), 118.8, 114.8 (arom C MP), 97.6 (C-1), 86.8 (Cq $\underline{C}Ph_3$), 75.2, 72.6, 71.9 (C-3, C-4, C-5), 63.6 (C-6), 55.8, 55.6 (C-2, O-$\underline{C}H_3$); m/z: calc= 657, found (LSI)= 680 (100%, $[M+Na]^+$).

***p*-Methoxyphenyl 3,4-Di-*O*-benzyl-2-deoxy-2-phtalimido-6-*O*-trityl-β-D-glucopyranoside (122)** – To a solution of *p*-Methoxyphenyl 2-Deoxy-2-phtalimido-6-*O*-trityl-β-D-glucopyranoside **(121)** (3.48 g, 5.29 mmol) in THF (75 mL) were added benzyl bromide (2.52mL, 21.2 mmol), sodium hydride (60% suspended in mineral oil, 0.50 g, 12.7 mmol) and TBAI (catalytic amount). The reaction mixture was heated under reflux for three hours, after which time it was directly poured into ice-water (200 mL). The aqueous layer was extracted with ethyl acetate (4 x 100 mL) and the combined organic layer was dried ($MgSO_4$), filtered and concentrated to dryness under reduced pressure. Purification by silica gel column chromatography of the oily residue (petroleum ether 40-60/CH_2Cl_2, 1/1, v/v, followed by petroleum ether 40-60/CH_2Cl_2, 1/2, v/v and CH_2Cl_2) gave compound **122** as a pale yellow foam (3.09 g, 70% yield); R_f (acetone/methylene

chloride, 1/99, v/v) = 0.83; $[\alpha]_d$ = +35.9 (methylene chloride, c=4.3 mg/mL, 25 ^oC); δ_H (300 MHz, $CDCl_3$): 7.90-6.82 (31H, m, arom H), 6.78-6.70 (2H, m, arom H MP), 5.67 (1H, d, $J_{1,2}$ = 8.5 Hz, H-1), 4.84 (1H, d, J_{gem}= 12.1 Hz, C$\underline{H}_2$ benzyl), 4.71 (1H, d, J_{gem}= 10.7 Hz, C$\underline{H}_2$ benzyl), 4.56-4.36 (3H, m, H-3, C$\underline{H}_2$ benzyl), 4.37 (1H, dd, $J_{2,3}$= 10.7 Hz, H-2), 4.00 (1H, dd, $J_{4,3}$= 8.8 Hz, $J_{4,5}$ = 9.6 Hz, H-4), 3.72 (3H, s, O-C$\underline{H}_3$), 3.75-3.60 (2H, m, H-6), 3.45-3.35 (1H, m, H-5); δ_C (75 MHz, $CDCl_3$): 167.9 (C=0), 155.1 (Cq MP), 143.9 (Cq trityl), 138.0, 137.7 (Cq benzyl), 133.9, 123.4 (arom C phthalimido), 131.7 (Cq phthalimido), 128.9-127.1 (arom C), 119.2, 114.5 (arom C MP), 97.2 (C-1), 86.6 (Cq $\underline{C}Ph_3$), 79.7, 79.2, 74.9 (C-3, C-4, C-5), 75.1 ($\underline{C}H_2$ benzyl), 62.2 (C-6), 56.2, 55.7 (C-2, O-$\underline{C}H_3$); m/z : calc= 837, found (LSI) 860 (100%, $[M+Na]^+$).

***p*-Methoxyphenyl 3,4-di-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (116)** - To a solution of *p*-Methoxyphenyl 3,4-di-O-benzyl-2-deoxy-2-phtalimido-6-O-trityl-β-D-glucopyranoside (**122**) (2.98 g, 3.55 mmol) in acetonitrile (40 mL), was added tetrafluoroboric acid (48% in water, 0.472 mL, 3.55 mmol) and the reaction mixture was stirred at room temperature for 30 min. The solution was neutralized with triethylamine and evaporated to dryness *in vacuo*. The oily residue was purified by silica gel column chromatography (acetone/CH_2Cl_2, 2/98, v/v) to give **116** as a yellow foam (1.89 g, 90% yield). R_f (acetone/ CH_2Cl_2, 1/49, v/v) = 0.29; $[\alpha]_D$ = +77.3 (methylene chloride, c = 10.3 mg/mL, 25 ^oC); δ_H (300 MHz, $CDCl_3$): 7.68 (4H, br-s, arom H phtalimido), 7.41-6.85 (10H, m, arom H), 6.82-6.75 (2H, m, arom H MP), 6.74-6.66 (2H, m, arom H MP), 5.71 (1H, d, $J_{1,2}$ = 8.1 Hz, H-1), 4.92, 4.84, 4.76 (3H, 3d, J_{gem}= 10.8 Hz, J_{gem}= 12.1 Hz, -C$\underline{H}_2$-benzyl), 4.48 (1H, d, -C$\underline{H}_2$- benzyl), 4.44, 4.43 (2H, 2dd, $J_{3,2}$= 10.7 Hz, H-3, H-2), 3.95

(1H, dd, $J_{6a,6b}$= 11.7 Hz, H-6a), 3.85-3.74 (2H, m, H-4, H-6b), 3.64 (1H, dd, $J_{5,6a}$ = 2.6 Hz, $J_{5,\ 6b}$ = 4.0 Hz, $J_{5,4}$ = 9.9 Hz, H-5), 3.72 (3H, s, O-C$\underline{H}_3$); δ_C (75 MHz, $CDCl_3$): 167.9 (C=0), 155.4, 150.7 (Cq -MP), 137.9 (Cq benzyl), 133.9, 123.4 (arom C phthalimido), 131.6 (Cq phthalimido), 128.6-127.5 (arom C), 118.4, 114.5 (arom C MP), 97.4 (C-1), 79.3, 79.0, 75.6 (C-3, C-4, C-5), 75.2, 75.0 ($\underline{C}H_2$ benzyl), 61.7 (C-6), 55.9, 55.6 (C-2, O$\underline{C}H_3$); m/z: calc= 595, found (LSI) 618 (100%, $[M+Na]^+$).

Methyl 2,3-Di-*O*-isopropylidene-α-D-mannopyranoside (133) - To a suspension of Methyl α-D-mannopyranoside (**132**) (10.0 g, 51.5 mmol) in acetone (70 mL) were added 2,2-dimethoxypropane (30.7 mL, 257mmol) and CSA (2.0 g, 10.3 mmol). The reaction mixture was stirred for 18 h, after which time water (10 mL) was added. After 75 min, the solution was neutralized and concentrated to dryness. Purification by silica gel column chromatography (methylene chloride/methanol, 98/2, v/v) gave the desired compound **133** as a white solid (8.18 g, 67%). R_f (methylene chloride/methanol, 90/10, v/v)= 0.56; $[\alpha]_d$ = +25.5 (methylene chloride, c=8.3 mg/mL, 25 ^oC) ; δ_H ppm (300 MHz, $CDCl_3$): 4.89 (1H, s, H-1), 4.12 (2H, m, H-2, H-4, 3.83 (2H, m, H-6), 3.68 (1H, m, H-3), 3.55 (1H, m, H-5), 3.46 (3H, s, O-C$\underline{H}_3$), 1.50, 1.42 (6H, 2s, C$\underline{H}_3$ methylidene); δ_C ppm (75 MHz, $CDCl_3$): 98.4 (C-1), 78.2, 75.4, 69.7, 69.5 (C-2, C-3, C-4, C-5), 62.5 (C-6), 55.2 (O-$\underline{C}H_3$), 27.9, 26.1 ($\underline{C}H_3$, methylidene); m/z: calc= 234, found (LSI) = 257 $[M+Na]^+$.

Methyl 6-*O*-Benzyl-2,3-di-*O*-isopropylidene-α-D-mannopyranoside (128) - To a solution of Methyl 2,3-Di-*O*-isopropylidene-α-D-mannopyranoside (**133**) (8.08 g, 33.5 mmol) in toluene (125 mL) was added *tert*-butyl tin dimethoxide (8.9 mL, 36.9 mmol). The reaction mixture was refluxed under Dean Stark conditions for 18 h. Benzyl bromide (4.8 mL, 40.2 mmol) and TBAI (18.5 g, 50.2 mmol) were added and the reaction mixture was refluxed for 18 h. The reaction mixture was allowed to cool down, filtered and the solvent was removed *in vacuo*. The residue was taken up into methylene chloride (200 mL) and washed with, successively, aqueous KF (1M, 2 × 200 mL) and water (200 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (toluene/acetone, 95/5, v/v, then 90/10, v/v) to give **128** as colorless oil (9.89 g, 91%). *R*f (methylene chloride/methanol, 95/5, v/v)= 0.88; $[\alpha]_d$ = +38.5 (methylene chloride, c=10.0 mg/mL, 25 ^oC) ; δ_H ppm (300 MHz, $CDCl_3$): 7.38-7.24 (5H, m, arom H), 4.90 (1H, s, H-1), 4.59 (2H, 2d, J_{gem}= 12.3 Hz, J_{gem}= 11.9 Hz, C$\underline{H}_2$ benzyls), 4.12 (2H, m, H-2, H-3), 3.73 (4H, m, H-4, H-5, H-6a, H-6b), 3.40 (3H, s, O-C$\underline{H}_3$), 1.71, 1.58 (6H, 2s, C$\underline{H}_3$ methylidene); δ_C ppm (75 MHz, $CDCl_3$): 128.6, 127.9, 127.9 (arom C), 109.7 (C_q methylidene), 98.6 (C-1), 78.4, 75.6, 70.7, 68.9 (C-2, C-3, C-4, C-5), 73.9, 70.5 (C-6, C$\underline{H}_2$ benzyl), 55.4 (O-$\underline{C}H_3$), 28.2, 26.4 ($\underline{C}H_3$, methylidene); m/z: calc= 324, found (Maldi-Tof)= 345 $[M+Na]^+$.

Methyl 6-*O*-benzyl-2,3-*O*-isopropylidene-α-D-lyxo-hexopyranosid-4-ulose (129) - To a cooled solution (-65 ^oC) of DMSO (4.3 mL, 60.4 mmol) in methylene chloride (40 mL) was added dropwise over 20 min a solution of TFAA (6.4 mL, 45.3 mmol) in methylene

chloride (20 mL). After stirring for 15 min, a solution of Methyl 6-*O*-Benzyl-2,3-di-*O*-isopropylidene-α-D-mannopyranoside (**128**) (9.79 g, 30.2 mmol) in methylene chloride (40 mL) was added dropwise over 20 min. The reaction mixture was stirred under argon at -65 ^{o}C for 30 more min. Et_3N (8 mL) was then added dropwise and the solution was stirred at -65 ^{o}C for 5 more min and the reaction mixture was allowed to warm up to room temperature. The reaction mixture was washed with water (100 mL) and the aqueous layer was extracted with methylene chloride (2×200 mL). The collected organic layer was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo*. Purification by silica gel column chromatography (methylene chloride/acetone, 99/1, v/v) afforded **129** as a colorless oil (7.78 g, 80%). *R*f (methylene chloride/acetone, 97/3,v/v)= 0.87; $[\alpha]_d$ = +46.8 (methylene chloride, c=5.8 mg/mL, 25 ^{o}C) ; δ_H ppm (300 MHz, $CDCl_3$): 7.38-7.22 (5H, m, arom H), 4.91 (1H, s, H-1), 4.62 (2H, 2d, J_{gem}= 12.3 Hz, C$\underline{H}_2$ benzyls), 4.46-4.31 (3H, m, H-2, H-3, H-5), 3.84 (2H, m, H-6a, H-6b), 3.42 (3H, s, O-C$\underline{H}_3$), 1.47, 1.35 (6H, 2s, C$\underline{H}_3$ methylidene); δ_C ppm (75 MHz, $CDCl_3$): 202.3 ($\underline{C}$=O), 138.0 (C_q arom.), 128.5, 127.9, 127.8 (arom C), 111.9 (C_q methylidene), 98.4 (C-1), 78.5, 75.8, 74.4 (C-2, C-3, C-5), 73.9, 69.6 (C-6, $\underline{C}H_2$ benzyl), 54.3 (O-$\underline{C}H_3$), 27.0, 27.8 ($\underline{C}H_3$, methylidene); m/z: calc= 322, found (Maldi-Tof)= 345 $[M+Na]^+$.

Methyl 6-*O*-Benzyl-2,3-di-*O*-isopropylidene-α-D-talopyranoside (134) - To a cooled solution (0 ^{o}C) of Methyl 6-*O*-Benzyl-2,3-*O*-isopropylidene-α-D-lyxo-hexopyranosid-4-ulose (**129**) (7.68 g, 23.9 mmol) in a mixture EtOH/water (7/2, v/v, 150 mL) was added $NaBH_4$ (0.54 g, 14.3 mmol). The reaction mixture was stirred for 30 min (0^{o}C to room temperature). The reaction was quenched with acetone, the solvent was removed *in vacuo*

and the residue was taken up into methylene chloride (150 mL). The resulting solution was washed with, successively, aqueous $NaHCO_3$ (saturated, 2×150 mL) and water (2 × 150 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography (methylene chloride/acetone, 99/1, v/v) to give compound **134** as a colorless oil (7.19 g, 93%). Rf (methylene chloride, 97/3, v/v)= 0.62; $[\alpha]_d$ = +60.6 (methylene chloride, c= 11.9 mg/mL, 25 ^oC) ; δ_H ppm (300 MHz, $CDCl_3$): 7.38-7.22 (5H, m, arom H), 4.98 (1H, s, H-1), 4.60 (2H, 2d, J_{gem}= 11.9 Hz, C$\underline{H}_2$ benzyls), 4.22 (1H, dd, $J_{3,2}$=$J_{3,4}$= 5.7 Hz, H-3), 4.06 (1H, d, H-2), 3.94-3.86 (1H, m, H-5), 3.82-3.72 (3H, m, H-4, H-6a, H-6b), 3.43 (3H, s, O-C$\underline{H}_3$), 1.59, 1.38 (6H, 2s, C$\underline{H}_3$ methylidene); δ_C ppm (75 MHz, $CDCl_3$): 128.6, 128.5, 127.8 (arom C), 109.6 (Cq methylidene), 98.7 (C-1), 73.9, 72.8, 68.2, 64.7 (C-2, C-3, C-4, C-5), 73.8, 70.2 (C-6, $\underline{C}H_2$ benzyl), 55.4 (O-$\underline{C}H_3$), 26.2, 25.5 ($\underline{C}H_3$, methylidene); m/z: calc= 324, found (Maldi-Tof)= 349 $[M+Na]^+$; Elemental analysis: Calc. C: 69.11, H: 6.16, Found: C: 69.32, H: 6.41.

Methyl 6-*O*-benzyl-2,3-di-*O*-isopropylidene-4-*O*-mesylate-α-D-talopyranoside (135) - To a cooled solution (0 ^oC) of methyl 6-O-benzyl-2,3-di-O-isopropylidene-α-D-talopyranoside (**134**) (7.09 g, 21.9 mmol) in methylene chloride (125 mL) were added Et_3N (6.10 mL, 43.8 mmol) and methanesulfonyl chloride (3.4 mL, 43.8 mmol). The reaction mixture was stirred under argon for 10 min and allowed to warm to room temperature. After 2 h, the reaction was quenched with aqueous $NaHCO_3$ (saturated, 10 mL). Methylene chloride (100 mL) was added. The organic phase was separated and washed with aqueous $NaHCO_3$ (saturated, 2×200 mL), then aqueous $CuSO_4$ (saturated,

2×200 mL) and water (2×200 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. Purification of the crude product by silica gel column chromatography (methylene chloride/acetone, 99/1, v/v) afforded product **135** as a colorless oil (6.60 g, 75%). *R*f (methylene chloride /acetone, 97/3, v/v)= 0.47; $[\alpha]_d$ = +35.6 (methylene chloride, c=7.8 mg/mL, 25 ^oC) ; δ_H ppm (300 MHz, $CDCl_3$): 7.38-7.22 (5H, m, arom. H), 4.92 (1H, s, H-1), 4.83 (1H, d, $J_{2,3}$,= 8.7 Hz, H-2), 4.58 (2H, dd, J_{gem}= 11.3 Hz, C$\underline{H}_2$ benzyls), 4.35 (1H, dd, $J_{3,4}$= 9.0 Hz, H-3), 4.11-3.98 (2H, m, H-4, H-5), 3.83-3.69 (2H, m, H-6a, H-6b), 3.42 (3H, s, O-C$\underline{H}_3$), 3.03 (3H, s, C$\underline{H}_3$ Ms), 1.60, 1.38 (6H, 2s, C$\underline{H}_3$ methylidene); δ_C ppm (300 MHz, $CDCl_3$): 128.6-127.5 (arom C), 104.8 (C-1), 78.4, 73.7, 69.8, 67.4, 65.0 (C-2, C-3, C-4, C-5, C-6), 72.4 (C$\underline{H}_2$ benzyl), 55.5 (O-C$\underline{H}_3$), 38.5 (C$\underline{H}_3$ Ms), 29.6 (C$\underline{H}_3$, methylidene); m/z: calc= 402, found (Maldi-Tof)= 426 $[M+Na]^+$.

Methyl 6-*O*-Benzyl-4-*O*-mesylate-α-D-talopyranoside (136) - To a solution of Methyl 6-*O*-Benzyl-2,3-di-*O*-isopropylidene-4-*O*-mesylate-α-D-talopyranoside (**135**) (6.50 g, 16.2 mmol) in DCM (64 mL) was added TFA (16 mL) and water (2 drops). The reaction mixture was stirred for 30 min. The reaction mixture was carefully neutralized using aqueous $NaHCO_3$ (saturated, 300 mL). The organic layer was separated, dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. The oily residue was purified by silica gel column chromatography (methylene chloride/methanol, 98/2, v/v) to afford **136** as a white foam (5.33 g, 91%). *R*f (methylene chloride/methanol, 95/5, v/v)= 0.69; $[\alpha]_d$ = +12.5 (methylene chloride, c=3.9 mg/mL, 25 ^oC) ; δ_H ppm (300 MHz, $CDCl_3$): 7.38-7.22 (5H, m, arom. H), 4.97 (1H, s, H-1), 4.81 (1H, s, H-4), 4.54 (2H, s,

C$\underline{H}_2$ benzyls), 4.03 (1H, dd, $J_{6a,6b}$=$J_{6a,5}$= 6.2 Hz, H-6a), 3.92 (1H, m, H-2), 3.79-3.63 (3H, m, H-3, H-5, H-6b), 3.38 (3H, s, O-C$\underline{H}_3$), 3.11 (3H, s, C$\underline{H}_3$ Ms), 2.46 (2H, s, O$\underline{H}$); δ_C ppm (75 MHz, $CDCl_3$): 137.8 (arom Cq), 128.6, 128.0 (arom C), 101.6 (C-1), 79.7, 70.0, 67.4, 65.4 (C-2, C-3, C-5, C-5), 73.9, 68.8 (C-6, $\underline{C}H_2$ benzyl), 55.7 (O-$\underline{C}H_3$), 39.0 ($\underline{C}H_3$, methylidene); m/z: calc= 362, found (Maldi-Tof)= 384 $[M+Na]^+$.

Methyl 6-*O*-Benzyl-4-deoxy-4-azido-α-D-mannopyranoside (137) - To a solution of methyl 6-*O*-benzyl-4-*O*-mesylate-α-D-talopyranoside (**136**) (5.23 g, 14.4 mmol) in DMSO (80 mL) was added NaN_3 (4.68 g, 72 mmol) and the reaction mixture was stirred under argon at 100°C for 18 h. The reaction mixture was poured into an ice/water bath (300 mL) and the aqueous phase was extracted with ethyl acetate (7×100 mL). The combined organic layer was dried ($MgSO_4$), filtered and the solvent was evaporated *in vacuo*. Purification of the crude product by flash silica gel column chromatography (methylene chloride/acetone, 95/5, v/v, then 90/10, v/v) gave compound **137** as colorless oil (2.91 g, 81%). R_f (methylene chloride/MeOH, 95/5, v/v)= 0.59; $[\alpha]_d$ = +25.7 (methylene chloride, c=8.4 mg/mL, 25 °C) ; δ_H ppm (300 MHz, $CDCl_3$): 7.38-7.22 (5H, m, arom. H), 4.75 (1H, s, H-1), 4.67, 4.54 (2H, 2d, C$\underline{H}_2$ benzyls), 3.89-3.77 (2H, m, H-6a, H-6b), 3.76-3.66 (3H, m, H-2, H-3, H-4), 3.59-3.50 (1H, m, H-5), 3.43 (3H, s, O-C$\underline{H}_3$); δ_C ppm (75 MHz, $CDCl_3$): 137.8 (arom Cq), 128.6, 128.1, 128.0 (arom C), 101.0 (C-1), 71.0, 70.3, 70.1 (C-2, C-3, C-5), 74.0, 69.4 (C-6, $\underline{C}H_2$ benzyl), 60.2 (C-4), 55.5 (O-$\underline{C}H_3$); m/z: calc= 309, found (Maldi-Tof)= 331 $[M+N a]^+$.

Methyl 2,6-Di-*O*-benzyl-4-deoxy-4-azido-α-D-mannopyranoside (117) - To a solution of Methyl 6-*O*-Benzyl-4-deoxy-4-azido-α-D-mannopyranoside (**137**) (2.81 g, 9.1 mmol) in DCM (60 mL) was added aqueous NaOH (30%, 60 mL), TBAI (0.85 g, 2.3 mmol) and benzyl bromide (1.2 mL, 10.0 mmol). The reaction mixture was stirred 18 h and washed with water (2 × 150 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was evaporated *in vacuo*. The residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then, 90/10, 80/20 and 70/30). Building block **117** was obtained as colorless oil (2.61 g, 72%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.61; $[\alpha]_d$ = +49.5 (methylene chloride, c= 12.7 mg/mL, 25 ^oC) ; δ_H ppm (300 MHz, $CDCl_3$): 7.40-7.22 (10H, m, arom H), 4.84 (1H, s, H-1), 4.76, 4.69 (2H, 2d, J_{gem}= 11.9 Hz, J_{gem}= 12.3 Hz, C$\underline{H}_2$ benzyls), 4.55 (2H, 2d, J_{gem}=11.4 Hz, J_{gem}= 11.0 Hz, C$\underline{H}_2$ benzyls), 3.87 (1H, dd, $J_{3,2}$= 9.7 Hz, $J_{3,4}$= 3.5 Hz, H-3), 3.78-3.64 (4H, m, H-2, H-4, H-6a, H-6b), 3.57-3.48 (1H, m, H-5), 3.37 (3H, s, O-C$\underline{H}_3$); δ_C ppm (75 MHz, $CDCl_3$): 138.3, 137.5 (arom Cq), 128.8, 128.5, 128.4, 128.1, 127.8 (arom C), 98.1 (C-1), 70.8, 70.3, 69.6 (C-2, C-3, C-5), 73.8, 73.1, 69.6 (C-6, $\underline{C}H_2$ benzyls), 61.1 (C-4), 55.4 (O-$\underline{C}H_3$); m/z: calc= 399, found (Maldi-Tof)= 421 $[M+Na]^+$; Elemental analysis: Calc. C: 63.14, H: 6.31, Found: C: 63.25, H: 6.45.

Methyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl -4-deoxy-4-azido-α-D-mannopyranoside (138) - To a stirred mixture of Methyl 2,6-Di-*O*-benzyl-4-deoxy-4-azido-α-D-mannopyranoside (**117**) (2.51 g, 6.3 mmol) and Ethyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-1-deoxy-1-thio-α-D-mannopyranoside (**79**) (4.04g, 7.5 mmol) 4 Å molecular sieves (4 g) in methylene chloride (100 mL) were added NIS (1.69

g, 7.5 mmol) and TMSOTf (136 μl, 0.75 mmol). The reaction mixture was stirred for 1h, filtered through celite and the filtrate was washed with aqueous $Na_2S_2O_3$ (15%, 2 × 200 mL) and water (200 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was evaporated *in vacuo*. The residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then, 90/10, 80/20 and 70/30) to afford disaccharide **138** as colorless oil (5.05 g, 92%). R_f (hexane/ethyl acetate, 70/30, v/v)= 0.47; $[\alpha]_d$ = +31.7 (methylene chloride, c= 9.3 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.42-7.12 (25H, m, arom H), 5.51 (1H, s, H-2'), 5.15 (1H, s, H-1'), 4.88 (1H, d, J_{gem}= 11.4 Hz, C$\underline{H}_2$ benzyls), 4.69-4.43 (10H, m, H-1, C$\underline{H}_2$ benzyls), 4.13-3.72 (3H, m, H-4, H-3', H-3), 3.71-3.63 (7H, m, H-2, H-4', H-5', H-6, H-6'), 3.56-3.46 (1H, m, H-5), 3.38 (3H, s, OC$\underline{H}_3$), 2.12 (3H, s, C$\underline{H}_3$-C=O); δ_C ppm (75 MHz, $CDCl_3$): 139.5, 139.3, 139.2 (arom Cq), 128.7-127.3 (arom C), 100.1, 99.5 (C-1, C-1'), 79.8, 79.7, 77.2, 75.3, 73.7, 72.5, 69.6 (C-2, C-3, C-5, C-2', C-3', C-4', C-5'), 76.8, 76.0, 72.3, 71.2, 70.0 (C-6, C-6', $\underline{C}H_2$ benzyls), 58.5 (C-4), 54.9 (O-$\underline{C}H_3$), 21.3 ($\underline{C}H_3$-C=O); m/z: calc= 873, found (Maldi-Tof)= 896 $[M+Na]^+$; Elemental analysis: Calc. C: 68.71, H: 6.34, Found: C: 68.90, H: 6.55.

Methyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl -4-deoxy-4-amino-α-D-mannopyranoside (139) - To a solution of methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-deoxy-4-azido-α-D-mannopyranoside (**138**) (4.95 g, 5.7 mmol) in a mixture of pyridine/water (3/1, v/v, 80 mL) were added 1,3-propanedithiol (5.7 mL, 57 mmol) and Et_3N (few drops). The reaction mixture was stirred for 6 h and the solvent was co-evaporated with toluene (4 ×

100 mL). The crude product was purified by silica gel column chromatography (methylene chloride/methanol, 99/1, v/v) to afford amine **139** as colorless oil (3.84 g, 80%). R_f (methylene chloride/methanol, 99/1, v/v)= 0.82; $[\alpha]_d$ = +67.0 (methylene chloride, c=6.79 mg/mL, 25 ^oC) ; δ_H ppm (500 MHz, $CDCl_3$): 7.38-7.14 (25H, m, arom H), 5.40 (1H, s, H-2'), 5.14 (1H, s, H-1'), 4.91-4.45 (11H, m, H-1, C$\underline{H}_2$ benzyls), 4.05-3.97 (2H, m, H-3', H-5'), 3.89-3.58 (9H, m, H-2, H-4', H-3, H-4, H-5, H-6a, H-6b, H-6a', H-6b'), 3.34 (3H, s, O-C$\underline{H}_3$), 2.23 (3H, s, C$\underline{H}_3$-C=O); δ_C ppm (500 MHz, $CDCl_3$): 128.6-126.9 (arom C), 100.3 (C-1'), 98.9 (C-1), 78.9, 78.2, 74.9, 72.8, 72.1, 68.7 (C-2, C-3, C-5, C-2', C-3', C-4', C-5'), 75.1-71.1 ($\underline{C}H_2$ benzyls), 76.0, 68.2 (C-6, C-6'), 69.5 (C-4), 54.4 (O-$\underline{C}H_3$), 22.4 ($\underline{C}H_3$-C=O); m/z: calc= 847, found (Maldi-Tof)= 848 $[M+H]^+$.

Methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside-*p*-methoxyphenyl 3,4-di-*O*-benzyl-2-deoxy-2-N-phthalimido-β-D-glucopyranosyl -4,6"-carbamate (140) - To a solution of methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-deoxy-4-amino-α-D-mannopyranoside (**139**) (2.48 g, 2.9 mmol) in methylene chloride (40 mL) were added Et_3N (0.49 mL, 3.5 mmol) and triphosgene (0.42 mL, 3.5 mmol). The reaction mixture was stirred for 20 min under argon after which time TLC analysis indicated complete conversion of the starting amine into a new compound, presumably an isocyanate (Rf (DCM/acetone, 95/5, v/v)= 0.86). A solution of *p*-methoxyphenyl 2-deoxy-3,4-di-*O*-benzyl-2-N-phthalimido-β-D-glucopyranoside (**116**) (1.74 g, 2.9 mmol) in methylene chloride (20 mL) was added and the reaction mixture was stirred under argon for 18 h. The solvent was removed under reduced pressure and the residue was

purified by silica gel column chromatography (methylene chloride/acetone, 98/2, v/v, then methylene chloride /methanol, 99/1, v/v) to afford trisaccharide **140** as a colorless oil (1.29 g, 30%). R_f (methylene chloride/acetone, 95/5, v/v)= 0.28; $[\alpha]_d$ = +45.6 (methylene chloride, c= 8.8 mg/mL, 25 ^oC) ; δ_H ppm (500 MHz, $CDCl_3$): 7.65-7.59 (4H, br-s, arom H phthalimido), 7.42-7.08 (35 H, m, arom H), 7.04-6.61 (4H, 4d, arom H PM), 5.60 (1H, d, $J_{1",2"}$= 4.0 Hz, H-1"), 5.31 (1H, s, H-2'), 5.02 (1H, s, H-1'), 4.88-4.77 (2H, m, C$\underline{H}_2$ benzyls), 4.71 (1H, s, H-1), 4.66-4.37 (13H, m, C$\underline{H}_2$ benzyls, H-2"), 4.35 (1H, dd, $J_{6a,6b}$= 5.3 Hz, $J_{6a,5}$= 11.9 Hz, H-6a), 4.23 (1H, d, $J_{3,4}$= 10.6 Hz, H-3), 4.05-3.62 (16H, m, H-4, H-6a", H-6b", H-5", H-3', H-2, H-5, H-6b, H-4', H-5', H-3", H-4", H-6a', H-6b', O-C$\underline{H}_3$ PM), 3.30 (3H, s, O-C$\underline{H}_3$), 2.04 (3H, s, C$\underline{H}_3$-C=O); δ_C ppm (75 MHz, $CDCl_3$): 138.9, 138.6, 138.4, 138.1, 137.9, 134.0, 131.8 (arom Cq), 128.6-114.6 (arom C), 100.0, 98.9, 97.9 (C-1, C-1', C-1"), 80.0, 79.4, 76.4, 74.7, 73.8, 72.5, 71.2, 69.0 (C-2, C-3, C-5, C-2', C-3', C-4', C-5', C-3", C-4", C-5"), 75.4, 75.2, 75.0, 73.7, 71.9, 70.2, 69.7, 63.8 (C-6, C-6', C-6", $\underline{C}H_2$ benzyls), 56.1, 55.8, 55.3, 50.7 (C-2", C-4, O-$\underline{C}H_3$, O-$\underline{C}H_3$ PM), 21.2 ($\underline{C}H_3$-C=O); m/z: calc= 1469, found (Maldi-Tof)= 1491 $[M+Na]^+$; Elemental analysis: Calc. C: 70.28, H: 6.03, Found: C: 70.51, H: 6.30.

Methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl -4-deoxy-α-D-mannopyranoside-acetyl 3,4-di-*O*-benzyl-2-deoxy-2-N-phtalimido-α/β-D-glucopyranosyl-4,6"-carbamate (141) - To a solution of methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside-*p*-methoxyphenyl 3,4-di-*O*-benzyl-2-deoxy-2-N-phtalimido-β-D-glucopyranosyl -4,6-carbamate (**140**) (1.19 g, 8.0 mmol) in a mixture toluene/acetonitrile/water (1/4/1, v/v/v,

50 mL) was added CAN (1.42 g, 24 mmol) and the reaction mixture was stirred under the exclusion of light for 4 h. The reaction was quenched with aqueous $NaHCO_3$ (10 mL). The solution was then washed with water (5×100 mL), dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The yellow residue was dissolved in a mixture pyridine/Ac_2O (1/1, v/v, 40 mL) and this mixture was stirred for 18 h. The solvent was removed *in vacuo* and the remainder was co-evaporated with, successively, toluene (5×50 mL), EtOH (50 mL) and methylene chloride (50 mL). The crude product was purified by silica gel column chromatography (methylene chloride/acetone, 97/3, v/v) to afford **141** as colorless oil (711 mg, 63%). R_f (methylene chloride /acetone, 95/5, v/v)= 0.67; $[\alpha]_d$ = +34.7 (methylene chloride, c=7.3 mg/mL, 25 ^oC) ; δ_H ppm (500 MHz, $CDCl_3$): 7.69-7.55 (4H, br-s, arom H phthalimido), 7.40-6.91 (35H, m, arom H), 6.33 (1H, d, $J_{1",2"}$= 8.8 Hz, H-1"), 5.38 (1H, s, H-2'), 5.11 (1H, s, H-1'), 4.93-4.84 (3H, 3d, J_{gem}= 11.2 Hz, J_{gem}= 11.7 Hz, C$\underline{H}_2$ benzyls), 4.80 (1H, s, H-1), 4.76-4.26 (16H, m, H-3", H-6"a, H-3, H-2", H-6"a), 4.10-3.64 (12H, m, H-4, H-3', H-5", H-2, H-5, H-6a, H-6b, H-4', H-5', H-6a', H-6b', H-4"), 3.34 (3H, s, O-C$\underline{H}_3$), 2.07, 1.85 (6H, 2s, C$\underline{H}_3$-C=O); δ_C ppm (500 MHz, $CDCl_3$): 134.5-122.9 (arom C), 99.5 (C-1'), 98.4 (C-1), 90.0 (C-1"), 78.9, 78.6, 77.6, 76.4, 75.7, 72.0, 70.7, 68.5 (C-2, C-3, C-5, C-2', C-3', C-4', C-5', C-3", C-4", C-5"), 75.6-70.7 ($\underline{C}H_2$ benzyls), 73.3, 69.2, 63.0 (C-6, C-6', C-6"), 54.5 (O$\underline{C}H_3$), 54.4 (C-2"), 50.0 (C-4), 20.5, 20.4 ($\underline{C}H_3$-C=O); m/z: calc= 1404, found (Maldi-Tof)= 1427 [$M+Na^+$].

Methyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl -4-deoxy-α-D-mannopyranoside-ethyl 3,4-di-*O*-benzyl-2-deoxy-2-N-phtha-limido-1-thio-α-D-glucopyranosyl-4,6"-carbamate (142) - To a cooled solution (0^oC) of Methyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside-acetyl 3,4-di-*O*-benzyl-2-deoxy-2-N-phtalimido-α/β-D-glucopyranosyl-4,6"-carbamate (**141**) (682 mg, 0.49 mmol) in methylene chloride (10 mL) were added ethanethiol (360 µl, 4.9 mmol) and TMSOTf (0.4 eq, 35 µl, 0.19 mmol). After stirring at room temperature at 0 ^oC for 1 h, the reaction mixture was washed with, successively, aqueous KF solution (1M, 2×20 mL), aqueous $NaHCO_3$ (saturated, 2×20 mL) and water (20 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo*. Purification by silica gel column chromatography (methylene chloride/acetone, 97/3, v/v) afforded trisaccharide **142** as a colorless oil (480 mg, 70%). R_f (methylene chloride/acetone, 95/5, v/v)= 0.72; $[\alpha]_d$ = +24.1 (methylene chloride, c= 8.6 mg/mL, 25 ^oC) ; δ_H ppm (500 MHz, $CDCl_3$): 7.44-6.82 (39H, m, arom H), 5.34 (1H, s, H-2'), 5.27 (1H, d, $J_{1",2"}$= 10.4 Hz, H-1"), 5.08 (1H, s, H-1'), 4.97-4.37 (17H, m, H-1, J_{gem}= 10.9 Hz, C$\underline{H}_2$ benzyls, H-6"a, H-3"), 4.35-4.20 (3H, m, H-3, H-4, H-2"), 4.05-3.63 (12H, m, H-3', H-5', H-2, H-4', H-5", H-5, H-6a, H-6b, H-6a', H-6b', H-6b"), 3.59 (1H, dd, $J_{4",3"}$=$J_{4",5"}$= 9.0 Hz, H-4"), 3.33 (3H, s, O-C$\underline{H}_3$), 2.69-2.52 (2H, m, S-C$\underline{H}_2$-CH_3), 2.07 (3H, s, C$\underline{H}_3$-C=O), 1.14 (3H, m, S-CH_2-C$\underline{H}_3$); δ_C ppm (500 MHz, $CDCl_3$): 129.6-126.1 (arom C), 99.8 (C-1'), 98.8 (C-1), 81.5 (C-1"), 80.4, 80.0, 77.8, 77.6, 76.7, 76.1, 74.4, 68.5 (C-2, C-3, C-5, C-2', C-3', C-4', C-5', C-3", C-4", C-5"), 76.2-70.7 (C$\underline{H}_2$ benzyls), 71.6, 70.1, 69.5 (C-6, C-6', C-6"), 64.0, 54.8 (C-4, C-2"), 54.8 (O$\underline{C}H_3$), 24.5, 24.0 (S-

$\underline{C}H_2$-CH_3, S-CH_2-$\underline{C}H_3$), 20.9 ($\underline{C}H_3$-C=O); m/z: calc= 1407, found (Maldi-Tof)= 1431 $[M+Na]^+$.

Methyl (3,4-Di-*O*-benzyl-2-deoxy-2-N-phthalimido-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside-4,6"-carbamate (143) - To a solution of methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside-ethyl 3,4-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-1-thio-α-D-glucopyranosyl-4,6"-carbamate (**142**) (446 mg, 0.31 mmol) in MeOH (15 mL) was added sodium metal (0.1 eq, 1 mg). The solution was stirred under argon for 4 h. The reaction mixture was neutralized using Dowex 200WH+, filtered and the solvent was removed *in vacuo*. The deacetylated residue was used without further purification and was dissolved in a mixture of methylene chloride/diethyl ether (6/4, v/v, 10 mL). 4 Å molecular sieves were added (500 mg) and the reaction mixture was stirred for 1 h. NIS (86 mg, 0.37 mmol) and TMSOTf (7 μl, 0.04 mmol) were added and the solution was stirred under argon for 1 h. The solution was filtered trough celite and diluted with methylene chloride (20 mL). The resulting solution was washed with, successively, aqueous $Na_2S_2O_3$ (15%, 2×40 mL), then water (2×40 mL) and brine (40 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography (methylene chloride/acetone, 95/5, v/v) to afford the cyclic trisaccharide **143** as white foam (312 mg, 76%, carbamate amide cis/trans-1/1). R_f (methylene chloride/methanol, 97/3, v/v)= 0.78; $[\alpha]_d$ = +59.7 (methylene chloride, c= 5.3 mg/mL, 25 ^oC) ; δ_H ppm (500 MHz, DMSO): 7.82-6.65 (78H, m, arom H), 5.71 (1H, d,

H-1”$_1$), 5.30 (1H, s, H-1’$_1$), 5.25 (1H, d, H-1”$_2$), 5.14 (1H, s, H-1’$_2$), 4.84-4.10 (38H, m, H-1$_1$, H-1$_2$, H-6a”$_1$, H-6a”$_2$, C$\underline{H}_2$ benzyls, H-2”$_1$, H-2’$_1$, H-2$_1$, H-2$_2$, H-2’$_2$, H-3”$_1$,), 4.08-3.07 (28H, m, H-2”$_2$, H-3”$_2$, H-4’$_1$, H-4’$_2$, H-4”$_1$, H-6a’$_1$, H-6b’$_1$, H-6b$_1$, H-6b”$_1$, H-6b”$_2$, H-3’$_1$, H-5”$_1$, H-4”$_2$, H-3$_1$, H-3$_2$, H-6a$_2$, H-3’$_2$, H-5”$_2$, H-5’$_1$, H-5’$_2$, H-4$_1$, H-6a’$_2$, H-6b’$_2$,H-4$_2$, H-6b$_2$, H-5$_1$, H-5$_2$,), 3.26, 3.21 (6H, 2s, O-C$\underline{H}_3$); δ_C ppm (500 MHz, DMSO): 156.8, 156.3 (C$\underline{}$=O), 139.1-138.2 (arom Cq), 129.1-126.6 (arom C), 100.6, 99.0, 98.8, 96.3, 96.1, 93.0, (C-1, C-1’, C-1”), 80.0, 79.9, 79.6, 79.1, 77.9, 77.6, 77.2, 76.3, 76.1, 74.9, 74.8, 74.7, 74.4, 74.3, 74.0, 73.6, 73.2, 73.1, 73.0, 72.8, 72.6, 72.4, 72.1, 71.9, 70.9, 70.5, 70.1, 69.9, 69.6, 69.4, 69.0, 63.5, 61.8, 57.5, 55.7, 55.0, 52.1 (C-2, C-3, C-4, C-5, C-6, C-2’, C-3’, C-4’, C-5’, C-6’, C-2”, C-3”, C-4”, C-5”, C-6”, C$\underline{H}_2$ benzyls), 50.9, 50.4 (O-C$\underline{H}_3$); m/z: calc= 1302, found (Maldi-Tof)= 1325 $[M+Na]^+$; Elemental analysis: Calc. C: 70.95, H: 6.03, Found: C: 71.12, H: 6.24.

4.4.2 Intramolecular Carbamate Bond Formation

2-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (146) - A solution of *p*-Methoxyphenyl 3,4-Di-O-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (**116**) (500 mg, 0.84 mmol) in a mixture acetic anhydride/pyridine (1/1, v/v, 10 mL) was stirred under argon for 5 h. Co-evaporation of the solvent with, respectively, toluene (5 × 20 mL), EtOH (20 mL) and methylene chloride (20 mL) afforded a pure acetylated intermediate **145** that was dissolved in a mixture toluene/acetonitrile/water (1/4/1, v/v/v, 20 mL). CAN (3 eq, 1.47 g, 2.52 mmol) was added and the reaction was vigorously stirred for 4 h. The solution was diluted with ethyl acetate (40 mL) and washed with, successively, aqueous $NaHCO_3$ (saturated, 40 mL), and water (4 × 40 mL).

The organic layer was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo*. The orange residue was purified by silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then 90/10, 80/20 and 70/30) to afford compound **146** as a yellow foam (415 mg, 93%, α/β~5/1). R_f (hexane/ ethyl acetate, 70/30, v/v)= 0.15; $[\alpha]_d$ = +10.7 (methylene chloride, c= 7.8 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.69 (4H, br-s, arom H phthalimido), 7.40-6.84 (10H, m, arom H), 5.39 (1H, dd, $J_{1,2}$= 7.1 Hz, H-1β), 5.31 (1H, m, H-1α), 4.98-4.59 (3H, 3d, J_{gem}= 10.9 Hz, C$\underline{H}_2$ benzyls), 4.56-4.39 (3H, m, H-3, H-4, C$\underline{H}_2$ benzyl), 4.13 (1H, dd, $J_{6a,6b}$= 12.1 Hz, $J_{6a,5}$= 4.5 Hz, H-6a), 4.08 (1H, dd, H-2), 3.81-3.62 (2H, m, H-5, H-6b), 3.11 (1H, d, $J_{OH,1}$= 6.3 Hz), 2.04 (3H, s, C$\underline{H}_3$-C=O); δ_C ppm (300 MHz, $CDCl_3$): 134.1-123.9 (arom C), 92.1 (C-1), 79.9 (C-6), 75.3, 73.8, 63.2, 63.0, 55.9 (C-2, C-3, C-4, C-5), 75.1, 75.0 (C$\underline{H}_2$ benzyls), 22.0 (C$\underline{H}_3$-C=O); m/z: calc= 531, found (Maldi-Tof)= 554 $[M+Na]^+$.

Trichloroacetimidate 2-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-2-phtalimido-β-D-gluco-pyranoside (144) - To a solution of 2-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-2-phtalimido-β-D-glucopyranoside (**146**) (365 mg, 0.69 mmol) in methylene chloride (10 mL) were added trichloroacetonitrile (0.90 mL, 9.0 mmol) and DBU (0.25 eq, 26 µl, 0.17 mmol). The reaction was stirred for 18 h under argon, after which time the solvent was evaporated. The crude product was purified by flash silica gel column chromatography (hexane/ethyl acetate, 70/30, v/v) to afford imidate **144** as a colorless oil (440 mg, 95%). R_f (hexane/ethyl acetate, 70/30, v/v) = 0.36; $[\alpha]_d$ = +54.9 (methylene chloride, c= 9.7 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.68 (4H, br-s, arom H phthalimido), 7.42-6.84 (10H, m, arom H), 6.41 (1H, d, $J_{1,2}$= 8.5 Hz, H-1), 4.97-4.64 (3H, 3d, J_{gem}= 12.0 Hz,

J_{gem}= 11.3 Hz, C<u>H</u>$_2$ benzyl), 4.58-4.25 (5H, H-3, H-2, H-6a, H-6b, C<u>H</u>$_2$ benzyl), 3.98-3.83 (1H, m, H-5), 3.79 (1H, dd, $J_{4,3}$=$J_{4,5}$= 6.7 Hz, H-4), 2.08 (3H, s, C<u>H</u>$_3$-C=O); m/z: calc= 676, found (Maldi-Tof) = 554 ([M+Na]$^+$-trichloroacetimidate).

Methyl (3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-4-azido-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside (147) - To a solution of Methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-4-azido-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside (**138**) (714 mg, 0.82 mmol) in MeOH (10 mL) and THF (0.5 mL) was added sodium methoxide (17 mg, 0.33 mmol). The reaction mixture was stirred for under argon 18 h. The solution was neutralized using Dowex 200WH+ resin, filtered and the solvent was removed *in vacuo*. The residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then 90/10, 80/20 and 70/30) to afford **147** as colorless oil (674 mg, 99%). R_f (hexane/ ethyl acetate, 70/30, v/v)= 0.15; $[\alpha]_d$ = +21.9 (methylene chloride, c=9.9 mg/mL, 25 ^oC) ; δ_H ppm (500 MHz, $CDCl_3$): 7.39-7.17 (25H, m, arom H), 5.19 (1H, s, H-1'), 4.88-4.49 (11H, m, H-1'), 4.88-4.49 (11H, m, H-1, C<u>H</u>$_2$ benzyls), 4.24 (1H, s, H-2'), 4.08-3.91 (5H, m, H-4, H-3, H-5, H-3', H-2), 3.81-3.61 (5H, H-4', H-6a', H-6b', H-6a, H-6b), 3.54 (1H, m, H-5'), 3.52 (3H, s, O-<u>C</u>H$_3$); δ_C ppm (500 MHz, $CDCl_3$): 129.2-127.0 (arom C), 101.9 (C-1'), 98.4 (C-1), 80.1, 79.1, 76.5, 74.4, 72.3, 70.9, 68.4 (C-2, C-3, C-5, C-2', C-3', C-4', C-5'), 74.9, 73.6, 73.4, 72.0, 71.8 (<u>C</u>H$_2$ benzyls), 69.5 (C-6, C-6'), 58.9 (C-4), 55.0 (O-<u>C</u>H$_3$); m/z: calc= 831, found (Maldi-Tof)= 853 [M+Na$^+$].

Methyl (6-*O*-Acetyl-3,4-di-*O*-benzyl-2-deoxy-2-*N*-phtalimido-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-4-azido-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside (148) - To a cooled solution (-20 ^{o}C) of Methyl (3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-4-azido-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside (**147**) (596 mg, 0.72 mmol), Trichloroacetimidate 6-*O*-Acetyl-3,4-di-*O*-benzyl-2-deoxy-2-*N*-phtalimido-β-D-glucopyranoside (**144**) (580 mg, 0.86 mmol) and 4 Å molecular sieves (0.5 g) in methylene chloride (10 mL) was added TMSOTf (39 µl, 0.26 mmol) and the reaction mixture was stirred under argon for 1 h. The solution was neutralized using Et_3N, filtered through celite and the solvent was removed *in vacuo*. Purification of the residue by flash silica gel column chromatography (methylene chloride/acetone, 97/3, v/v) afforded trisaccharide **148** as colorless oil (820 mg, 85%, α/β, 1/5). R_f (methylene chloride/acetone, 97/3, v/v)= 0.51; $[\alpha]_d$ = +54.7 (methylene chloride, c=6.7 mg/mL, 25 ^{o}C) ; δ_H ppm (500 MHz, $CDCl_3$): 7.57-6.63 (35H, m, arom H), 5.21 (1H, d, $J_{1",2"}$= 8.4 Hz, H-2"), 4.96-4.93 (2H, m, H-1" α, H-1'α), 4.78-4.17 (17H, H-1, H-1', H-1α, J_{gem}= 12.2 Hz, J_{gem}= 11.2 Hz, C$\underline{H}_2$ benzyls), 4.15-4.01 (2H, m, H-2', $J_{3,2}$= 11.7 Hz, $J_{3,4}$= 4.8 Hz, H-3), 3.89-3.44 (13H, m, H-4", H-6a', H-6b', H-3', H-3", H-2, H-4, H-5, H-5', H-2", H-5", H-6a", H-6b"), 3.37-3.26 (3H, m, H-6a, H-6b, H-4'), 3.08 (3H, s, O-C$\underline{H}_3$), 1.84 (3H, s, C$\underline{H}_3$-C=O); δ_C ppm (500 MHz, $CDCl_3$): 133.7-123.3 (arom C), 101.6, 100.5 (C-1"α, C-1'α), 99.2 (C-1'), 98.6 (C-1), 98.2 (C-1α), 96.7 (C-1"), 79.2, 79.1, 77.9, 76.0, 74.2, 73.3, 72.9, 70.1, 69.1 (C-2, C-3, C-5, C-2', C-3', C-4', C-5', C-3", C-4", C-5"), 76.0-69.7 (C$\underline{H}_2$ benzyls), 72.9, 70.3, 63.0 (C-6, C-6', C-6"), 58.8 (C-4), 55.3 (C-2"), 54.9 (O-$\underline{C}H_3$), 53.5 (C-2"α), 20.6 ($\underline{C}H_3$-C=O); m/z: calc= 1344, found (Maldi-

Tof)= 1369 $[M+Na]^+$; Elemental analysis: Calc. C: 71.46, H: 6.31, Found: C: 71.61, H: 6.50.

Methyl (3,4-di-*O*-benzyl-2-deoxy-2-*N*-phtalimido-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-4-amino-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside (149) - To a solution of methyl (6-*O*-acetyl-3,4-di-*O*-benzyl-2-deoxy-2-*N*-phtalimido-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-4-azido-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside (**148**) (790 mg, 0.59 mmol) in MeOH (50 mL) and THF (5 mL) was added potassium *tert*-butoxide (68 mg, 0.59 mmol) and the reaction mixture was stirred under argon for 18 h. The solution was then neutralized with Dowex 200WH+ resin, filtered and the solvent was removed *in vacuo*. The residue was dissolved in a mixture pyridine/water (3/1, v/v, 20 mL), and 1,3-propanedithiol (10 eq, 0.35 mL, 5.9 mmol) and Et_3N (few drops) were added. After stirring for 18 h TLC analysis indicted the complete conversion of the starting material to 2 new compounds, a major, presumably **149** and its α-anomer from previous glycosilation step. The solvent was co-evaporated with toluene (5×100 mL) and the residue was purified by silica gel preparative TLC (methylene chloride/acetone, 95/5, v/v) to afford the desired trisaccharide **149** as a white foam (352 mg, 47%). R_f (methylene chloride/methanol, 97/3, v/v)= 0.26; $[\alpha]_d$ = +39.4 (methylene chloride, c=9.4 mg/mL, 25 ^oC) ; δ_H ppm (500 MHz, DMSO): 7.42-6.99 (39H, m, arom H), 5.23 (1H, d, $J_{1",2"}$= 8.4 Hz, H-1"), 4.85-4.23 (17H, m, H-1, H-1', H-3", J_{gem}= 11.2 Hz, J_{gem}= 11.9 Hz, C$\underline{H}_2$ benzyls), 4.45-3.19 (17H, m, H-2", H-3, H-2', H-4", H-3', H-4, H-4', H-5", H-2, H-5', H-5, H-6, H-6', H-6"), 3.24 (3H, s, O-C$\underline{H}_3$); δ_C ppm (500 MHz, DMSO):

135.2-124.1 (arom C), 99.8 (C-1'), 98.5 (C-1), 96.8 (C-1"), 81.0, 79.9, 79.0, 77.8, 76.0, 73.5, 72.2, 70.1, 69.9 (C-2, C-3, C-5, C-2', C-3', C-4', C-5', C-3", C-4", C-5"), 60.9 (C-4), 56.2 (C-2"), 75.1-69.3 ($\underline{C}H_2$ benzyls), 54.8 (O-$\underline{C}H_3$); m/z: calc= 1277, found (Maldi-Tof)= 1299 $[M+Na]^+$; Elemental analysis: Calc. C: 69.63, H: 5.99, Found: C: 69.85, H: 6.23.

Methyl (3,4-di-*O*-Benzyl-2-deoxy-2-*N*-phthalimido-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside-4,6"-carbamate (143) - To a solution of Methyl (3,4-di-*O*-Benzyl-2-deoxy-2-*N*-phthalimido-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-manno-pyranosyl)-(1→3)-4-amino-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside (**149**) (322 mg, 0.25 mmol) in methylene chloride (15 mL) were added Et_3N (42 µl, 0.3 mmol) and phosgene (2M in Toluene, 150 µl, 3 mmol) and the reaction mixture was stirred under argon at room temperature for 1 h. The solvent was then removed in vacuo. The residue was dissolved in toluene (20 mL) and refluxed for 2 days. The solvent was removed *in vacuo* and the residue was taken up into methylene chloride (10 mL). The resulting solution was washed with, successively aqueous $NaHCO_3$ (saturated, 2×10 mL) and water (10 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The crude product was purified by flash silica gel column chromatography (methylene chloride/acetone, 95/5, v/v) to afford the cyclic trisaccharide **143** as a colorless oil (247 mg, 69%, carbamate amide *cis*/*trans*, 1/1). R_f (methylene chloride/methanol, 97/3, v/v)= 0.71; $[\alpha]_d$ = +56.9 (methylene chloride, c= 8.5 mg/mL, 25 ^oC); δ_H ppm (500 MHz, d^6-DMSO): 7.82-6.65 (78H, m, arom H), 5.71 (1H, d,

H-1”$_1$), 5.30 (1H, s, H-1’$_1$), 5.25 (1H, d, H-1”$_2$), 5.14 (1H, s, H-1’$_2$), 4.84-4.10 (38H, m, H-1$_1$, H-1$_2$, H-6a”$_1$, H-6a”$_2$, C$\underline{H}_2$ benzyls, H-2”$_1$, H-2’$_1$, H-2$_1$, H-2$_2$, H-2’$_2$, H-3”$_1$,), 4.08-3.07 (28H, m, H-2”$_2$, H-3”$_2$, H-4’$_1$, H-4’$_2$, H-4”$_1$, H-6a’$_1$, H-6b’$_1$, H-6b$_1$, H-6b”$_1$, H-6b”$_2$, H-3’$_1$, H-5”$_1$, H-4”$_2$, H-3$_1$, H-3$_2$, H-6a$_2$, H-6b$_2$, H-3’$_2$, H-5”$_2$, H-5’$_1$, H-5’$_2$, H-4$_1$, H-6a’$_2$, H-6b’$_2$, H-4$_2$, H-6b$_2$, H-5$_1$, H-5$_2$,), 3.26, 3.21 (6H, 2s, O-$\underline{C}H_3$); δ_C ppm (500 MHz, d^6-DMSO): 156.8, 156.3 ($\underline{C}$=O carbamate, C=O phthalimido), 139.1-138.2 (arom Cq), 129.1-126.6 (arom C), 100.6, 99.0, 98.8, 96.3, 96.1, 93.0, (C-1, C-1’, C-1”), 80.0, 79.9, 79.6, 79.1, 77.9, 77.6, 77.2, 76.3, 76.1, 74.9, 74.8, 74.7, 74.4, 74.3, 74.0, 73.6, 73.2, 73.1, 73.0, 72.8, 72.6, 72.4, 72.1, 71.9, 70.9, 70.5, 70.1, 69.9, 69.6, 69.4, 69.0, 63.5, 61.8, 57.5, 55.7, 55.0, 52.1 (C-2, C-3, C-4, C-5, C-6, C-2’, C-3’, C-4’, C-5’, C-6’, C-2”, C-3”, C-4”, C-5”, C-6”, $\underline{C}H_2$ benzyls), 50.9, 50.4 (O-$\underline{C}H_3$); m/z: calc= 1302, found (Maldi-Tof)= 1325 $[M+Na]^+$.

Methyl (2-*N*-acetyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside-4,6”-carbamate (150) - To a solution of methyl (3,4-di-*O*-benzyl-2-deoxy-2-*N*-phtalimido-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-manno-pyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside-4,6”-carbamate (**143**) (227 mg, 0.17 mmol) in EtOH (10 mL) was added hydrazine monohydrate (0.4 mL, 8.5 mmol) and the reaction mixture was refluxed for 18 h. The solvent was then co-evaporated with toluene (5×20 mL) and the residue was dissolved in a mixture pyridine/Ac_2O (1/1, v/v, 10 mL). The reaction mixture was stirred for 18 h and the solvent was then removed and co-evaporated with, successively, toluene (5×20 mL), EtOH (20 mL) and methylene chloride

(20 mL). The crude product was purified by silica gel column chromatography (methylene chloride/methanol, 99/1, v/v) to give **150** as white foam (186 mg, 86%, cis/trans, 1:1). R_f (methylene chloride/methanol, 99/1, v/v)= 0.23; $[\alpha]_d$ = +32.5 (methylene chloride, c= 8.5 mg/mL, 25 ^oC) ; δ_H ppm (500 MHz, d^6-DMSO): 8.04-7.08 (70H, m, arom H), 5.31 (1H, s, H-1'$_1$), 5.09 (1H, s, H-1'$_2$), 4.99 (1H, d, H-1"$_1$), 4.86 (1H, d, H-1"$_2$), 4.78 (2H, s, H-1$_1$, H-1$_2$), 4.82-4.26 (32H, m, H-2$_2$, H-3$_1$, H-2'$_1$, H-2'$_2$, C$\underline{H}_2$ benzyls), 3.99-3.40 (32H, m, H-2$_1$, H-3"$_1$, H-4'$_1$, H-4'$_2$, H-3'$_1$, H-4"$_1$, H-2"$_1$, H-5"$_1$, H-3'$_2$, H-3"$_2$, H-6"$_1$, H-4"$_2$, H-2"$_2$, H-3$_2$, H-4$_1$, H-4$_2$, H-5$_1$, H-5$_2$, H-6a$_1$, H-6b$_1$, H-6a$_2$, H-6b$_2$, H-5'$_1$, H-5'$_2$, H-6a'$_1$, H-6b'$_1$, H-6a'$_2$, H-6b'$_2$, H-5"$_2$, H-6a"$_2$, H-6b"$_2$), 3.39, 3.35 (6H, 2s, O-C$\underline{H}_3$), 1.76, 1.42 (6H, 2s, C$\underline{H}_3$-C=O); δ_C ppm (500 MHz, d^6-DMSO): 130.6-126.30 (arom C), 101.1 (C-1'$_2$), 98.6 (C-1), 98.0 (C-1"$_1$), 96.7 (C-1'$_1$), 95.3 (C-1"$_2$), 82.2, 78.0, 77.9, 76.9, 75.9, 75.2, 74.1, 73.0, 72.1, 69.8 (C-2, C-3, C-5, C-6, C-2', C-3', C-4', C-5', C-6', C-3", C-4", C-5"), 75.7-67.9 ($\underline{C}H_2$ benzyls), 63.1, 61.4 (C-6"$_1$, C-6"$_2$), 54.7, 50.3 (O-$\underline{C}H_3$), 52.6, 49.7 (C-4$_1$, C-4$_2$), 23.5, 23.2 ($\underline{C}H_3$-C=O); m/z: calc= 1215, found (Maldi-Tof)= 1238 $[M+Na]^+$.

Methyl (2-*N*-Acetyl-2-deoxy-α-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6"-carbamate (72) - Methyl (2-*N*-Acetyl-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-deoxy-α-D-mannopyranoside-4,6"-carbamate (**150**) (166 mg, 0.14 mmol) in ethanol (4 mL) was hydrogenated (H_2, $Pd(OAc)_2$, 10%, 17 mg) for 18 h. The mixture was filtered through celite and concentrated to give the final cyclic trisaccharide **72** as a white solid (80 mg, 100%, cis(1)/trans(2), 1/1). R_f (methylene

chloride/methanol, 70/30, v/v)= 0.12; $[\alpha]_d$ = +12.4 (methylene chloride, c= 5.3 mg/mL, 25 ^oC) ; δ_H ppm (500 MHz, D_2O): 5.09 (1H, s, H-1'$_1$), 5.06 (1H, s, H-1'$_2$), 4.87 (1H, d, $J_{1",2"}$= 8.8 Hz, H-1"$_2$), 4.79 (1H, dd, $J_{6a",6b"}$= 11.8 Hz, H-6a"$_2$), 4.69-4.58 (5H, m, H-1$_1$, H-1$_2$, H-1"$_1$, H-3$_2$, H-6a"$_1$), 4.14-4.08 (2H, m, H-5'$_2$, H-2'$_2$), 4.05-3.99 (2H, m, H-2'$_1$, H-6b"$_1$), 3.94-3.81 (4H, m, H-2$_2$, H-2"$_2$, H-6b"$_2$, H-2$_1$), 3.75-3.30 (21H, m, H-3$_1$, H-3'$_1$, H-5$_1$, H-5'$_2$, H-3'$_2$, H-6a'$_1$, H-6b'$_1$, H-6a$_1$, H-2"$_2$, H-4$_1$, H-6b$_1$, H-5'$_1$, H-6a'$_2$, H-6b'$_2$, H-4"$_1$, H-4'$_2$, H-3"$_1$, H-4'$_1$, H-3"$_2$, H-5"$_1$, H-4"$_2$), 3.30-3.26 (6H, m, O-C<u>H</u>$_3$), 3.16 (1H, $J_{4,3}$=$J_{4,5}$= 10.8 Hz, H-4$_2$), 1.92, 1.88 (6H, C<u>H</u>$_3$-C=O); δ_C ppm (500 MHz, D_2O): 104.4 (C-1'$_2$, $J_{C1',H1'}$= 177.0 Hz), 103.4 (C-1$_2$, $J_{C1,\ H1}$= 173.3 Hz), 103.0 (C-1$_1$, $J_{C1,H1}$= 177.3 Hz), 101.4 (C-1"$_2$, $J_{C1",H1"}$= 167.0 Hz), 100.6 (C-1'$_1$, $J_{C1',H1'}$= 172.6 Hz), 98.9 (C-1"$_1$, $J_{C1",H"}$= 166.3 Hz), 78.7, 77.4, 76.6, 76.1, 75.7, 75.5, 75.1, 74.2, 73.3, 72.9, 72.3, 72.1, 71.8, 71.4, 71.3, 71.0, 69.5, 59.5, 54.2, 52.0, 51.4 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2", C-3", C-4", C-5"), 69.5, 63.4, 63.1 (C-6, C-6', C-6"), 57.2 (O-<u>C</u>H$_3$), 24.7, 24.5 (<u>C</u>H$_3$-C=O); m/z: calc= 584, found (FAB)= 585 $[M+H]^+$, 607 $[M+Na]^+$.

4.5. Synthesis of Methyl (2-N-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (**73**)

3,4,6-*O*-Tri-acetyl-*N*-2-phthalimido-2-deoxy-D-glucopyranoside (153) - To a solution of Acetyl 3,4,6-Tri-*O*-acetyl-2-*N*-phthalimido-2-deoxy-D-glucopyranoside (**118**) (10 g, 21.0 mmol) in DMF (250 mL) was added hydrazine acetate (2.9 g, 31.4 mmol) and the reaction mixture was stirred under argon at room temperature 18 h. The solvents were removed under reduced pressure and the residue was taken up into dichloromethane (100 mL). The resulting solution was washed with, successively, aqueous $NaHCO_3$ (saturated,

2 × 150 mL) and water (150 mL). The aqueous layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (acetone/methylene chloride, 5/95, v/v) to afford **153** as a pale yellow oil (5.10 g, 56%). R_f (acetone/methylene chloride, 5/95, v/v)= 0.69; $[\alpha]_d$ = +51.1 (methylene chloride, c=12.9 mg/mL, 25 ^oC); δ_H ppm (300 MHz, $CDCl_3$): 7.30 (1H, m, aromatic H phtalimido), 6.16 (1H, dd, $J_{3\alpha,4\alpha}$=11.4 Hz, $J_{3\alpha,2\alpha}$= 9.2 Hz, H-3α), 5.85 (1Hβ, dd, $J_{3\beta,4\beta}$= 10.7 Hz, $J_{3\beta,2\beta}$= 9.2 Hz, H-3β), 5.64 (1H, dd, $J_{1\beta,OH}$= 7.5 Hz, H-1β), 5.36 (1H, d, $J_{1\alpha,J2\alpha}$= 3.3 Hz, H-1α), 5.18 (1H, $J_{4\beta,5\beta}$= 10.3 Hz, H-4β), 4.66 (1H, dd, $J_{2\alpha,3\alpha}$= 11.4 Hz, H-2α), 4.48 (1H, m, H-5α), 4.37 (1H, dd, $J_{6a\alpha,5\alpha}$= 4.0 Hz, $J_{6a\alpha,6b\alpha}$= 11.6 Hz, H-6aα), 4.33-4.24 (2H, m, H-2β, H-6aβ), 4.19 (1H, dd, $J_{6b\beta,5\beta}$= 2.4 Hz, H-6bβ), 3.96 (1H, m, H-5β), 3.46 (1H, d, O<u>H</u>), 2.11, 2.04, 1.87 (9H, 3s, C<u>H</u>$_3$-C=O); δ_C ppm (75 MHz, $CDCl_3$): 172.2, 170.4, 169.8, 168.0 (CH_3-<u>C</u>=O), 135.0, 134.6, 124.2, 123.9 (aromatic C), 131.5 (Cq phthalimido), 92.8 (C-1), 72.1, 70.8, 69.1, 56.2 (C-2, C-3, C-4, C-5), 62.3 (C-6), 21.0, 20.9, 20.7 (<u>C</u>H_3-C=O); m/z: calc= 435, found (LSI)= 458 (100%, $[M+Na]^+$).

Trichloroacetimidate 3,4,6-Tri-*O*-acetyl-*N*-2-phtalimido-2-deoxy-β-D-glucopyranoside (151) - To a solution of 3,4,6-*O*-Tri-acetyl-*N*-phtalimido-2-deoxy-D-glucopyranoside (**153**) (4.3 g, 9.9 mmol) in methylene chloride (100 mL) were added trichloroacetonitrile (12.91 mL, 128.6 mmol) and DBU (0.36 mL, 2.5 mmol). The reaction mixture was stirred under argon at room temperature 18 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (acetone/methylene chloride, 2/98, v/v) to give **151** as a white solid (2.92 g, 51%). R_f (acetone/methylene chloride, 3/97, v/v)= 0.35; $[\alpha]_d$ = +57.0 (methylene

chloride, c=13.4 mg/mL, 25 ^{o}C); δ_H ppm (300 MHz,$CDCl_3$): 8.68 ppm (1H, s, C(N<u>H</u>)CCl_3), 7.78 (5H, m, aromatic H phtalimido), 6.62 (1H, d, $J_{1,2}$= 8.8 Hz, H-1), 5.91 (1H, dd, $J_{3,2}$= 10.7 Hz, $J_{3,4}$= 8.8 Hz, H-3), 5.29 (1H, m, H-4), 4.63 ppm (1H, dd, $J_{2,1}$= 8.8 Hz, H-2), 4.39 (1H, dd, $J_{6a,5}$= 4.4 Hz, $J_{6a,6b}$= 12.5 Hz, H-6a), 4.20 (1H, dd , $J_{6b,5}$=2.2 Hz, H-6b), 4.08 (1H, m, H-5), 2.12, 2.05, 1.89 (9H, 3s, C<u>H</u>$_3$-C=O); δC ppm (75 MHz, $CDCl_3$): 170.7, 170.1, 169.5 (CH_3-<u>C</u>=O acetyls), 167.4 (<u>C</u>=O, phtalimido), 160.5 (OC(NH)<u>C</u>Cl_3), 134.5 (aromatic C), 131.2, 123.7 (aromatic Cq), 93.6 (C-1), 72.8, 70.4, 68.4, 53.6 (C-2, C-3, C-4, C-5), 61.6 (C-6), 20.8, 20.7, 20.5 (<u>C</u>H_3-C=O); m/z: 578, found (LSI)= 603.0 (25%, $[M+Na]^+$).

Methyl 4,6-Di-*O*-benzyl-2,3-di-*O*-isopropylidene-α-D-mannopyranoside (154) – To a cooled solution (0 ^{o}C) of Methyl 2,3-Di-*O*-isopropylidene-α-D-mannopyranoside (**133**) (8.07 g, 34.5 mmol) in DMF (100 mL) was added sodium hydride (60% suspended in mineral oil, 3.15 g, 105.4 mmol) and the reaction mixture was stirred for 15 min. The ice bath was removed, benzyl bromide (8.31 mL, 69.9 mmol) was added dropewise and the solution was stirred under argon at room temperature for 18 h. After destroying the excess of NaH with methanol, the solvent was removed under reduced and the residue was dissolved in diethyl ether (100 mL). The resulting solution was washed with water (100 mL) and the aqueous layer was extracted with diethyl ether (3 × 100 mL). The combined organic layer was dried ($MgSO_4$), filtered and the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography (methylene chloride/acetone, 98/2, v/v) to afford compound **154** as a colorless oil (13.6 g, 95%). R_f (methylene chloride/acetone, 98/2,v/v)= 0.75; $[\alpha]_d$ = +23.2 (methylene chloride,

c=10.4 mg/mL, 25 ^{o}C); δ_H ($CDCl_3$, 300 MHz): 7.37-7.22 (10H, m, arom H), 4.96 (1H, s, H-1), 4.86, 4.64, 4.55, 4.53 (4H, 4d, J_{gem}= 11.9 Hz, J_{gem}= 12.2 Hz, C$\underline{H}_2$ benzyl), 4.31 (1H, dd, $J_{4,3}$=$J_{4,5}$= 6.4 Hz, H-4), 4.12 (1H, dd, $J_{2,1}$= 0.6 Hz, $J_{2,3}$= 5.9 Hz, H-2), 3.80-3.64 (3H, m, H-3, H-5, H-6b), 3.57 (1H, dd, $J_{6a,6b}$= 9.8 Hz, $J_{6a,5}$= 6.9 Hz, H-6a), 3.40 (3H, s, O-C$\underline{H}_3$), 1.51, 1.37 (6H, 2s, C$\underline{H}_3$ isopropylidene); δ_C ($CDCl_3$, 75 MHz): 138.6, 138.5 (Cq benzyls), 128.5-127.7 (arom C), 109.5 (Cq isopropylidene), 98.5 (C-1), 79.2, 76.0, 75.9, 68.4 (C-2, C-3, C-4, C-5), 73.7, 73.0 (C$\underline{H}_2$ benzyls), 69.5 (C-6), 55.1 (O-C$\underline{H}_3$), 28.2, 26.5 (C$\underline{H}_3$ isopropylidene); m/z: calc= 414, found (Maldi-Tof)= 437 $[M+Na]^+$.

Methyl 4,6-Di-*O*-benzyl-α-D-mannopyranoside (155) - A solution of Methyl 4,6-Di-*O*-benzyl-2,3-di-*O*-isopropylidene-α-D-mannopyranoside (**154**) (13.55 g, 32.7 mmol) in acetic acid/water (4/1, v/v, 150 mL) was stirred at 50 ^{o}C for 18 h. Removal of the solvent, followed by co-evaporation with toluene (3 × 200 mL), ethanol (200 mL) and methylene chloride (200 mL) afforded the desired compound **155** as a white foam (12.3 g, 100%). R_f (methylene chloride/methanol, 95/5, v/v)= 0.61; $[\alpha]_d$ = +27.2 (methylene chloride, c=9.6 mg/mL, 25 ^{o}C); δ_H ($CDCl_3$, 300 MHz): 7.40-7.21 (10H, m, arom H), 4.75 (1H, s, H-1), 4.71, 4.68, 4.57, 4.55 (4H, 4d, J_{gem}= 11.5 Hz, J_{gem}= 12.5 Hz, C$\underline{H}_2$ benzyls), 3.95-3.66 (6H, m, H-2, H-3, H-4, H-5, H-6), 3.38 (3H, s, O-C$\underline{H}_3$), 2.43, 2.38 (2H, 2 br-s, O$\underline{H}$); δ_C ($CDCl_3$, 75 MHz): 138.4, 137.9 (Cq benzyl), 128.5-127.8 (arom C), 100.7 (C-1), 75.8, 72.1, 71.0, 70.6 (C-2, C-3, C-4, C-5), 74.6, 73.6 (C$\underline{H}_2$ benzyls), 68.9 (C-6), 54.9 (O-C$\underline{H}_3$); m/z: calc= 374, found (Maldi-Tof)= 397 $[M+Na]^+$.

Methyl 2-*O*-benzyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (152) - Methyl 4,6-di-*O*-benzyl-α-D-mannopyranoside (**155**) (12.2 g, 32.5 mmol) was dissolved in methylene chloride (150 mL) and aqueous NaOH (30%, 45 mL) was added. Benzyl bromide (4.25 mL, 35.8 mmol) and TBAI (3.0 g, 8.1 mmol) were added and the reaction mixture was vigorously stirred for 24 h. The solution was diluted with methylene chloride (100 mL) and washed with water (2 × 200 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The residue was purified by silica gel column chromatography (methylene chloride/acetone, 98/2, v/v) to afford building block **152** as a colorless oil (9.80 g, 65%); R_f (methylene chloride/acetone, 97/3, v/v)= 0.59; $[\alpha]_d$ = +23.0 (methylene chloride, c=5.9 mg/mL, 25 ^oC); δ_H ($CDCl_3$, 300 MHz): 7.40-7.20 (15H, m, arom H), 4.85 (1H, d, J_{gem}= 11.0 Hz, C$\underline{H}_2$ benzyl), 4.82 (1H, d, $J_{1,2}$= 1.4 Hz, H-1), 4.76, 4.68, 4.57, 4.58, 4.51 (5H, 5d, J_{gem}= 11.5 Hz, J_{gem}= 12.2 Hz, C$\underline{H}_2$ benzyls), 4.02-3.92 (1H, m, H-3), 3.83-3.64 (5H, m, H-2, H-4, H-5, H-6), 3.36 (3H, s, O-C$\underline{H}_3$), 2.33 (1H, d, $J_{OH,3}$= 9.6 Hz, O$\underline{H}$); δ_C ($CDCl_3$, 300 MHz): 138.6, 138.4, 137.9 (Cq benzyls), 128.6-127.0 (arom C), 98.0 (C-1), 78.4, 76.7,71.9, 70.9 (C-2, C-3, C-4, C-5), 74.8, 73.5, 72.9 (C$\underline{H}_2$ benzyls), 69.3 (C-6), 54.9 (O-$\underline{C}H_3$); m/z: calc= 464, found (Maldi-Tof)= 487 $[M+Na]^+$.

Methyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)- 2,4,6-tri-*O*-benzyl -α-D-mannopyranoside (156) – To a solution of Compound **152** (2.44 g, 5.26 mmol) and **79** (3.10 g, 5.79 mmol) and 4 Å molecular sieves (5.5 g) in methylene chloride (50 mL) was added a solution of NIS (1.42g, 6.31 mmol) in a mixture of diethyl ether/methylene chloride (6/4, v/v, 64mL), containing TMSOTf (0.112 mL, 0.63 mmol).

The reaction mixture was stirred under argon for 18 h. The solution was neutralised with Et_3N, filtered and washed with, successively aqueous $Na_2S_2O_3$ (15%, 2 × 100 mL) and water (150 mL). The organic layer was dried ($MgSO_4$), filtered, and the solvents were removed under reduced pressure. The brown residue was purified by silica gel column chromatography (acetone/dichloromethane 1/99, v/v then 2/98) to give **156** as a yellow oil (4.85 g, 98%). R_f= 0.82 (acetone/methylene chloride, 2/98, v/v); $[\alpha]_d$ = +21.7 (methylene chloride, c=7.8 mg/mL, 25 °C); δ_H ppm (500 MHz,$CDCl_3$): 7.37-7.14 (30H, m, aromatic H), 5.51 (1H, dd, $J_{2',3'}$=1.8 Hz, $J_{2',3'}$= 3.3 Hz, H-2'), 5.19 (1H, d, H-1'), 4.88 (1H, d, $J_{6a,6b}$= 11.0 Hz, CH_2 benzyl), 4.76-4.43 (6H, m, J_{gem}= 11.0, J_{gem}= 12.0 Hz, C$\underline{H}_2$ benzyls, H-1), 4.14 (1H, dd, $J_{3,2}$= 3.1 Hz, $J_{3,4}$= 9.3 Hz, H-3), 4.04-3.95 (2H, m, H-4, H-3'), 3.91-3.87 (1H, m, H-5'), 3.82-3.77 (2H, m, H-2, H-4'), 3.76-3.66 (5H, m, H-5, H-6a', H-6b', H-6a, H-6b), 3.30 (3H, s, O-CH_3), 3.10 (3H, s, C$\underline{H}_3$-C=O); δ_C ppm (75 MHz, $CDCl_3$): 170.2 (CH_3-$\underline{C}$=O), 138.7-137.9 (arom Cq), 128.7-127.5 (arom C), 99.6, 98.4 (C-1, C-1'), 78.1, 77.3, 75.3, 74.4, 72.2, 71.7, 68.8 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 75.0, 73.4. 72.3, 71.9, 69.2, 69.1 (C-6, C-6', $\underline{C}H_2$ benzyls), 54.9 (O-$\underline{C}H_3$), 21.1 (CH_3-C=O); m/z: calc= 938, found (LSI)= 961 (100%, $[M+Na]^+$).

Methyl (3,4,6-Tri-O-benzyl-α-D-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (157) - To a solution of methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-*α*-D-mannopyranosyl)-(1→3)- 2,4,6-tri-*O*-benzyl -α-D-mannopyranoside (**156**) (4.73 g, 5.04 mmol) in methanol (100 mL) was added potassium *tert*-butoxide (0.59 g, 5.04 mmol) and the reaction mixture was stirred under argon at room temperature for 18 h. The solution

was neutralized with dowex-50WXA-H^+ resin, filtered and the solvent removed under reduced pressure. Compound **157** was obtained as a yellow oil which was used without further purification(3.85 g, 85%). R_f= 0.55 (acetone/methylene chloride, 3/97, v/v); $[\alpha]_d$ = +27.4 (methylene chloride, c=8.4 mg/mL, 25 ^oC); δ_H ppm (500 MHz,$CDCl_3$): 7.35-7.12 (30H, m, aromatic H), 5.24 (1H, d, $J_{1',2'}$= 1.5 Hz, H-1'), 4.85 (1H, d, J_{gem}= 11.0 Hz, C$\underline{H}_2$ benzyl), 4.73 (1H, d, $J_{1,2}$= 1.8 Hz, H-1), 4.72-4.43 (11H, m, C$\underline{H}_2$ benzyls), 4.13 (1H, dd, $J_{3,2}$= 3.1 Hz, $J_{3,4}$= 9.4 Hz, H-3), 3.99 (2H, m, H-2', H-4), 3.91-3.88 (2H, m, H-5, H-3'), 3.84 (1H, dd, $J_{2,1}$= 1.8 Hz, $J_{2,3}$= 2.9 Hz, H-2), 3.82-3.61 (6H, m, H-4', H-5', H-6a, H-6b, H-6a', H6b'), 3.30 (3H, s, O-C$\underline{H}_3$), 2.32 ppm (1H, d, OH); δC ppm (75 MHz, $CDCl_3$): 138.6, 138.4, 138.2, 138.0 (arom Cq), 128.5-127.6 (arom C), 101.3, 98.5 (C-1, C-1'), 80.1, 78.5, 77.5, 75.3, 74.5, 72.0, 71.8 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 75.0, 74.9, 73.6, 73.5, 72.2, 72.1 ($\underline{C}H_2$ benzyls), 69.3, 69.1 (C-6, C-6'), 54.9 (O-$\underline{C}H_3$).

Methyl (3,4,6-Tri-*O*-acetyl-*N*-2-phtalimido-2-deoxy-β-D-glucopyranoside)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosil)-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (158) - To a cooled solution (-20 °C) of Methyl (3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-α-D-mannopyranoside (**157**) (2.24 g, 2.5 mmol), Trichloroacetimidate 3,4,6-Tri-*O*-acetyl-*N*-phtalimido-2-deoxy-β-D-glucopyranoside (**151**) (1.73 g, 3.0 mmol) and 4 Å molecular sieves (4 g) in methylene chloride (30 mL) was added $BF_3.OEt_2$ (70 µl, 0.5 mmol) and the reaction mixture was stirred under argon for 1 h. The solution was neutralized with triethylamine, filtered and diluted with methylene chloride (50 mL). The resulting mixture was washed with, successively, aqueous $NaHCO_3$ (saturated, 150 mL), brine (150 mL) and water (150 mL). The organic

layer was dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (acetone/toluene, 10/90, v/v) to afford trisaccharide **158** (3.55 g, 121%) was obtained as a pale yellow oil (some disaccharide remained after the column). R_f (acetone/toluene, 20/80, v/v)= 0.73; $[\alpha]_d$ = +42.5 (methylene chloride, c=7.3 mg/mL, 25 ^oC); δ_H ppm (500 MHz,$CDCl_3$): 7.52-7.40 (34H, m, aromatic H), 5.64 (1H, dd, $J_{3",4"}$= 9.2 Hz, $J_{3",2"}$= 11.0 Hz, H-3"), 5.14 (1H, d, $J_{1",2"}$= 8.5 Hz, H-1"), 5.06 (1H, dd, $J_{4",5"}$= 10.3 Hz, H-4"), 4.97 (1H, d, $J_{1',2'}$= 1.1 Hz, H-1'), 4.87 (1H, J_{gem}= 11.0 Hz, C$\underline{H}_2$ benzyl), 4.69 (10H, m, H-1, C$\underline{H}_2$ benzyls), 4.59 (1H, dd, $J_{2,1}$= 8.4 Hz, $J_{2,3}$= 10.7 Hz, H-2), 4.46-3.84 (13H, m, H-1, H-6a", H-3, H-2', H-4, H-3, H-6b", H-5, H-2, H-6a', H-6b', H-6a, C$\underline{H}_2$ benzyls), 3.76-3.37 (2H, m, H-6b, H-4'), 2.84 (1H, dd, *J*5',4'= 6.6 Hz, *J*5',6'= 10.7 Hz, H-5'), 2.68 (1H, m, H-5"), 3.29 (3H, s, O-C$\underline{H}_3$), 2.20, 1.90, 1.85 (9H, 3s, C$\underline{H}_3$-C=O); δ_C ppm (75 MHz, $CDCl_3$): 170.2, 169.4 (C$\underline{O}$ Ac), 138.8, 138.2, 133.8 (arom Cq), 129.2 (Cq phthalimido), 129.1-125.4 (arom C), 123.3 (arom C phtalimido), 98.8-96.2 (C-1, C-1', C-1"), 77.9, 77.5, 74.4, 73.6, 72.6, 71.8, 70.6, 68.7 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-3", C-4", C-5"), 61.8 (C-6"), 54.9, 54.3 (C-2", O-C$\underline{H}_3$), 21.5, 20.8 (C$\underline{H}_3$-C=O); m/z: calc= 1313, found (Maldi-Tof)= 1337 $[M+Na]^+$.

Methyl (2-*N*-acetyl-amino-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosil)-(1→3)- 2,4,6-tri-*O*-benzyl-α-D-manno-pyranoside (159) - To a solution of Methyl (3,4,6-Tri-*O*-acetyl-*N*-2-phthalimido-2-deoxy-β-D-glucopyranoside)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosil)-(1→3)-2,4,6-tri-*O*-benzyl-α-D-manno-pyranoside (**158**) (3.45 g, 2.6 mmol) in methanol (40 mL)

was added potassium *tert*-butoxide (0.19 g, 1.59 mmol) and the reaction mixture was stirred under argon for 18 h. The solution was neutralized with Dowex-50WAX-[H^+] resin, filtered and the solvent was removed under reduced pressure. The residue was dissolved in ethanol (15 mL) and hydrazine hydrate (1.2 mL, 24.7 mmol) was added. The reaction mixture was heated under reflux for 18 h. The solvent was evaporated under reduced pressure and the residue was co-evaporated with toluene (5 × 50 mL). The residue was dissolved in a mixture of pyridine (20 mL) and acetic anhydride (20 mL). And the reaction mixture was stirred for 4 h. The solvents were co-evaporated with, successively, toluene (5 × 100 mL), ethanol (100 mL) and methylene chloride (100 mL). The crude product was purified by silica gel column chromatography (methanol/methylene chloride, 1/99, v/v, then 2/98) to afford compound **159** as a colorless oil (481 mg, 79%). R_f = 0.64 (methanol/methylene chloride, 5/95, v/v); $[\alpha]_d$ = +23.7 (methylene chloride, c=8.3 mg/mL, 25 ^oC); δ_H ppm (500 MHz,$CDCl_3$): 7.41-7.15 (30H, m, arom H), 5.12 (1H, dd, $J_{3",2"}$=$J_{3",4"}$= 8.1 Hz, H-3"), 5.07 (1H, d, $J_{1',2'}$= 1.5 Hz, H-1'), 4.93 (1H, d, $J_{1,2}$=1.5 Hz, H-1), 4.70-4.43 (12H, m, C$\underline{H}_2$ benzyls), 4.25 (1H, d, $J_{1",2"}$= 8.5 Hz, H-1"), 4.16 (1H, dd, $J_{3,2}$= 2.9 Hz, $J_{3,4}$= 9.6 Hz, H-3), 4.03 (1H, dd, $J_{6a",5"}$= 4.8 Hz, $J_{6a",6b"}$= 12.5 Hz, H-6a"), 4.00-3.88 (4H, m, H-3', H-4, H-2', H-6b"), 3.82-3.56 (9H, m, H-6b', H-2, H-5, H-6a, H-6b, H-4', H-2", H-5', H-6a"), 3.29 (3H, s, O-C$\underline{H}_3$), 2.80 (1H, m, H-5"), 2.44 (3H, s, NHCOC$\underline{H}_3$), 2.03, 1.99, 1.98 (9H, 3s, C$\underline{H}_3$-C=O); δ_C ppm (75 MHz, $CDCl_3$): 170.7 (CH_3-$\underline{C}$=O), 138.1 (arom Cq benzyl), 128.6-126.1 (arom C), 98.9, 98.8 (C-1, C-1', C-1"), 77.9, 77.3, 74.4, 71.8, 68.6 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-3", C-4", C-5"), 74.9, 73.4, 69.0, 62.1 ($\underline{C}H_2$ benzyls, C-6, C-6', C-6"),

54.9, 54.6 (O-$\underline{C}H_3$, C-2”), 20.7 ($\underline{C}H_3$-C=O); m/z: calc= 1225, found (LSI)= 1248 (100%, $[M+Na]^+$).

Methyl (2-*N*-acetyl-amino-2-deoxy-β-D-glucopyranoside)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (73) - Methyl (2-*N*-acetyl-amino-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)- 2,4,6-tri-*O*-benzyl -α-D-mannopyranoside (**159**) (696 mg, 0.57 mmol) in ethanol (5 mL) was hydrogenated (palladium (II) acetate, 10%, 70 mg) for 18 h. The reaction mixture was filtered through celite and the solvent was removed under reduced pressure to afford **160** (464 mg) as a white solid. Compound **160** (464 mg, 0.83 mmol) was dissolved in a mixture of pyridine (20 mL) and acetic anhydride (20 mL) and the solution was stirred for 18 h. The solvents were co-evaporated with, successively, toluene (5 × 75 mL), ethanol (75 mL) and methylene chloride (75 mL). The residue was then applied onto a Sephadex LH20 column (methanol/methylene, 50/50, v/v). The appropriate fraction was collected and concentrated under reduced pressure. The residue was then dissolved in dry methanol (10 mL) and potassium *tert*-butoxide was added (48.5 mg, 0.41 mmol) and the reaction mixture was stirred under argon for 3 h. The solution was neutralized with Dowex-50WXA-H^+ resin, filtered and the solvent was removed under reduced pressure to afford trisaccharide **73** as a white solid (178 mg, 47%). $[\alpha]_d$ = +37.5 (methanol, c=3.4 mg/mL, 25 ^oC); δ_H ppm (500 MHz, D^4-MeOH): 5.06 (1H, d, $J_{1”,2”}$= 1.1 Hz, H-1”), 4.64 (1H, d, $J_{1’,2’}$= 1.5 Hz, H-1’), 4.42 (1H, d, $J_{1,2}$= 8.1 Hz, H-1), 4.06 (1H, dd, $J_{2”,3”}$= 3.3 Hz, H-2”), 4.02 (1H, dd, $J_{2’,3’}$= 2.6 Hz, H-2’), 3.88-3.58 (11H, m, H-6a, H-6a”, H-5’, H-3”, H-3’, H-5”, H-6a’, H-6b, H-2, H-6b’, H-6b”), 3.54-3.49 (2H, m, H-4”, H-4’), 3.45 (1H, dd, $J_{3,2}$= 8.5 Hz, $J_{3,4}$= 10.3 Hz, H-3), 3.38 (3H, s, O-C$\underline{H}_3$), 3.33-3.25 (2H, m, H-

4, H-5), 2.00 (3H, s, C<u>H</u>$_3$-C=O); δ_C ppm (125 MHz, D^4-MeOH): 174.0 (CH$_3$-<u>C</u>=O), 102.8 (C-1', $J_{C\text{-}1',H\text{-}1'}$= 168.8 Hz), 101.8 (C-1, $J_{C\text{-}1,H\text{-}1}$= 169.1 Hz), 101.1 (C-1", $J_{C\text{-}1",H\text{-}1"}$= 158.8 Hz), 80.7, 78.9, 78.0, 75.4, 75.2, 74.7, 71.6, 71.4, 69.2, 67.6 (C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2", C-3", C-4", C-5"), 63.2, 62.8, 62.5 (C-6, C-6', C-6"), 57.1, 55.2 (O-<u>C</u>H$_3$, C-2), 23.3 (<u>C</u>H$_3$-C=O); m/z: calc= 559, found (LSI)= 582 (100%, $[M+Na]^+$).

4.6. Synthesis of 3-amino-propyl (2-*N*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6" methylidene acetal (**74**) and (2-*N*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (**75**)

4.6.1 Synthesis of 3-Amino-propyl (2-N-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6" methylidene acetal (**74**)

3-Chloropropyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside (163) - To a cooled solution (0 ^oC) of Acetyl 2,3,4,6-tetra-*O*-acetyl mannopyranoside (**162**) (20g, 51.2 mmol) in methylene chloride (400mL) were added 3-chloro-propan-1-ol (6.4 mL, 76.8 mmol) and $BF_3.OEt_2$ (32.4 mL, 256 mmol). The reaction mixture was stirred under argon at room temperature for 96 h. The solution was neutralized using an aqueous $NaHCO_3$ (saturated, 20 mL). The organic layer was collected, dried ($MgSO_4$), filtered and the filtrate was evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (methylene chloride /acetone, 98/2, v/v) to afford **163** as a pale yellow oil (17.4 g, 80%). R_f (methylene chloride /acetone, 97/3, v/v)= 0.69; $[\alpha]_d$ = +37.3 (methylene chloride, c=11.7 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 300 MHz): 5.36-5.21 (3H, m, H-3, H-4, H-2), 4.82 (1H, s, H-1), 4.28 (1H, dd, $J_{6a,6b}$= 12.3 Hz, $J_{6a,5}$= 6.3 Hz, H-6a), 4.12 (1H, dd, $J_{6b,5}$= 2.0 Hz, H-6b), 3.99 (1H, m, H-5), 3.95-3.87 (1H, m, O-

C<u>H</u>$_2$ linker), 3.70-3.53 (3H, m, CH$_2$-C<u>H</u>$_2$-CH$_2$ linker), 2.21-1.96 (14H, 4s+m, C<u>H</u>$_3$-C=O, C<u>H</u>$_2$-N linker); δ_C ppm ($CDCl_3$, 300 MHz): 97.8 (C-1), 69.6, 68.7, 66.5 (C-2, C-3, C-4), 69.0 (C-5), 62.7 (C-6), 64.7, 41.6, 32.1 (C linker), 20.6 (<u>C</u>H$_3$-C=O); m/z: calc= 424, found (Maldi-Tof)= 446 $[M+Na]^+$.

3-Azidopropyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside (**164**) - To a solution of 3-Chloropropyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside (**163**) (17,3g, 40.8 mmol) in DMF (300 mL) was added KI (13.6 g, 81.6 mmol) and the reaction mixture was heated at 50 ^oC until all KI was dissolved. NaN_3 (9.8 g, 408 mmol) was added and the reaction mixture was heated under argon at 100 ^oC for 18 h. The solution was poured in ice-water (500mL) and the aqueous layer was extracted with a mixture of ethyl acetate/diethyl ether (1/1, v/v, 5×200 mL). The collected organic layer was dried ($MgSO_4$), filtered and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then 90/10, 80/20, 70/30 and 50/50) to afford **164** as a colorless oil (14.9 g, 85%). R_f (methylene chloride /acetone, 97/3, v/v)= 0.65; $[\alpha]_d$ = +24.9 (methylene chloride, c=7.9 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 300MHz): 5.38-5.21 (3H, m, H-3, H-4, H-2), 4.82 (1H, d, $J_{1,2}$= 1.3, H-1), 4.28 (1H, dd, $J_{6a,6b}$=12.36 Hz, $J_{6a,5}$=5.5 Hz, H-6a), 4.11 (1H, dd, $J_{6b,5}$= 5.49 Hz), 3.97 (1H, m, H-5), 3.88-3.74 (1H, m, O-C<u>H</u>$_2$ linker), 3.57-3.49 (1H, m, O-C<u>H</u>$_2$ linker), 3.48-3.37 (2H, m, CH$_2$-C<u>H</u>$_2$-CH$_2$ linker), 2.20-1.96 (12H, 4s, CH$_3$-C=O), 1.95-1.83 (2H, m, C<u>H</u>$_2$-N linker); δ_C ppm ($CDCl_3$, 300 MHz): 98.3 (C-1), 69.7, 69.5, 68.7, 66.6 (C-2, C-3, C-4, C-5), 63.1 (C-6), 65.6, 48.7, 29.3 (C linker), 21.3 (<u>C</u>H$_3$-C=O); m/z: calc= 431, found (Maldi-Tof)= 453 $[M+Na]^+$.

3-Azidopropyl α-D-mannopyranoside (**165**) – To a solution of 3-Azidopropyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranoside (**164**) (14.8 g, 34.3 mmol) in methanol (200 mL) was sodium methoxide (catalytic amount) and the reaction mixture was stirred for under argon 18 h. The reaction mixture was neutralized using Dowex 50WH+ resin, filtered and the solvent was evaporated to dryness under reduced pressure to give **165** as a colorless oil, which was used without further purification (9.0 g, 100%). R_f (methylene chloride /methanol, 80/20, v/v)= 0.47; δ_H ppm (CD_3OD, 300MHz): 4.83 (1H, s, H-1), 4.03-3.60 (6H, m, H-2, H-3, H-4, H-5, H-6), 3.57-3.45 (2H, m, O-C$\underline{H}_2$ linker), 3.42-3.34 (2H, m, CH_2-C$\underline{H}_2$-CH_2 linker), 1.92-1.79 (2H, m, C$\underline{H}_2$-N linker); δ_C ppm (CD_3OD, 300 MHz): 100.5 (C-1), 71.3, 71.1, 68.2, 67.3, 61.7 (C-2, C-3, C-4, C-5, C-6), 73.2, 63.8, 28.5 (C linker); m/z: calc= 263, found (Maldi-Tof)= 284 $[M+Na]^+$.

3-Azidopropyl 4,6-*O*-Benzylidene-α-D-mannopyranoside (**166**) - To a solution of 3-Azidopropyl α-D-mannopyranoside (**165**), (8.9 g, 33.8 mmol) in DMF (180 mL, 5% w/v) was added benzaldehyde dimethylacetal (5.3 mL, 35.5 mmol) and tetrafluoroboric acid (52% in diethyl ether, 5.7 mL). The reaction mixture was stirred under argon for 18 h. The solution was neutralized with Et_3N and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (methylene chloride/acetone, 95/5, v/v, then 90/10) to afford **166** as a colorless oil (7.8 g, 66%). R_f (methylene chloride /methanol, 95/5, v/v)= 0.48; $[\alpha]_d$ = +56.9 (methylene chloride, c=10.0 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 300MHz): 7.28-7.12 (5H, m, arom H), 5.58 (1H,

s, C<u>H</u> benzylidene), 4.88 (1H, s, H-1), 4.29 (1H, dd, $J_{6a,6b}$= 12.36 Hz, H-6a), 4.08 (1H, s, H-2), 3.92 (1H, dd, $J_{4,3}$=$J_{4,5}$= 9.83 Hz, H-4), 4.88-4.77 (4H, m, H-3, H-5, H-6b, O-C<u>H</u>$_2$ linker), 3.58-3.49 (1H, m, O-C<u>H</u>$_2$ linker), 3.47-3.35 (2H, m, CH_2-C<u>H</u>$_2$-CH_2 linker), 1.95-1.82 (2H, m, C<u>H</u>$_2$-N linker); δ_C ppm ($CDCl_3$, 300 MHz): 131.5-125.1 (C aromatic), 102.5 (<u>C</u>H benzylidene), 100.3 (C-1), 76.2, 70.0, 69.8, 63.2 (C-2, C-3, C-4, C-5), 68.4 (C-6), 64.8, 48.5, 29.4 (C linker); m/z: calc= 351, found (Maldi-Tof)= 373 $[M+Na]^+$.

3-Azidopropyl 4,6-*O*-Bensylidene-3-*p*-methoxybenzyl-α-D-mannopyranoside (**167**) - To a solution of 3-Azidopropyl 4,6-*O*-Bensylidene-α-D-mannopyranoside (**166**) (7.7 g, 21.9 mmol) in benzene (150 mL) was added *tert*-butyl tin dimethoxide (3.7 mL, 24.1 mmol) and the reaction mixture was refluxed under Dean Stark conditions for 2 h. TBAI (12.8 g, 32.9 mmol) and PMBCl (3.3 mL, 24.1 mmol) were added and the reaction mixture was refluxed for 2 h. The solution was then allowed to cool down to room temperature and filtered. The solvent was removed and the residue was taken up into methylene chloride (100 mL). The obtained solution was washed with, successively aqueous KF (1M, 2×100 mL) and water (100 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (methylene chloride /acetone, 99/1, v/v) and **167** was obtained as slightly pink oil (9.3g, 90%). R_f (methylene chloride /Acetone, 97/3, v/v)= 0.38; $[\alpha]_d$ = +39.6 (methylene chloride, c= 9.4 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 300MHz): 7.55-6.85 (9H, m, arom H), 5.62 (1H, s, C<u>H</u> benzylidene), 4.85 (1H, s, H-1), 4.79 (1H, d, J_{gem}= 11.0 Hz, C<u>H</u>$_2$ *p*-methoxybenzyl), 4.65 (1H, d, J_{gem}= 11.4 Hz, C<u>H</u>$_2$ *p*-methoxybenzyl), 4.17 (1H, m, H-6a), 4.12-3.99 (2H, m, $J_{2,3}$=2.2 Hz, H-6b, H-2), 3.93-

3.71 (7H, m, H-3, H-4, H-5, O-C$\underline{H}_2$ linker, O-C$\underline{H}_3$ *p*-methoxybenzyl), 3.55-3.47 (1H, m, O-C$\underline{H}_2$ linker), 3.42-3.31 (2H, m, CH_2-C$\underline{H}_2$-CH_2 linker), 1.95-1.82 (2H, m, C$\underline{H}_2$-N linker); δ_C ppm ($CDCl_3$, 300 MHz): 129.7, 128.7, 126.5, 113.9 (arom C), 101.7 (C$\underline{H}$ benzylidene), 100.2 (C-1), 78.9, 75.4, 70.0, 68.9, 65.1 (C-2, C-3, C-4, C-5, C-6), 73.1 ($\underline{C}H_2$ PMB), 64.7, 48.3, 28.8 (C linker), 55.0 (O-$\underline{C}H_3$); m/z: calc= 471, found (Maldi-Tof)= 493 $[M+Na]^+$.

3-Azidopropyl 2-*O*-Acetyl-4,6-*O*-bensylidene-3-*p*-methoxybenzyl-α-D-mannopyranoside (**167Ac**) – A solution of 3-Azidopropyl 4,6-*O*-Bensylidene-3-*p*-methoxybenzyl-α-D-mannopyranoside (**167**) (50 mg, 0.1 mmol) in a mixture pyridine/Ac_2O (1/1, v/v, 5 mL) was stirred under argon at room temperature for 3 h. The solvent was co-evaporated with, successively, toluene (5×5 mL), ethanol (5 mL) and methylene chloride (5 mL) to afford **167Ac** as pale yellow oil (54 mg, 100%). R_f= 0.72 (methylene chloride/acetone, 99/1, v/v); δ_H ppm ($CDCl_3$, 300MHz): 7.55-6.78 (9H, m, arom. H), 5.62 (1H, s, C$\underline{H}$ benzylidene), 5.35 (1H, s, H-2), 4.78 (1H, s, H-1), 4.60 (2H, 2d, C$\underline{H}_2$ PMB), 4.25 (1H, d, $J_{6a,6b}$= 10.25 Hz, H-6a), 4.10-3.92 (2H, m, H-6b, $J_{3,2}$= 9.0 Hz, $J_{3,4}$= 3.7 Hz, H-3), 3.87-3.76 (6H, m, H-4, H-5, O-C$\underline{H}_2$ linker, O-C$\underline{H}_3$ PMB), 3.41-3.30 (2H, m, CH_2-C$\underline{H}_2$-CH_2 linker), 1.95-1.81 (2H, m, C$\underline{H}_2$-N linker).

3-Azidopropyl 2-*O*-Benzyl-4,6-*O*-bensylidene-3-*p*-methoxybenzyl-α-D-mannopyranoside (**168**) - To a cooled solution (0 ^oC) of 3-Azidopropyl 4,6-*O*-Benzylidene-3-*p*-methoxybenzyl-α-D-mannopyranoside (**167**) (9.2g, 19.5 mmol) in DMF (100 mL) was added NaH (50% in mineral oil, 1.17 g, 29.3 mmol) and the reaction mixture was stirred

for 15 min. The ice bath was removed and benzyl bromide (2.8 mL, 23.4 mmol) was added slowly. The reaction mixture was stirred under argon at room temperature for 5 h. The excess of NaH was destroyed with methanol and the solvent was removed under reduced pressure. The residue was taken up into diethyl ether (150 mL) and was washed with water (2×150 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent removed under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then 90/10 and 80/20) to afford **168** (10.4 g, 95%) as a colorless oil. R_f (methylene chloride /Acetone, 97/3, v/v)= 0.47; $[\alpha]_d$ = +31.7 (methylene chloride, c=13.3 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 300MHz): 7.58-6.80 (14H, m, aromatic H), 5.63 (1H, s, C<u>H</u> benzylidene), 4.89-4.66 (4H, H-1, C<u>H</u>$_2$ benzyl, C<u>H</u>$_2$ PMB), 4.60 (1H, d, J_{gem}= 11.8 Hz, C<u>H</u>$_2$ PMB), 4.29-4.18 (2H, m, H-4, H-6a), 3.97-3.68 (8H, m, H-3, H-2, H-5, H-6b, O-C<u>H</u>$_3$ PMB, O-C<u>H</u>$_2$ linker), 3.47-3.25 (3H, m, O-C<u>H</u>$_2$ linker, CH_2-C<u>H</u>$_2$-CH_2 linker), 1.88-1.75 (2H, m, C<u>H</u>$_2$-N); δ_C ppm ($CDCl_3$, 300 MHz): 128.9, 128.5, 125.5, 113.8 (arom C), 101.5 (CH benzylidene), 99.5 (C-1), 78.9, 76.4, 76.2, 69.1, 68.5 (C-2, C-3, C-4, C-5, C-6), 73.3, 72.8 (<u>C</u>H_2 benzyl, <u>C</u>H_2 PMB), 64.6, 48.1, 28.8 (C linker), 55.5 (O-<u>C</u>H_3 PMB); m/z: calc= 561, found (Maldi-Tof)= 583 $[M+Na]^+$.

3-Azidopropyl 2-*O*-Benzyl-4,6-*O*-bensylidene-α-D-mannopyranoside (**161**) - To a cooled solution (0 ^oC) of 3-Azidopropyl 2-*O*-Benzyl-4,6-*O*-bensylidene-3-*p*-methoxy-benzyl-α-D-mannopyranoside (**168**) (10.3 g, 18.4 mmol) in a mixture methylene chloride/water (20/1, v/v, 100 mL), was added DDQ (8.3 g, 36.8 mmol) and the reaction mixture was stirred for 5 h. The reaction was quenched with aqueous $NaHCO_3$ (saturated,

20 mL). The solution was washed with water (3×200 mL), and the organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by flash silica gel column chromatography (toluene/acetone, 97/3, v/v) to afford **161** as a colorless oil (7.4 g, 92%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.67; $[\alpha]_d$ = +55.3 (methylene chloride, c=10.7 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 300MHz): 7.57-7.27 (10H, m, arom. H), 5.59 (1H, s, C<u>H</u> benzylidene), 4.83 (1H, s, H-1), 4.75 (2H, dd, J_{gem}= 12.2 Hz, C<u>H</u>$_2$ benzyl), 4.25 (1H, dd, $J_{6a,6b}$= 9.4 Hz, $J_{6a,5}$= 3.8 Hz, H-6a), 4.08 (1H, m, H-3), 3.97-3.70 (5H, m, $J_{4,3}$=$J_{4,5}$= 9.2 Hz, H-2, H-4, H-5, H-6b, O-C<u>H</u>$_2$ linker), 3.57-3.29 (3H, m, O-C<u>H</u>$_2$ linker, CH_2-C<u>H</u>$_2$-CH_2 linker), 2.31 (1H, d, O<u>H</u>), 1.92-1.79 (2H, m, C<u>H</u>$_2$-N linker); δ_C ppm ($CDCl_3$, 300 MHz): 130.5-125.3 (arom C), 102.3 (<u>C</u>H benzylidene), 98.5 (C-1), 79.5, 78.5, 68.8, 68.7, 64.2 (C-2, C-3, C-4, C-5, C-6), 73.9 (<u>C</u>H_2 benzyl), 64.3, 48.2, 28.6 (C linker); m/z: calc= 441, found (Maldi-Tof)= 463 $[M+Na]^+$; Elemental analysis: Calc. C: 62.57, H: 6.16, Found: C: 62.74, H: 6.36.

3-Azidopropyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2-*O*-benzyl-4,6-*O*-bensylidene-α-D-mannopyranoside (169) - To a stirred solution of 3-Azidopropyl 2-*O*-Benzyl-4,6-*O*-bensylidene-α-D-mannopyranoside (**161**) (1.58 g, 3.6 mmol), Ethyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-1-deoxy-1-thio-*α*-D-mannopyranoside (**79**) (2.69 g, 5.0 mmol) and 4 Å molecular sieves (1g) in methylene chloride (50 mL) were added NIS (1.04 g, 5.0 mmol) and TMSOTf (84 µl, 0.5 mmol). The reaction mixture was stirred under argon at room temperature for 1h before being filtered through celite. The resulting solution was diluted with methylene chloride (100 mL) and washed with aqueous $Na_2S_2O_3$ (25% in water, 2 ×200 mL). The aqueous layer was dried ($MgSO_4$),

filtered and the solvent was removed under reduced pressure. The residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then 90/10 and 80/20) to afford **169** (2.46 g, 75%) as colorless oil. R_f (hexane/ethyl acetate, 70/30, v/v)= 0.47; $[\alpha]_d$ = +44.7 (methylene chloride, c=11.4 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 300MHz): 7.56-7.17 (25H, m, arom H), 5.66 (1H, s, C<u>H</u> benzylidene), 5.62 (1H, s, H'-2), 5.33 (1H, s, H'-1), 4.90 (1H, d, J_{gem}= 10.3 Hz, C<u>H</u>$_2$ benzyl), 4.82-4.63 (5H, m, H-1, J_{gem}= 12.5 Hz, C<u>H</u>$_2$ benzyl), 4.51-4.44 (3H, m, C<u>H</u>$_2$ benzyl), 4.32-4.20 (3H, m, H-6a, H'-6a, H-3), 4.01 (1H, dd, H'-3), 3.93-3.64 (8H, m, H-2, H-4, H-5, H-6b, H'-4, H'-5, H'-6b, O-C<u>H</u>$_2$ linker), 3.47-3.38 (1H, m, O-C<u>H</u>$_2$ linker), 3.33-3.24 (2H, m, CH_2-C<u>H</u>$_2$-CH_2 linker), 2.11 (C<u>H</u>$_3$-C=O), 1.82-1,74 (2H, m, C<u>H</u>$_2$-N linker); δ_C ppm ($CDCl_3$, 75 MHz): 138.8, 138.5, 138.0, 137.9, 137.5 (arom Cq), 129.4-126.1 (arom C), 101.5 (<u>C</u>H benzylidene), 99.5, 99.1 (C-1, C'-1), 79.2, 78.2, 75.3, 72.5, 68.5 (C-2, C-3, C-4, C-5, C'-2, C'-3, C'-4, C'-5), 75.3, 73.9, 73.6, 71.8, 69.3, 68.5 (C-6, C'6, <u>C</u>H$_2$ benzyls), 64.6, 48.7, 29.1 (C linker), 21.4 (<u>C</u>H$_3$-C=O); m/z: calc= 915, found (Maldi-Tof)= 938 $[M+Na]^+$; Elemental analysis: Calc. C: 68.18, H: 6.27, Found: C: 68.41, H: 6.54

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3-Azidopropyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside (170) - To a solution of 3-Azidopropyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2-*O*-benzyl-4,6-*O*-bensylidene-α-D-mannopyranoside (**169**) (2.40 g, 2.6 mmol) in THF (50 mL) were added 4 Å molecular sieves (1g), $NaBH_3CN$ (1.65g, 26 mmol) and methyl orange (catalytic amount). A 2N solution of HCl in diethyl ether was added until the pH was acidic and the reaction mixture was stirred at room temperature for 2 h. The solution was filtered through celite

and washed with, successively, aqueous $NaHCO_3$ (saturated, 2×100 mL) and water (100 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 100/0, then 90/10, 80/20 and 70/30) to give **170** as a colorless oil (2.14 g, 89%). R_f (hexane/ethyl acetate, 70/30, v/v)= 0.32; $[\alpha]_d$ = +32.9 (methylene chloride, c=9.4 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 500MHz): 7.44-7.18 (20H, m, arom H), 5.50 (1H, dd, $J_{2',3'}$= 1.7 Hz, H'-2), 5.39 (1H, s, H'-1), 4.89 (1H, d, J_{gem}= 10.8 Hz, C$\underline{H}_2$ benzyl), 4.83 (1H, s, H-1), 4.75 (1H, d, J_{gem}= 11.2 Hz, C$\underline{H}_2$ benzyl), 4.69-4.47 (6H, m, C$\underline{H}_2$ benzyl), 4.13 (1H, dd, $J_{6'a,6'b}$=$J_{6'a,5'}$= 9.5 Hz, H'-6a), 4.06-3.96 (3H, m, H-3, H'-3, H'-6b), 3.82-3.65 (8H, m, H-2, H'-4, H'-5, H-4, H-5, H-6a, H-6b, O-C$\underline{H}_2$ linker), 3.48-3.39 (1H, m, O-C$\underline{H}_2$ linker), 3.46-3.27 (2H, m, CH_2-C$\underline{H}_2$-CH_2 linker), 2.12 (3H, s, C$\underline{H}_3$-C=O), 1.83-1.77 (2H, m, C$\underline{H}_2$-N linker); δ_C ppm ($CDCl_3$, 75 MHz): 138.7, 138.2, 138.1, 138.0 (arom Cq), 128.6-127.7 (arom C), 98.9,97.9 (C'-1, C-1), 78.4, 78.2, 77.9, 74.8, 71.7, 69.1, 68.6 (C-2, C-3, C-4, C-5, C'-2, C'-3, C'-4, C'-5), 75.2, 73.9, 73.7, 72.8, 72.1, 71.0, 69.7 (C-6, C'-6, $\underline{C}H_2$ benzyls), 64.6, 48.6, 29.2 (C linker), 21.4 ($\underline{C}H_3$-C=O); m/z: calc= 917, found (Maldi-Tof)= 940 $[M+Na]^+$.

3-Azidopropyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2,6-di-*O*-benzyl-4-*O*-methyl-thio-methyl-α-D mannopyranoside (171) - To a solution of 3-Azidopropyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside (**170**) (2.04 g, 2.2 mmol) in acetonitrile (30 mL) was added Me_2S (1.7 mL, 22 mmol). Under the exclusion of light, the reaction mixture was cooled to 0 ^oC and benzoyl peroxide (2.15 g, 8.8 mmol) was added portion wise over a period of

45 min. The temperature was allowed to rise to room temperature very slowly and the reaction mixture was stirred under argon for 18 h. The reaction mixture was diluted with ethyl acetate (60 mL) and the solution was washed with aqueous $NaHCO_3$ (Saturated, 2×100 mL) and water (100 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The residue was purified by flash silica gel column chromatography (hexane/ethyl acetate, 100/0, then 90/10, 80/20 and 70/30) to afford **171** was obtained as a colorless oil (1.89 g, 87%). R_f (hexane/ethyl acetate, 70/30, v/v)= 0.49; $[\alpha]_d$ = +27.3 (methylene chloride, c=9.9 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 500MHz): 7.39-7.17 (25H, m, arom H), 5.37 (1H, s, H-2'), 5.13 (1H, s, H-1'), 4.92-4.46 (13H, m, H-1, J_{gem}= 10.8 Hz, J_{gem}= 11.3 Hz, C$\underline{H}_2$ benzyl, C$\underline{H}_2$ MTM), 4.07 (1H, dd, $J_{3,2}$= 9.2 Hz, $J_{3,4}$= 2.9 Hz, H-3), 4.01 (1H, dd, $J_{3',2'}$= 9.0 Hz, $J_{3',4'}$= 3.1 Hz, H-3'), 3.95-3.63 (10H, m, H-5', H-4, H-4', H-2, H-6', H-5, H-6, O-C$\underline{H}_2$ linker), 3.47-3.39 (1H, m, O-C$\underline{H}_2$ linker), 3.38-3.23 (2H, m, CH_2-C$\underline{H}_2$-CH_2 linker), 2.16, 2.02 (6H, 2s, C$\underline{H}_3$-C=O, C$\underline{H}_3$ MTM), 1.92-1.86 (2H, m, C$\underline{H}_2$-N linker); δ_C ppm ($CDCl_3$, 500MHz): 129.3-126.8 (arom. C), 100.0 (C-1'), 97.5 (C-1), 79.1, 77.8, 77.4, 74.3, 72.2, 71.6, 69.1 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 77.2, 74.8, 74.7, 73.4, 71.9 (C$\underline{H}_2$ benzyls), 74.1, 69.4 (C-6, C-6'), 64.4, 48.3, 28.6 (C linker), 20.8 (C$\underline{H}_3$-C=O), 14.5 (C$\underline{H}_3$ MTM); m/z: calc= 977, found (Maldi-Tof)= 1000 $[M+Na]^+$.

3-Azidopropyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside *p*-methoxyphenyl 3",4"-di-*O*-benzyl-2-deoxy-2"-*N*-phtalimido-β-D-glucopyranoside-4,6"-methylidene acetal (172) - In a separate reaction vessel, NIS (428 mg, 1.9 mmol) was dissolved in a mixture DCE/THF (1/1, v/v, 18.4

mL). TfOH (16 μl, 0.18 mmol) was added and the resulting solution was stirred for 30 seconds. The resulting mixture was added to a stirred cooled solution (0 ^{o}C) of 3-Azidopropyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2,6-di-*O*-benzyl-4-*O*-methyl-thio-methyl-α-D-mannopyranoside (**171**) (1.20 g, 1.2 mmol), *p*-methoxyphenyl 3",4"-di-*O*-benzyl-2"-deoxy-2"-*N*-phtalimido-β-D-glucopyranoside (**116**) (1.10 g, 1.9 mmol) and 4 Å molecular sieves (1 g). The reaction mixture was stirred under argon for 30 min. The solution was filtered through celite and the filtrate was washed with $Na_2S_2O_3$ solution (20% in water, 2×80 mL), aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The crude product was purified by flash silica gel column chromatography (methylene chloride/acetone, 97/3, v/v) to afford the desired trisaccharide **172** as a colorless oil (1.22 g, 73%). R_f (methylene chloride /acetone, 99/1, v/v)= 0.62; $[\alpha]_d$ = +19.8 (methylene chloride, c=8.5 mg/mL, 25 ^{o}C); δ_H ppm ($CDCl_3$, 500MHz): 7.75-7.62 (4H, br-s, arom H phthalimido), 7.41-6.84 (35H, m, arom H), 6.82 (2H, d, H PM), 6.69 (2H, d, H PM), 5.61 (1H, d, $J_{1",2"}$=5.2 Hz, H"-1), 5.31 (1H, s, H'-2), 5.12 (1H, s, H'-1), 4.91-4.79 (5H, m, H-1, O-C$\underline{H}_2$-O, J_{gem}= 10.8 Hz, J_{gem}= 12.1 Hz, C$\underline{H}_2$ benzyl), 4.73 (2H, dd, J_{gem}= 10.8 Hz, J_{gem}=11.2 Hz, C$\underline{H}_2$ benzyl), 4.67-4.45 (10H, m, C$\underline{H}_2$ benzyls), 4.43 (2H, m, H"-2, H"-6a), 4.07-3.61 (19H, m, H-3, H'-3, H-4, H'-4, H-2, H'-5, H-5, H-6a, H-6b, H'-6a, H'-6b, H"-3, H"-4, H"-5, H"-6b, O-C$\underline{H}_2$ linker, O-C$\underline{H}_3$ MP), 3.45 (1H, m, O-C$\underline{H}_2$ linker), 3.30 (2H, m, CH_2-C$\underline{H}_2$-CH_2 linker), 2.12 (3H, s, C$\underline{H}_3$-C=O), 1.88 (2H, m, C$\underline{H}_2$-N linker); δ_C ppm ($CDCl_3$, 500 MHz): 134.0-114.7 (arom C), 99.1 (C'-1), 98.03 (C-1), 97.8 (C"-1), 97.3 (O-$\underline{C}H_2$-O), 78.9, 77.9, 77.5, 75.2, 74.6, 74.0, 72.2, 71.9, 69.2 (C-2, C-3, C-4, C-5, C'-3, C'-4, C'-5, C"-3, C"-4, C"-5), 75.2, 74.9, 73.6,

73.0, 72.0 (C-6, C'-6, C"-6, $\underline{C}H_2$ benzyls), 69.3 (C'-2), 55.9 (C"-2), 64.5, 48.4, 28.9 (C linker), 55.6 (O-$\underline{C}H_3$ MP), 21.0 ($\underline{C}H_3$-C=O); m/z: calc= 1525, found (Maldi-Tof)= 1548 $[M+Na]^+$; Elemental analysis: Calc. C: 69.28, H: 6.08, Found: C: 69.42, H: 6.26.

3-Azidopropyl (3,4,6-Tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside-3",4"-di-*O*-benzyl-2"-deoxy-2"-*N*-phtalimido-β-D-gluco-pyranoside-4,6"-methylidene acetal (173) - To solution of 3-Azidopropyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside -*p*-methoxyphenyl 3",4"-di-*O*-benzyl-2-deoxy-2"-*N*-phtalimido-β-D-glucopyranoside-4,6"-methylidene acetal (**172**) (1.22 g, 0.79 mmol) in methanol (20 mL), was added potassium *tert*-butoxide (89 mg, 0.79 mmol) and the reaction mixture was stirred under argon at room temperature for 1h. The solution was neutralized using Dowex 200WH+ resin, filtered and the solvent was removed under reduced pressure. The residue was used without further purification and was dissolved in a mixture of toluene/acetonitrile/water, 1/4/1, v/v/v (40 mL). CAN (1.39 g, 2.37 mmol) was added and the reaction mixture was stirred in the dark at room temperature for 4 h. The solution was diluted with ethyl acetate and washed with aqueous $NaHCO_3$ (saturated, 2×100 mL) and brine (100 mL). The organic phase was dried ($MgSO_4$), filtered and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (methylene chloride/methanol, 99/1, v/v) to afford the desired **173** as colorless oil (600.2 mg, 55%). R_f (methylene chloride/methanol, 97/3, v/v)= 0.27; $[\alpha]_d$ = +31.1 (methylene chloride, c=5.5 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 500MHz): 7.75-7.62 (4H, br-s, arom H phthalimido), 7.60-6.84 (35H, m, arom H), 5.33 (1H, s, H'-1), 5.26

(1H, d, $J_{1",2"}$= 8.2 Hz , H"-1), 4.90-4.78 (3H, m, H-1, C<u>H</u>$_2$ benzyls), 4.76-4.39 (15H, m, H'-3, O-C<u>H</u>$_2$-O, C<u>H</u>$_2$ benzyls), 4.36 (1H, s, H'-2), 4.18-4.04 (2H, m, H"-2, H-3), 4.02-3.57 (15H, m, H'-3, H-2, H"-4, H'-4, H-4, H-5, H-6a, H-6b, H'-5, H'-6a, H'-6b, H"-5, H"-6a, H"-6b, O-C<u>H</u>$_2$ linker), 3.44-3.24 (3H, m, O-C<u>H</u>$_2$ linker, CH_2-C<u>H</u>$_2$-CH_2 linker), 1.92-1.87 (2H, m, C<u>H</u>$_2$-N linker); δ_C ppm ($CDCl_3$, 500 MHz): 134.0-123.4 (arom C), 100.8 (C'-1), 97.7 (C-1), 97.7 (O-<u>C</u>H_2-O), 93.1 (C"-1), 79.9, 79.4, 79.2, 78.3, 77.3, 75.8, 69.6, 69.2 (C-2, C-3, C-4, C-5, C'-3, C'-4, C'-5, C"-3, C"-4, C"-5), 78.9, 73.6, 72.0 (<u>C</u>H_2 benzyls), 74.6, 71.7, 66.8 (C-6, C'-6, C"-6), 68.3, 57.7 (C'-2, C"-2), 64.5, 48.4, 28.9 (C linker); m/z: calc= 1377, found (Maldi-Tof)= 1400 $[M+Na]^+$.

3-Azidopropyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2,6-di-*O*-benzyl-4-*O*-methyl-thio-methyl-α-D-mannopyranoside-trichloroacetimidate 3",4"-di-*O*-benzyl-2-deoxy-2"-*N*-phtalimido-β-D-glucopyranoside-4,6"-methylidene acetal (174) - To a solution of 3-azidopropyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-manno-pyranoside)-(1→3)-2,6-di-*O*-benzyl-4-*O*-methyl-thio-methyl-α-D-mannopyranoside-3",4"-di-*O*-benzyl-2"-deoxy-2"-*N*-phtalimido-β-D-glucopyranoside-4,6"-methylidene acetal (**173**) (416.2 mg, 0.30 mmol) in methylene (9 mL) were added trichloroacetonitrile (182 µl, 1.8 mmol) and DBU (4.5 µl, 0.015 mmol). The reaction mixture was stirred under argon and followed by TLC analysis (methylene chloride/acetone, 95/5, v/v). After 20 min, it showed no more progress in the conversion of the starting material to the desired imidate, the reaction was then stopped. The solvent was concentrated *in vacuo* and the oily residue purified by silica gel chromatography (methylene chloride/acetone, 95/5, v/v) to afford **174** as a white foam (193.2 mg, 43%). R_f (methylene

chloride/Acetone, 95/5, v/v)= 0.43; δ_H ppm ($CDCl_3$, 500MHz): 7.65 (4H, br-S, arom H phthalimido), 7.46-6.84 (35H, m, arom H), 6.37 (1M, d, $J_{1",2"}$= 7.8 Hz, H-1"), 5.16 (1H, S, H-1'), 4.90-4.38 (17H, m, J_{gem} = 10.8, J_{gem} = 11.0, H-1, H-3", C$\underline{H}_2$ benzyls, H-4"), 4.17-3.78 (9H, m, H-2', H-4, H-4', H-2, H-6", H-3', H-6), 3.76-3.56 (6H, m, H-6', H-5', H-5", H-5, 1H linker, O-CH_2 linker), 3.45-3.35 (1H, m, O-C$\underline{H}_2$ linker), 3.31-3.19 (2H, m, CH_2-C$\underline{H}_2$-CH_2 linker), 1.82-1.68 (2H, m, C$\underline{H}_2$-N linker); δ_C ppm ($CDCl_3$, 500 MHz): 122.7-120.4 (arom C), 95.6 (C-1'), 91.0 (C-1), 87.5 (C-1"), 73.0, 72.3, 68.5, 68.1, 65.2, 63.2, 62.7, 61.6, 60.4, 48.4 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2", C-3", C-4", C-5"), 68.3, 68.1, 66.6, 65.6 (C$\underline{H}_2$ benzyls), 63.3, 62.6, 60.7 (C-6, C-6', C-6"), 57.9, 41.6, 22.0 (C-linker).

3-Azido-propyl (3,4-Di-*O*-benzyl-2-deoxy-2-*N*-phtalimido-β-D-glucopyranoside)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside-4,6"-methylidene acetal (175) - To a solution of 3-Azidopropyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2,6-di-*O*-benzyl-4-*O*-methyl -thio-methyl-α-D-mannopyranoside-trichloroacetimidate 3",4"-di-*O*-benzyl-2-deoxy-2"-*N*-phtalimido-β-D-glucopyranoside-4,6"-methylidene acetal (**174**) (183 mg, 0.12 mmol) in methylene chloride (6 mL) were added 4 Å molecular sieves and the reaction mixture was stirred for 45 min under argon. The solution was cooled to -20 ^oC. TMSOTf (1.1 µl, 0.006 mmol) was added and the reaction mixture was stirred under argon for 20 min. The solution was neutralized with Et_3N, filtered through celite and the solvent was removed under reduced pressure. The crude product was purified by flash silica gel column chromatography (methylene chloride/acetone, 99/1, v/v) to afford cyclic trisaccharide

175 as a colorless oil (133 mg, 80%). R_f (methylene chloride /acetone, 97/3, v/v)= 0.62; $[\alpha]_d$ = +57.4 (methylene chloride, c=4.6 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 500MHz): 7.56-7.35 (4H, br-s, arom H phthalimido), 7.32-6.75 (35H, m, arom H), 5.63 (2H, s, H'-1, H"-1), 4.97 (1H, d, J_{gem}= 3.9 Hz, O-C$\underline{H}_2$-O), 4.80 (1H, d, J_{gem}= 11.2 Hz , C$\underline{H}_2$ benzyls), 4.74-4.32 (13H, m, H-1, O-C$\underline{H}_2$-O, C$\underline{H}_2$ benzyls), 4.30-4.18 (4H, m, H'-2, H"-2, C$\underline{H}_2$ benzyls), 4.06-3.99 (2H, m, H-3, C$\underline{H}_2$ benzyls), 3.96-3.84 (2H, m, H-6a, H'-6a), 3.75-3.37 (13H, m, H'-3, H"-3, H"-4, H-6b, H'-4, H-2, H-5, H'-5, H'-6b, H"-5, H"-6, O-C$\underline{H}_2$ linker), 3.35-3.23 (1H, m, O-C$\underline{H}_2$ linker), 3.21-3.05 (2H, m, CH_2-C$\underline{H}_2$-CH_2 linker), 1.64-1.59 (2H, m, C$\underline{H}_2$-N linker); δ_C ppm ($CDCl_3$, 75 MHz): 139.3-131.9 (arom Cq), 133.6-123.2 (arom C), 100.2, 98.3, 94.6 (C-1, C'-1, C"-1), 97.5 (O-$\underline{C}H_2$-O), 79.6, 79.3, 76.4, 75.9, 75.3, 74.6, 72.6, 72.3 (C-2, C-3, C-4, C-5, C'-2, C'-3, C'-4, C'-5, C"-3, C"-4, C"-5), 75.3, 75.2, 73.8, 73.4, 72.5, 69.4, 69.1, 66.9 (C-6, C'-6, C"-6, $\underline{C}H_2$ benzyls), 64.4, 48.6, 29.1 (C linker), 54.5 (C"-2); m/z: calc= 1359, found (Maldi-Tof)= 1382 $[M+Na]^+$; Elemental analysis: Calc. C: 69.79, H: 6.08, Found: C: 70.00, H: 6.33.

3-Azidopropyl (2-*N*-Acetyl-2-deoxy-3,4-di-*O*-benzyl-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside-4,6"-methylidene acetal (176) - To a solution of 3-azidopropyl (3,4-di-*O*-benzyl-2-deoxy-2-*N*-phtalimido-β-D-glucopyranoside)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranoside)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside-4,6"-methylidene acetal (**175**) (132 mg, 0.10 mmol) in ethanol (5 mL) was added hydrazine monohydrate (237 µl, 5.0 mmol) and the reaction mixture was refluxed for 18 h. The solvent was removed *in vacuo* and co-evaporation of the residue with toluene (5 × 5 mL) afforded the amino

compound in quantitative yield. It was subsequently dissolved in a mixture pyridine/Ac_2O (1/1, v/v, 5 mL) and the solution was stirred at room temperature for 4 h. The mixture was concentrated and co-evaporated with toluene (5×20 mL), ethanol (20 mL) and methylene chloride (20 mL). Purification of the crude product by silica gel column chromatography (methylene chloride/acetone, 98/2, v/v) gave trisaccharide **176** as colorless oil (118 mg, 95%). R_f (methylene chloride /acetone, 95/5, v/v)= 0.87; $[\alpha]_d$ = +44.8 (methylene chloride, c=5.0 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 500MHz): 7.43-7.17 (35H, m, arom H), 5.48 (1H, d, N<u>H</u>), 5.41 (1H, s, H-1'), 5.23 (1H, d, $J_{1",2"}$= 8.7 Hz, H-1"), 4.82 (1H, s, H-1), 4.92-4.48 (17H, m, H-3", O-C<u>H</u>$_2$-O, C<u>H</u>$_2$ benzyls), 4.24 (1H, s, H-2'), 4.06 (2H, m, H-3, H-4), 3.90-3.52 (14H, m, H-4', H-3', H-6'a, H-6, H-2, H-4", H-4, H-5', H-5, H-6'b, H-5", H-6", O-C<u>H</u>$_2$ linker), 3.51-3.45 (1H, m, O-C<u>H</u>$_2$ linker), 3.33-3.22 (2H, m, CH_2-C<u>H</u>$_2$-CH_2 linker), 2.88 (1H, m, H-2"), 1.79-1.68 (2H, m, C<u>H</u>$_2$-N linker), 1.58 (3H, s, O-C<u>H</u>$_3$); δ_C ppm ($CDCl_3$, 500 MHz): 129.5-127.3 (arom C), 103.1 (C-1'), 97.8 (C-1), 96.5 (C-1"), 97.3 (O-<u>C</u>H_2-O), 79.0, 78.4, 78.1, 77.7, 76.7, 76.3, 76.1, 70.9, 69.3 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-3", C-4", C-5"), 75.2, 74.9, 74.6, 73.6, 72.7, 72.3 (C-6, C-6', C-6", <u>C</u>H_2 benzyls), 64.7, 48.5, 28.8 (C linker), 56.4 (C-2"), 23.4 (O-<u>C</u>H_3); m/z: calc= 1271, found (Maldi-Tof)= 1294 $[M+Na]^+$.

3-Amino-propyl (2-*N*-Acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(-α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6"-methylidene acetal (**74**) - 3-Azido-propyl (2-*N*-acetyl-2-deoxy-3,4-di-*O*-benzyl-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside-4,6"-methylidene acetal (**176**) (47 mg, 0.04 mmol) in EtOH (2 mL) and AcOH (0.2 mL) was hydrogenated

(H_2, $Pd(OAc)_2$, catalytic amount) for 48 h. The mixture was filtered through celite and the solvent was removed *in vacuo* to afford target trisaccharide **74** was obtained as a white solid (21 mg, 95%). R_f (methylene chloride/methanol, 70/30, v/v)= 0; $[\alpha]_d$ = +21.8 (methanol, c= 3.3 mg/mL, 25 ^oC); δ_H ppm (D_20, 500MHz): 5.48 (1H, s, H-1'), 4.96, 4.83 (2H, 2d, J_{gem}=5.2 Hz, J_{gem}=4.8 Hz, O-C$\underline{H}_2$-O), 4.74 (1H, d, $J_{1",2"}$= 8.5 Hz, H-1"), 4.69 (1H, s, H-1), 4.16 (1H, d, $J_{2',3'}$= 4.2 Hz, H-2'), 3.94-3.88 (3H, m, H-3, H-2, H-6a), 3.84-3.39 (16H, m, H-4, H-6b, H-4', H-2", H-3', H-5", H-6', H-4", H-5', H-5, H-6", H-3", O-C$\underline{H}_2$ linker), 3.14 (2H, t, CH_2-C$\underline{H}_2$-CH_2 linker), 1.93 (3H, s, O-C$\underline{H}_3$), 1.70 (2H, t, C$\underline{H}_2$-N linker); δ_C ppm (D_2O, 500 MHz): 100.1 (C-1, $J_{C1,H1}$= 172.3 Hz), 99.6 (C-1', $J_{C1',H1'}$= 177.6 Hz), 97.7 (C-1", $J_{C1",H1"}$= 167.3 Hz), 98.3 (O-C$\underline{H}_2$-O), 76.3, 75.5, 74.6, 73.9, 73.2, 72.5, 70.4, 66.9, 60.5, 60.1, (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-3", C-4", C-5"), 69.2, 68.1, 60.9 (C-6, C-6', C-6"), 65.4, 46.9, 28.5 (C linker), 54.1 (C-2"), 22.7 (O-C$\underline{H}_3$).

*4.6.2 Synthesis of 3-aminopropyl (2-N-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (**75**)*

3-Azidopropyl (3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (177) - To a solution of 3-Azidopropyl (2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**169**) (542 mg, 0.59 mmol) in methanol (25 mL) was added potassium *tert*-butoxide (67 mg, 0.59 mmol) and the reaction mixture was stirred under argon for 18 h. The reaction mixture was neutralized using Dowex 50WH+, filtered and the solvent was removed under reduced pressure to afford **177** as a colorless oil that was

used without any further purification (517 mg, 100%). R_f (methylene chloride/acetone, 97/3, v/v)= 0.85; $[\alpha]_d$ = +27.3 (methylene chloride, c=9.0 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 300MHz): 7.52-7.18 (25H, m, arom H), 5.57 (1H, s, C<u>H</u> benzylidene), 5.31 (1H, s, H-1'), 4.88-4.49 (9H, m, H-1, C<u>H</u>$_2$ benzyls), 4.34-4.10 (4H, m, H-6a', H-3, H-2', H-4), 3.94-3.68 (9H, m, H-3', H-2, H-5, H-6, H-4', H-5', H-6b', O-C<u>H</u>$_2$ linker), 3.51-3.41 (1H, m, O-C<u>H</u>$_2$ linker), 3.34-3.24 (2H, m, CH_2-C<u>H</u>$_2$-CH_2 linker), 1.82-1.72 (2H, m, C<u>H</u>$_2$-N linker); δ_C ppm ($CDCl_3$, 75 MHz): 138.7, 138.5, 138.0, 137.9, 137.5 (arom Cq), 128.7-126.2 (arom C), 101.8 (<u>C</u>H benzylidene), 100.8, 99.4 (C-1, C-1'), 80.1, 79.2, 77.9, 74.6, 72.3, 68.4, 64.5 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 75.2, 73.8, 73.7, 69.4, 69.1 (C-6, C-6', <u>C</u>H_2 benzyls), 64.8, 48.6, 29.1 (C linker); m/z: calc= 873, found (Maldi-Tof)= 897 $[M+Na]^+$.

3-Azidopropyl (3,4,6-Tri-*O*-acetyl-2-deoxy-2-*N*-phtalimido-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside (178) - To a cooled (-20 ^oC) stirred solution of 3-Azidopropyl (3,4,6-Tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**177**) (484 mg, 0.37 mmol), Trichloroacetimidate 3,4,6-Tri-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (**151**) (260 mg, 0.45 mmol) and 4 Å molecular sieves (0.5 g) in methylene chloride was added TMSOTf (6 µl, 0.05 mmol) and the reaction mixture was stirred under argon for 1h. The solution was neutralized using Et_3N, filtered through celite and the solvent was removed under reduced pressure. The crude product was purified by flash silica gel column chromatography (hexane/ethyl acetate, 100/0, v/v, then, 90/10, 80/20, 70/30 and 50/50) to afford trisaccharide **178** as a

colorless oil (451 mg, 63%). R_f= 0.36 (hexane/ethyl acetate, 50/50, v/v); $[\alpha]_d$ = +47.4 (methylene chloride, c=8.9 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 500MHz): 7.92-6.94 (29H, m, arom H), 5.64-5.56 (2H, H-3", C<u>H</u> benzylidene), 5.13-5.00 (3H, m, H-1', H-1", H-4"), 4.84 (1H, d, J_{gem}= 11.2 Hz, C<u>H</u>$_2$ benzyls), 4.79 (1H, s, H-1), 4.64 (2H, 2d, J_{gem}= 11.3 Hz, J_{gem}= 12.3 Hz, C<u>H</u>$_2$ benzyls), 4.58 (1H, d, J_{gem}= 12.3 Hz, C<u>H</u>$_2$ benzyls), 4.47-4.30 (3H, m, H-2", C<u>H</u>$_2$ benzyls), 4.27-3.80 (12H, m, H-3, H-2', H-6", H-3', H-4, H-5, H-6, J_{gem}= 11.3 Hz, J_{gem}=11.2 Hz, C<u>H</u>$_2$ benzyls), 3.72-3.66 (2H, m, H-2, O-C<u>H</u>$_2$ linker), 3.62 (1H, m, H-5'), 3.56-3.33 (3H, m, H-4', H-6a', O-C<u>H</u>$_2$ linker), 3.27 (2H, m, CH_2-C<u>H</u>$_2$-CH_2 linker), 2.88 (1H, m, H-6b', 2.30 (1H, m, H-5"), 2.09, 2.00, 1.96 (9H, 3s, C<u>H</u>$_3$-C=O), 1.77 (2H, m, C<u>H</u>$_2$-N linker); δ_C ppm ($CDCl_3$, 500 MHz): 134.5-123.6 (arom C), 102.7 (<u>C</u>H benzylidene), 99.6 (C-1), 98.6 (C-1'), 95.8 (C-1"), 79.5, 77.7, 77.5, 74.9, 74.3, 72.6, 72.1, 71.2, 70.7, 69.3, 68.9 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-3", C-4", C-5"), 75,1, 73.8, 72.9, 70.7 (<u>C</u>H$_2$ benzyls), 70.5 (C-6'), 69.1 (C-6), 61.6 (C-6"), 64.8, 48.5, 28.7 (C linker), 54.3 (C-2"), 20.7, 20.5 (<u>C</u>H$_3$-C=O); m/z: calc= 1291, found (Maldi-Tof)= 1315 $[M+Na]^+$; Elemental analysis: Calc. C: 65.11, H: 5.78, Found: C: 65.30, H: 6.01.

3-Azidopropyl (2-*N*-Acetyl-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside (179) - To a solution of 3-Azidopropyl (3,4,6-Tri-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside (**178**) (402 mg, 0.31 mmol) in ethanol (8 mL) was added hydrazine monohydrate (0.74 mL, 15.5 mmol) and the reaction mixture

was refluxed for 18 h. The solvent was removed in vacuo and co-evaporation of the residue with toluene (5 × 10 mL) afforded the amino compound in quantitative yield. The amine was subsequently dissolved in a mixture pyridine/acetic anhydride (1/1, v/v, 10 mL) and the solution was stirred at room temperature for 4 h. The mixture was concentrated and co-evaporated with toluene (5×30 mL), ethanol (30 mL) and methylene chloride (30 mL). Purification of the crude product by silica gel column chromatography (methylene chloride/methanol, 99/1, v/v) gave trisaccharide **179** as a white foam (285 mg, 76%). R_f= 0.57 (methylene chloride/methanol, 95/5, v/v); $[\alpha]_d$ = +22.9 (methylene chloride, c=10.3 mg/mL, 25 ^oC); δ_H ppm ($CDCl_3$, 500MHz): 7.63-7.18 (24H, m, arom. H), 5,58 (1H, s, C<u>H</u> benzylidene), 5.17 (1H, s, H-1'), 5.06-4.99 (2H, m, $J_{3",2"}$=$J_{3",4"}$= 9.8 Hz, H-3", N<u>H</u>Ac), 4.95-4.88 (2H, m, H-5", J_{gem}= 12.1 Hz, C<u>H</u>$_2$ benzyls), 4.78 (1H, s, H-1), 4.74-4.44 (7H, m, C<u>H</u>$_2$ benzyls), 4.30-4.21 (2H, H-1", H-3), 4.19-4.11 (2H, m, H-6b, H-2'), 4.02 (1H, dd, $J_{6a",5"}$= 4.2 Hz, $J_{6a",6b"}$= 12.6 Hz, H-6a"), 3.97-3.62 (12H, m, H-6b", H-3', H-4', H-2, H-5', H-5, H-4, H-2", H-6a, H-6', O-C<u>H</u>$_2$ linker), 3.44 (1H, m, O-C<u>H</u>$_2$ linker), 3.30 (2H, m, CH_2-C<u>H</u>$_2$-CH_2 linker), 2.10, 2.05, 2.04 (9H, 3s, C<u>H</u>$_3$-C=O), 1.78 (2H, m, C<u>H</u>$_2$-N linker), 1.75 (3H, s, C<u>H</u>$_3$-C=O NHAc); δ_C ppm ($CDCl_3$, 500MHz): 135.5-126.8 (arom C), 102.4 (C<u>H</u> benzylidene), 99.2 (C-1), 98.5 (C-1"), 98.3 (C-1'), 79.2, 77.7, 77.4, 74.1, 73.8, 72.6, 72.1, 64.0 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 74.7, 73.4, 72.9, 70.9 (C<u>H</u>$_2$ benzyls), 71.8 (C-3"), 71.0 (C-4"), 69.9, 69.0, 61.5 (C-6, C-6', C-6"), 68.5 (C-5"), 64.5, 48.3, 28.3 (C linker), 54.6 (C-2"), 20.4, 20.1 (C<u>H</u>$_3$-C=O); m/z: calc= 1202, found (Maldi-Tof)= 1226 $[M+Na]^+$.

3-Aminopropyl (2-*N*-Acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside (75) - To a solution of 3-Azidopropyl (2-*N*-Acetyl-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-*O*-benzyl-α-D-mannopyranoside (**179**) (79 mg, 0.07 mmol) in methanol (3 mL) was added potassium carbonate (catalytic amount) and the mixture was stirred under argon for 18 h. The solution was neutralized using Dowex 200WH+ resin, then filtered and the solvent removed. The obtained deacetylated compound was dissolved in ethanol (3 mL) and AcOH (0.3 mL) and was hydrogenated (H_2, $Pd(OAc)_2$, catalytic amount) for 72 h. The mixture was filtered through celite and the solvent was removed in vacuo. Purification of the crude product by sephadex G15 column chromatography using water as eluent afforded the target trisaccharide **75** as a white solid (24 mg, 61%). R_f (methylene chloride/methanol, 70/30, v/v)= 0; δ_H ppm (D_2O, 500MHz): 5.10 (1H, s, H-1'), 4.72 (1H, s, H-1), 4.43 (1H, d, $J_{1",2"}$= 8.7 Hz, H-1"), 4.07 (1H, bs, H-2'), 3.96 (1H, bs, H-2), 3.85-3.76 (3H, m, H-3, H-3', H-6a"), 3.71-3.30 (16H, m, H-4, H-5, H-6, H-4', H-5', H-6', H-2", H-3", H-4", H-5", H-6b", O-C$\underline{H}_2$ linker), 2.73 (2H, m, $CH_2C\underline{H}_2CH_2$ linker), 1.93 (3H, s, C$\underline{H}_3$ acetyl), 1.09 (2H, m, C$\underline{H}_2$-N linker). δ_C ppm (D_2O, 500MHz): 100.1, 99.9, 99.8 (C-1, C-1', C-1", $J_{C1,H1}$= 172.4 Hz, $J_{C1',H1'}$= 172.5 Hz, $J_{C1",H1"}$= 162.1 Hz), 78.2, 76.5, 75.8, 72.4, 69.9, 68.9, 67.3, 65.8, 55.2 (C-2, C-3, C-4, C-5, C-2', C-3', C-4', C-5', C-2", C-3", C-4", C-5"), 73.9, 65.9, 61.8 (C-6, C-6', C-6"), 55.9, 48.3, 16.2 (C linker), 22.0 ($\underline{C}H_3$-C=O).

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APPENDIX

Synthesis and Conformational Analysis of a Conformationally Constrained Trisaccharide, and Complexation Properties with Concanavalin A

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Abstract: The trisaccharide β-D-GlcNAc(1 $\rightarrow$2)α-D-Man(1 $\rightarrow$3)D-Man is a fragment of a biantennary glycan that is recognized by α-D-Man-specific lectins such as concanavalin A (ConA), *Lathyrus ochrus* lectin, lentil lectin, and can adopt several conformations upon binding. To probe the importance of loss of flexibility of a saccharide during binding with ConA this trisaccharide has been synthesized in a conformationally constrained form where a methylene acetal bridge mimics a GlcNAc – O-6$''$ $\cdots$ Man – O-4 intramolecular hydrogen bond. Microcalorimetry measurements revealed that the conformationally constrained compound has a more favorable entropy term but this term is offset by a smaller enthalpy term. NMR spectroscopic studies have shown that the cyclic compound is indeed considerably less flexible than the linear compound and both compounds adopt mainly one conformation. SYBYL software together with energy parameters appropriate for carbohydrates was used for a systematic conformational search. The linear compound is very flexible. A clustering method determined seven main conformational families. Six possible conformational families were identified for the cyclic compound when considering the orientations of the βGlcNAc(1 $\rightarrow$2)Man and $\alpha\beta$Man-(1 $\rightarrow$3)Man glycosidic bonds. The central mannose residue was docked in the binding site of ConA and the complex was refined. The results are compared with crystal structures of legume lectin – oligosaccharide complexes and with the NMR and thermodynamic data.

Keywords: calorimetry · carbohydrates · lectins · molecular modeling · oligosaccharides

Introduction

Protein – carbohydrate interactions are implicated in many essential biological processes. Examples include embryogenesis, fertilization, neuronal development, hormonal activities, and cell proliferation and organization into specific tissues. These interactions are also important in health science, and are involved in the invasion and attachment of pathogens, inflammation, metastasis, blood group immunology, and xenotransplantation. Among protein – carbohydrate complexes those involving lectins are of considerable interest because of the high specificities of their interactions.

The molecular basis of lectin – carbohydrate interactions has been widely studied[1] but the thermodynamics of these interactions are complex and not well understood.[2] Consequently, the prediction of binding constants is still a difficult and unreliable process. While counterexamples exist, protein – carbohydrate association is typified by a favorable enthalpy term, which is offset by an unfavorable entropy contribution. It is widely accepted that the enthalpy term arises from a large number of hydrogen bonds and extensive van der Waals interactions. Furthermore, it has been proposed that the dynamic rearrangement of water may also contribute to the enthalpy of complexation. The entropy term has been considered to arise either from solvation effects[3] or from the loss of conformational flexibility of the carbohydrate ligand.[4]

The majority of oligosaccharides are flexible and in many cases a lectin binds a saccharide in a secondary rather than a global minimum conformation. For example, the βGlcNAc-

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(1→2)αMan(1→3)Man trisaccharide is known to adopt different conformations when crystallized in different crystal forms of *Lathyrus ochrus* lectin.[5] Another different conformation is observed when it is complexed with animal S-lectin.[6] It has been shown that many of these conformations do not correspond to the global lowest energy conformation of the uncomplexed trisaccharide but to secondary minima.[7] This trisaccharide has not been cocrystallized with ConA but has been the subject of a systematic conformational search when docked in the lectin-binding site.[8] It has been predicted that several conformations can be complexed in the lectin-binding site.

In order to study both conformational and thermodynamic aspects of protein–carbohydrate interactions, two cyclic trisaccharides have been designed (Figure 1). The design of

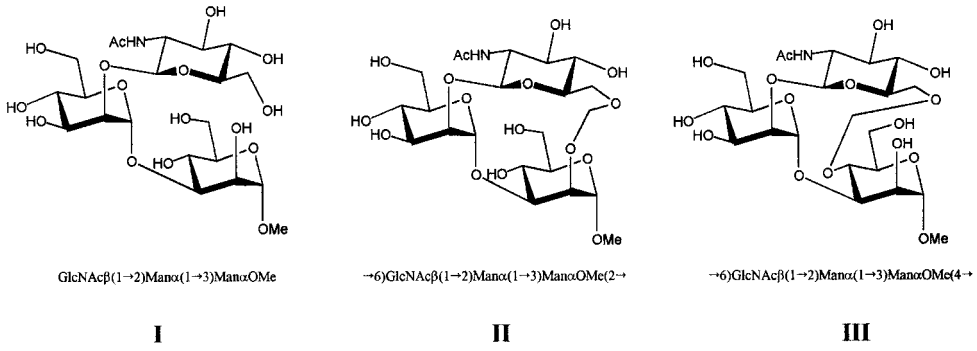


Figure 1. Schematic representation of the linear trisaccharide and the cyclic analogues.

trisaccharide **II**[9] is based on mimicking an intramolecular hydrogen bond O2···O6″ which is present in the crystalline lectin–oligosaccharide complex[5] by a methylene acetal. The O2···O6″ hydrogen bond is also present in a predicted docking mode of the trisaccharide to ConA.[8] Trisaccharide **II** has been recently synthesized[9] and its binding properties are currently under investigation.

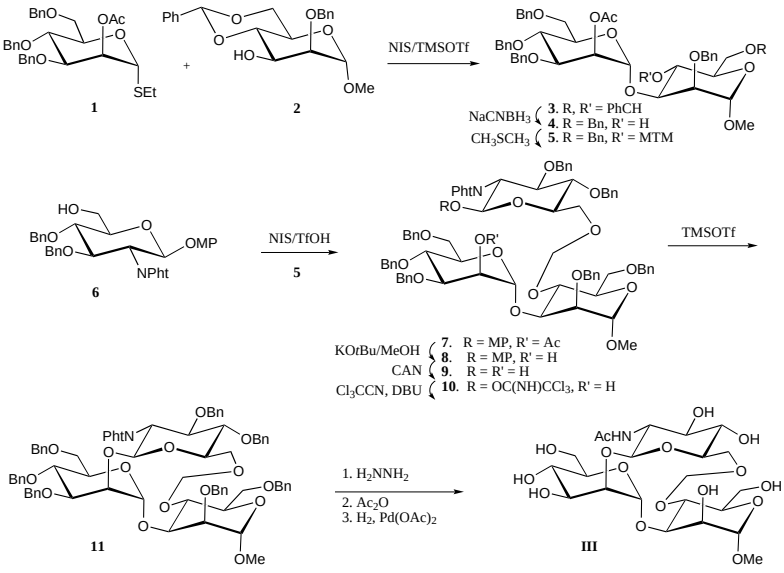
Here, we present the synthesis of trisaccharide **III**, which contains a methylene acetal that mimics an O-4···O-6″ intramolecular bond as was predicted to occur in another possible docking mode of βGlcNAc(1→2)αMan(1→3)Man with ConA.[8] Molecular modeling and NMR studies have demonstrated that the introduction of the methylene tether does not distort the conformation of the cyclic compound, although its flexibility is significantly reduced. On the basis of these results, it was anticipated that the conformationally constrained trisaccharide **III** would lose less conformational flexibility than the corresponding linear trisaccharide and therefore the entropic barrier associated with loss of flexibility of the cyclic oligosaccharide was expected to be smaller. Indeed, the measured thermodynamic data revealed a more favorable entropy of binding of compound **III**, which surprisingly was offset by a less favorable enthalpy term.

Results and Discussion

Synthesis of the cyclic trisaccharide:

Macrocyclization is a very challenging aspect in the preparation of the conformationally restricted trisaccharide **III**. A successful approach is outlined in Scheme 1. The cyclization was achieved by an intramolecular glycosylation utilizing the precursor **10**, which already possessed a methylene acetal linker. This approach was more fruitful than macrocyclization by methylene acetal formation.

The precursor **10** for the macrocyclization was assembled from the monomeric building blocks **1**,[10] **2**,[11] and **6**.[12] These compounds were readily available by standard carbohydrate protecting-group interconversion strategies. Thus, coupling of glycosyl donor **1** with glycosyl acceptor **2** in dichloromethane/ether in the presence of *N*-iodosuccinimide



Scheme 1. Synthesis of trisaccharide **III**.

(NIS)/catalytic trimethylsilyl triflate (TMSOTf)[13] afforded the α-linked disaccharide **3** in an excellent yield (96%). The benzylidene group of **3** was selectively opened by treatment with sodium cyanoborohydride, followed by HCl in ether[14] to give compound **4** (93%). The methylthiomethyl protecting group was introduced[15] with dimethyl sulfide and benzoyl peroxide in acetonitrile to yield compound **5** (87%). The methylene acetal functionality was introduced by coupling of

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the compounds **5** and **6** in the presence of NIS/triflic acid (TfOH), and the required saccharide **7** was obtained in a yield of 76%. Next, methylene-linked compound **7** had to be converted into a glycosyl donor suitable for an intramolecular glycosylation. To this end, the protecting group at the anomeric center of the 2-deoxy-2-phthalimidoglycosyl moiety of compound **7** had to be converted into a suitable leaving group and the 2-OH had to be deprotected. We selected an anomeric trichloroacetamido functionality as the anomeric leaving group.[16] Thus, cleavage of the acetyl group of **7** with potassium *tert*-butoxide in methanol gave **8** and the *p*-methoxyphenyl group (MP) of **8** was removed by reaction with cerium ammonium nitrate (CAN)[17] to afford **9**. Treatment of **9** with trichloroacetonitrile and DBU resulted in the formation of the trichloroacetimidate **10**. The regioselectivity of the latter reaction was achieved by virtue of the higher acidity of the anomeric hydroxyl group; however, care had to be taken to avoid bis(trichloroacetimidate) formation. In the following step, TMSOTf-mediated intramolecular glycosylation of **10** gave the macrocyclic compound **11** in a very good yield of 82%. Interestingly, a similar cyclization for the preparation of **II** resulted in a low yield of cyclic compound. Compound **11** was deprotected as follows: conversion of the phthalimido functionality into an NHAc moiety was accomplished by treatment with hydrazine monohydrate[18] followed by acetylation with acetic anhydride. The benzyl ether protecting groups were removed by catalytic hydrogenation over palladium acetate to afford the requisite saccharide **III**.

The structural integrity of compound **III** was confirmed by NMR spectroscopy and FAB mass spectrometry (594, $[M+Na]^+$). The ^1H and ^{13}C NMR signals were unambiguously assigned by two-dimensional homonuclear correlation spectroscopy (COSY, TOCSY). The assignments were aided by heteronuclear, proton–carbon chemical shift correlation experiments (δ (^{13}C) = 103.1 ($J_{C1,\,H1}$ = 173.5 Hz, C-1), 102.0 ($J_{C1',\,H1'}$ = 173.3 Hz, C-1′), 99.6 ($J_{C1'',\,H1''}$ = 166.4 Hz, C-1).

Molecular modeling of the uncomplexed trisaccharides:

A four-dimensional systematic conformational search performed on the Φ and Ψ torsion angles of the βGlcNAc-(1→2)αMan(1→3)Man trisaccharide **I** yielded 26712 conformations in a 10 kcal mol^{-1} energy window. A cluster analysis[8] gave seven families, and the lowest energy conformation of each of those families was fully optimized. The resulting geometries are described in Table 1. All seven conformations retained present very different geometries. The Φ and Ψ values of these conformers have been reported

Table 1. Low-energy conformations of linear trisaccharide **I** and structural characteristics (torsion angle values in ° and relative energy value in kcal mol^{-1}).

Family	Φ_{1-2}	Ψ_{1-2}	Φ_{1-3}	Ψ_{1-3}	ΔE
Linear_AA	– 50.7	– 171.7	68.7	99.4	0.0
Linear_AB	– 51.8	– 174.5	81.8	– 44.6	0.8
Linear_BC	44.4	143.7	164.6	174.5	1.6
Linear_BA	44.1	146.7	68.9	104.3	2.0
Linear_BB	46.4	149.5	92.5	– 39.9	2.5
Linear_BD	56.7	159.1	133.6	66.2	3.3
Linear_DC	– 104.0	71.4	152.9	160.1	3.6

on the energy maps of both linkages (Figure 2) which were calculated recently[19] with the MM3 program.[20] For both disaccharides, the main low-energy region has been labeled A, whereas the second low-energy region has been given the label B. Regions C and D correspond to remote parts of the main low-energy region that could be considered as secondary minima. The conformations in Table 1 have been identified by the orientation of each linkage: Linear_AB corresponds to a conformation with the βGlcNAc(1→2)Man linkage in conformation A and the αMan(1→3)Man linkage in conformation B of their respective energy map.

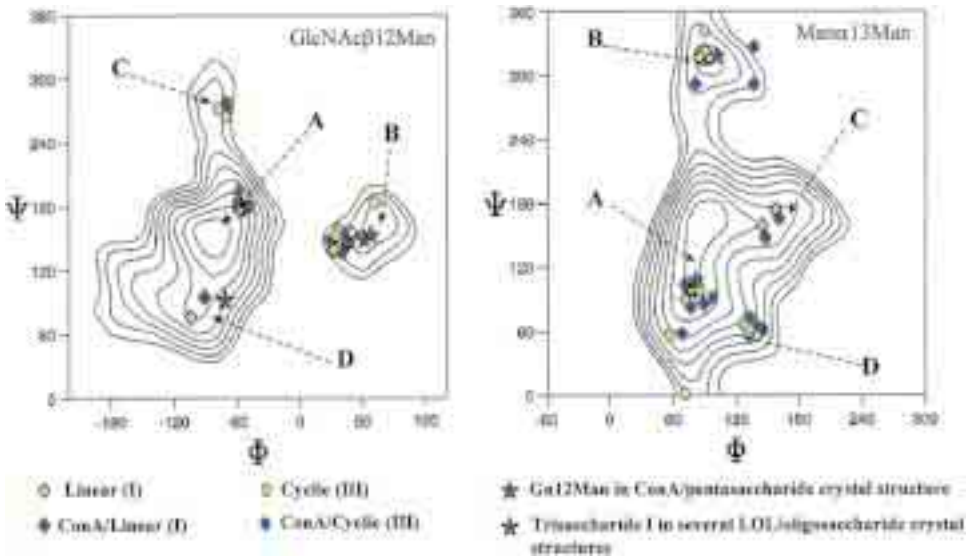

Figure 2. Superimposition of the conformations predicted for the isolated and ConA-bond trisaccharide **I** and **III** on the energy maps of each linkage, as calculated with the MM3 program.[19] The conformations observed in some crystal structures of lectin–oligosaccharide complexes are also depicted.

The lowest energy conformation, Linear_AA, corresponds to the one predicted by NMR and molecular modeling studies.[21] However, some of the other conformations are not much higher in energy. More particularly, the conformations Linear_BA and Linear_BB correspond to those observed in *N*-glycan fragments when cocrystallized with *Lathyrus ochrus* lectin.[5, 6] The occurrence of a conformation (B on each map) that greatly differs from the absolute minimum is a characteristic of oligosaccharide flexibility. The occurrence of a small percentage of these *anti* conformers has also been demonstrated by high-resolution nuclear magnetic resonance spectroscopy (NMR). For the α-linkage, the secondary minimum corresponds to the *gauche* orientation of the Ψ angle, which has been proven to exist in solution in mannobiose[22] and

maltose.[23] For the β-linkages, the secondary minimum is the *gauche* conformation about the ψ angle that has been referred to as the *gauche*–*gauche* or alternate *exo*-anomeric effect. Independent NMR studies on the $\beta(1\rightarrow3)$ linkage,[24] the $\beta(1\rightarrow2)$ linkage,[25] and the *C*-analogue of the $\beta(1\rightarrow4)$ linkage[26] in solution demonstrated the existence of the alternate conformation in addition to the usual *syn* conformer.

A systematic conformational search was run on nine of the torsion angles of the 13-membered-ring cyclic trisaccharide **III**. The Φ and Ψ torsion angles of both glycosidic linkages as well as five torsion angles of the methylene acetal bridge were rotated, resulting in 1203 conformations. After partial optimization and rejection of conformers with incorrect stereochemistry or van der Waals conflicts, 792 conformations were considered in an energy window of 10 kcal mol^{-1}. As a result of convergence during the last step of geometry optimization, only 23 conformations presented different geometries in terms of the 13-membered-ring shape and were stored. Since the acetal bridge appears to be the most flexible part of this macrocycle, the 23 conformations can be classified in six families, based on the conformation of the two glycosidic linkages (Table 2).

The conformations, grouped in six families, are displayed in Figure 3. Despite the cyclization constraints, both linkages can adopt several very different conformations in an energy window of 10 kcal mol^{-1}. For each family, the methylene acetal bridge moves freely. In order to evaluate the influence of cyclization on the conformational behavior of the glycosidic linkages, the six conformations have been reported on the energy maps of both linkages (Figure 2). From this comparison it can be seen that the cyclization does not force the linkages into high-energy regions: all the calculated conformations are encountered by the most external isoenergy contour (8 kcal mol^{-1}). The trisaccharide is still rather flexible since many areas of the energy maps are spanned by the calculated conformations. However, there is a significant reduction of flexibility when compared with the linear trisaccharide, and, for example, the AB conformation cannot be adopted by the constrained compound.

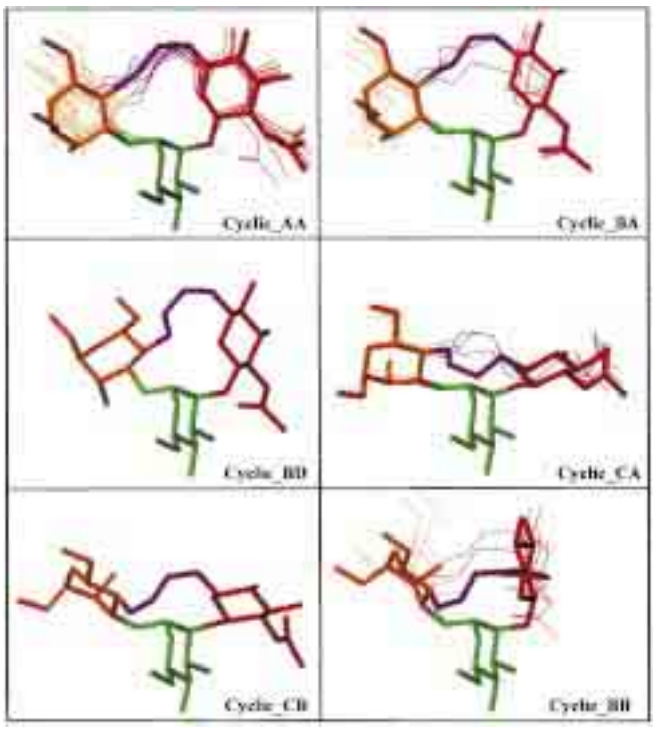

Figure 3. Graphical representation of the 23 low-energy conformers of compound **III** distributed in six families, based on the conformations of the glycosidic linkages. The lowest energy conformation of each family is represented by sticks. Color-coding is as follows: red for the βGlcNAc, green for the central αMan, and yellow for the αManOMe; the tether is colored violet.

Table 2. Low-energy conformations of cyclic trisaccharide **III**, and their classification in conformational families based on the conformation at the two glycosidic linkages (torsion angle values in ° and relative energy value in kcal mol^{-1}).

Family	Φ_{1-2}	Ψ_{1-2}	Φ_{1-3}	Ψ_{1-3}	ω_1	ω_2	ω_3	ω_4	ω_5	ΔE
Cyclic_AA	−44.4	−169.4	75.9	89.3	76.1	−74.5	128.6	159.6	−118.7	0.0
	−43.3	−168.8	62.9	84.5	56.9	80.7	−117.8	−85.9	−95.8	1.3
	−49.4	−163.7	77.8	117.5	42.3	69.1	−161.9	166.3	57.7	2.6
	−38.4	−170.7	77.5	146.1	61.0	−94.5	−162.6	−72.4	122.5	3.9
	−37.6	175.7	67.6	138.2	73.2	−44.1	−176.6	179.9	154.9	4.0
	−29.1	−172.1	97.1	86.3	−42.2	76.2	24.2	155.0	−103.8	4.2
	−47.7	−163.3	123.2	101.8	47.3	−97.5	142.5	51.8	40.2	4.8
	−38.7	−166.1	122.8	81.7	−42.6	112.2	−50.3	175.5	−37.1	8.2
	−38.6	−156.6	143.4	60.6	−44.5	107.2	−88.5	173.7	40.1	8.4
Cyclic_BA	43.0	150.4	74.0	95.3	−71.7	70.6	57.8	156.0	−129.0	0.3
	47.5	150.2	79.2	113.4	−59.7	107.0	−145.7	177.7	56.5	2.2
	66.3	−172.3	57.6	75.7	−77.2	85.8	−126.3	−168.5	64.4	3.4
	45.8	142.1	70.1	134.9	−60.6	68.2	151.1	−176.9	134.3	3.6
	45.7	147.8	68.0	93.9	−60.1	144.0	−78.6	−125.2	−89.5	3.8
Cyclic_BD	52.0	148.1	140.5	55.0	−65.6	73.4	−95.4	−144.7	66.0	2.7
Cyclic_CA	−59.6	−86.5	54.2	53.1	69.7	−131.5	57.6	142.5	52.7	3.3
	−57.0	−83.0	52.8	46.4	−56.7	76.6	40.9	165.7	−102.7	4.4
	−51.6	−77.2	51.9	63.7	−45.3	120.3	−132.8	166.7	57.7	6.4
	−54.7	−84.5	52.0	48.0	−45.4	139.5	−58.2	−134.7	−80.0	7.3
Cyclic_CB	−68.0	−96.2	74.0	1.5	49.3	75.4	−452.8	−23.9	−98.1	5.6
Cyclic_BB	69.0	−167.0	87.4	−16.3	−63.7	112.9	−148.7	−2.3	−99.2	5.8
	68.6	179.2	124.5	−30.8	−66.0	101.5	−160.9	38.9	−118.9	6.2
	65.1	161.1	141.5	−26.7	−58.5	101.1	−153.7	32.4	−107.8	6.4

When comparing the conformational behavior of the linear and cyclic trisaccharides, the global minimum conformation of each oligosaccharide appears to be almost identical. However, the secondary minima do not appear in the same order.

Conformational analysis by NMR spectroscopy:

The ^{1}H NMR and ^{13}C NMR spectra were completely assigned by a combination of homonuclear COSY, TOCSY, and heteronuclear HMQC, HMBC, and HMQC-TOCSY techniques. The latter two techniques were crucial to resolve the final ambiguities. The corresponding ^{1}H and ^{13}C NMR chemical shifts of compounds **I** and **III** are listed in Table 3.

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Table 3. 500 MHz ^{1}H and ^{13}C NMR chemical shifts for trisaccharides **I** and **III** at 303 K.

Proton	Trisaccharide **I**	Trisaccharide **III**
GlcNAc H-1	4.52/101.7	4.92/99.6
GlcNAc H-2	3.68/57.8	3.95/56.6
GlcNAc H-3	3.53/75.8	3.61/76.4
GlcNAc H-4	3.49/72.2	3.53/71.6
GlcNAc H-5	3.47/78.3	3.71/78.7
GlcNAc H-6A	3.92/63.0	4.10/70.4
GlcNAc H-6B	3.79/63.0	4.02/70.4
Manα(1→3) H-1	5.12/100.3	5.68/102.0
Manα(1→3) H-2	4.16/78.7	4.38/75.0
Manα(1→3) H-3	3.89/71.9	4.01/71.8
Manα(1→3) H-4	3.52/69.6	3.71/69.3
Manα(1→3) H-5	3.69/76.0	3.72/75.7
Manα(1→3) H-6A	3.91/63.6	3.97/63.4
Manα(1→3) H-6B	3.64/63.6	3.86/63.4
ManαOMe H-1	4.72/102.2	4.82/103.1
ManαOMe H-2	4.06/72.0	4.10/72.5
ManαOMe H-3	3.85/80.5	4.11/77.7
ManαOMe H-4	3.75/68.2	3.84/76.9
ManαOMe H-5	3.65/75.0	3.77/74.4
ManαOMe H-6A	3.95/63.0	3.95/62.7
ManαOMe H-6B	3.78/63.0	3.84/62.7
pro-R CH_2	–	5.17/100.4
pro-S CH_2	–	5.03/100.4

The existence of molecular motion around the glycosidic linkages of oligosaccharides has now been firmly established.[27–29] In addition, recent investigations have revealed that the rates of overall and internal motions of small and medium-size oligosaccharides may occur on similar time scales.[30] Since NMR parameters are essentially time-averaged, the information that can be deduced from NOE experiments corresponds to a time-averaged conformation in solution.

For both trisaccharides, the pyranoid rings can be described as essentially monoconformational $^4C_1$, as deduced from the proton–proton coupling patterns and intraresidue NOE data. Small couplings are observed for both Man anomeric protons (<2 Hz) and H-2 Manα(1→3) (<4 Hz), while an 8.4 Hz coupling is measured for the GlcNAc analogue. In fact, for the GlcNAc residue all proton chemical shifts (H-1→H-6$_{(S,R)}$) can be obtained through a TOCSY experiment as expected for an all-axial conformation of the ring protons. In addition, medium-strong H-1–H-3 and H-1–H-5 cross-peaks support the usual chair conformation. For both Man residues, the TOCSY transfer from H-1 stops at H-2 (two small consecutive $J_{H1,H2}$ and $J_{H2,H3}$), although all cross-peaks H-2→H-6$_{(S,R)}$ can be deduced from H-2 Manα(1→3), and H-2→H-5 connectivities from H-2 ManαOMe, as expected for the Man residues adopting the $^4C_1$ conformation. In addition, medium-strong H-1–H-2 cross-peaks are observed for both Man anomeric protons. The NOESY and ROESY experiments were used to estimate proton–proton interresidue distances qualitatively.[31] NOESY cross-peaks are positive at 299 K and 500 MHz. (Figures 4 and 5) ^{1}H NMR cross-relaxation rates[31, 32] (σ_{ROESY} and σ_{NOESY}) were obtained from the 2D-NOESY and 2D-ROESY experiments. $\sigma_{ROESY}/\sigma_{NOESY}$ ratios[33] are independent of interproton distances and allow us to estimate specific correlation times and therefore interproton distances (see Experimental Section). For both compounds, overall correlation times around 0.2–0.3 ns were obtained. This fact indicates that both molecules tumble almost isotropically in solution and that the isolated-spin-pair approximation (ISPA) can be safely applied to deduce proton–proton distances. In fact, the results for the distances given in Tables 4–6 are very similar to those estimated by the ISPA method. The corresponding H-1–H-2 intraresidue signals were used as reference (2.4 Å) for the α-linkages, and the H-1–H-3 and H-1–H-5 intensities for the unique β-linkage. The distances calculated by molecular mechanics are also shown in Tables 4–6.

For the acyclic trisaccharide **I**, the experimentally deduced NMR distances arise from different conformational isomers (Table 4). Indeed, the observed NOEs for the Manα(1→3)Man linkage (weak NOE for Manα(1→3) H-1–

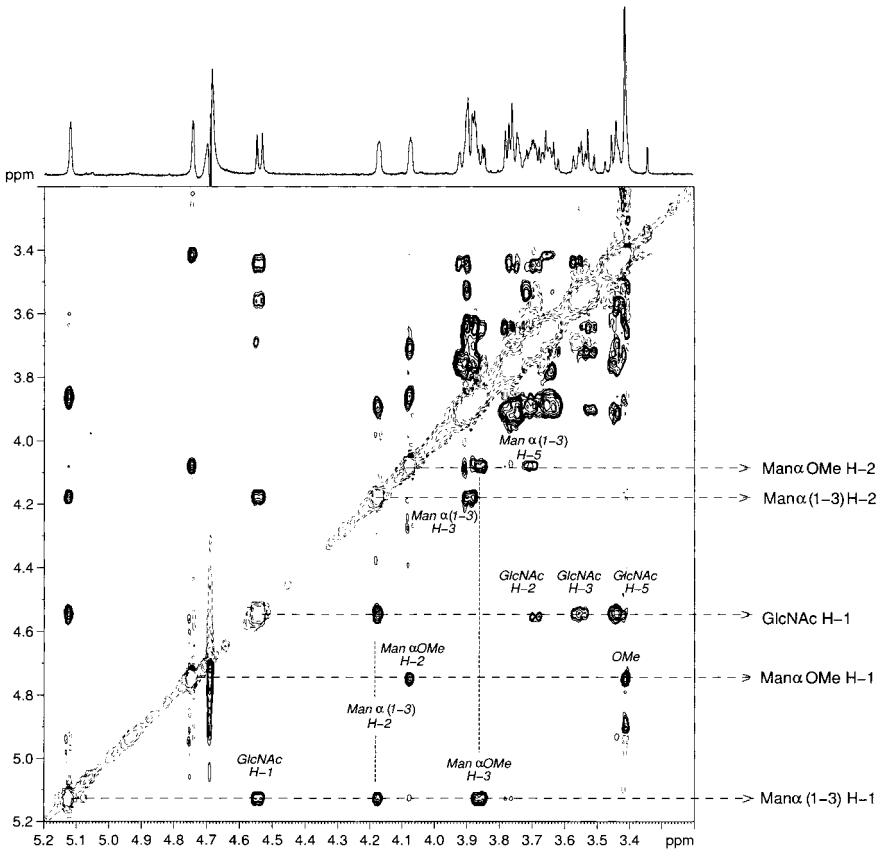

Figure 4. NOE spectrum of compound **I**.

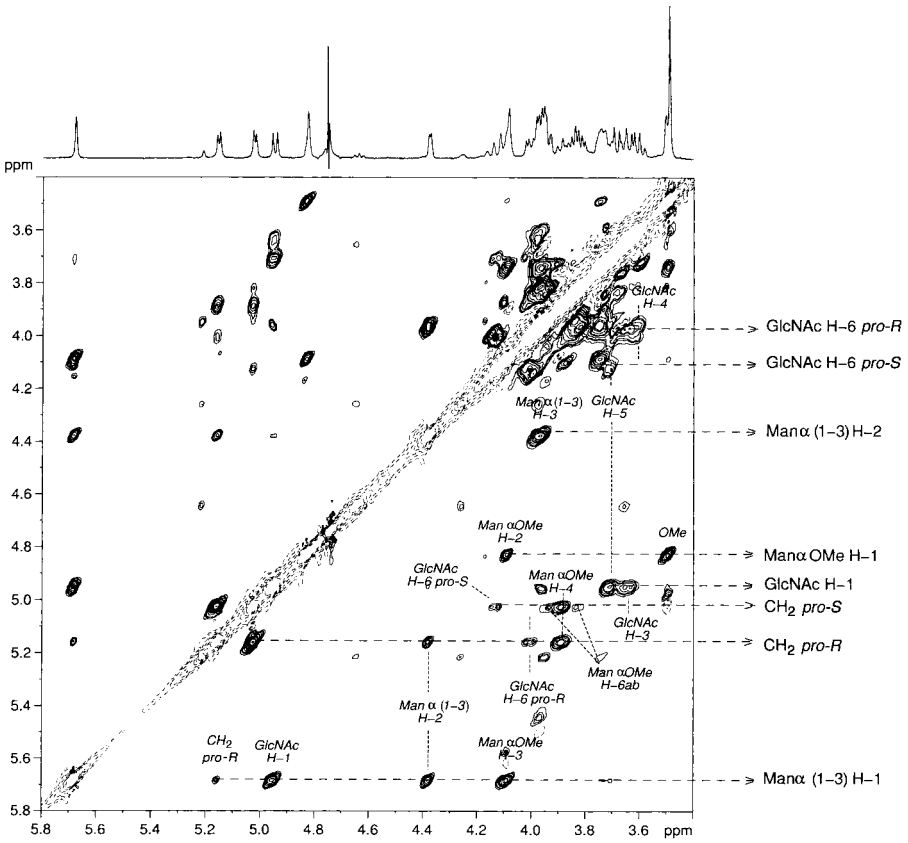

Figure 5. NOE spectrum of compound **III**.

Table 4. Experimental and calculated proton – proton distances for trisaccharide **I**. The experimental distances have been calculated from the NOE and ROE cross-relaxation rates as described in the experimental part. The calculated distances correspond to the representative structure for every local minimum. Estimated errors in the experimental distances are smaller than 5%.

H – H Pair	GlcNAcβ(1 →2)Manα linkage Conformer				Manα(1 →3)ManαOMe linkage Conformer					
Exp	A	B	C	D	Exp	A	B	C	D	
1 – 1	2.4	2.1	3.6		4.1	nd[a]	6.0	4.7	4.5	5.9
1 – 2	2.6	3.3	3.6		2.3	3.3	4.3	3.5	2.1	4.2
1 – 3	nd	4.8	5.3		3.8	2.2	2.4	3.6	2.9	2.4
1 – 4	nd	3.9	4.2		3.8	3.5	3.7	2.3	4.3	3.5
2 – 1	ov[b]	4.7	4.2		4.3	nd	7.1	6.0	5.3	6.2
2 – 2	nd	4.1	2.2		4.2	nd	5.2	3.9	3.3	5.0
5 – 2	nd	4.2	4.6		4.5	2.7	2.4	5.1	5.0	3.9

[a] nd = not determined. [b] ov = overlapping signal.

ManαOMe H-2, strong NOE for Manα(1 →3) H-1 – ManαOMe H-3, and medium NOE for Manα(1 →3) H-5 – ManαOMe H-2) are basically identical to those observed for the disaccharide indicating that the GlcNAc residue does not have a significant effect on the conformational behavior of this linkage. The conformation around this glycosidic linkage may be described as a conformational equilibrium around conformer A, with minor excursions towards the regions defined by conformers C and D, and in all cases the values of the Φ and Ψ angles are positive. The NOE data obtained for the GlcNAcβ(1 →2)Man linkage unequivocally indicate that the conformation of this linkage can be described by a conformational equilibrium between conformers A and D, since both GlcNAcβ H-1 – Manα H-1 and GlcNAcβ H-1 – Manα H-2 NOE contacts are fairly strong. Since no GlcNAcβ H-2 – Manα H-2 NOE is observed, conformer B is less than 5% present in solution at this linkage, within experimental error. In addition, no conformer can simultaneously satisfy the corresponding two close distances, and therefore the presence of conformational averaging between conformers A and D is firmly confirmed.

The observed NOEs for cyclic compound **III** (Tables 5 and 6) are fairly distinct for the GlcNAcβ(1 →2)Man linkage and now the interresidue GlcNAcβ H-1 – Manα H-2 distance is appreciably larger than the corresponding GlcNAcβ H-1 – Manα H-1, as can be deduced from the relative intensities of the NOEs. Again, no GlcNAcβ H-2 – Manα H-2 is observed; this indicates that conformer B does not participate in the conformational equilibrium. According to these results, the cyclization rigidifies the GlcNAcβ(1 →2)Man linkage and conformer A is basically the only one in solution. On the other hand, the NOEs for the Manα(1 →3)Man linkage are identical to the linear compound. Although there is overlapping between

Table 5. Experimental and calculated proton – proton distances for trisaccharide **III**.

H – H Pair	GlcNAcβ(1 →2)Manα linkage Conformer			Manα(1 →3)Manα linkage Conformer			
Exp	AA	BA	CA, CB	Exp	AA	BB	BD	
1 – 1	2.2	2.1	3.6	3.3	nd[a]	6.0	4.7	5.9
1 – 2	3.2	3.3	3.6	3.6	ov[b]	4.3	3.5	4.2
1 – 3	nd	4.8	5.3	3.6	2.2	2.4	3.6	2.4
1 – 4	nd	3.9	4.2	2.0	3.3	3.7	2.3	3.5
2 – 1	nd	4.7	4.2	4.6	nd	7.1	6.0	6.2
2 – 2	nd	4.1	2.2	4.5	nd	5.2	3.9	5.0
5 – 2	nd	4.2	4.6	5.4	ov	2.4	5.1	3.9

[a] nd = not determined. [b] ov = overlapping signal.

Table 6. Experimental and calculated proton – proton distances for the bridge methylene protons of trisaccharide **III**.

Pair	Exp	AA	BA	BB	BD	CA	CB
pro-R CH_2 – Manα(1 →3) H-1	2.8	2.6	2.6	4.2	3.4	4.2	4.2
pro-R CH_2 – Manα(1 →3) H-2	2.6	2.5	2.3	6.6	4.5	6.6	6.6
pro-R CH_2 – GlcNAcβ H-6*S*	3.2	3.6	3.1	3.4	4.0	3.1	2.7
pro-R CH_2 – GlcNAcβ H-6*R*	2.9	2.5	3.8	2.8	3.3	2.7	3.7
pro-R CH_2 – ManαOMe H-4	2.7	2.7	2.8	3.9	3.6	3.8	3.2
pro-S CH_2 – GlcNAcβ H-6S	2.7	3.8	3.7	3.4	3.5	3.9	2.6
pro-S CH_2 – GlcNAcβ H-6R	2.9	3.1	4.1	2.2	2.2	3.6	3.6
pro-S CH_2 – ManOMe H-6AB	3.3	2.6	3.0	3.8	4.1	3.4	3.7
pro-S CH_2 – ManαOMe H-4	2.8	2.3	2.1	3.3	3.9	3.6	3.5

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ManαOMe H-3 and H-2 protons, the NOE cross-peak intensity between these resonances and Manα(1→3)Man H-1 can be ascribed exclusively to the ManαOMe H-3–Manα(1→3)Man H-1 cross-peak. In fact, according to the modeling results, and despite the extensive search performed (see below), no conformer with a ManαOMe H-2–Manα(1→3)Man H-1 distance below 3.82 Å could be detected. This is due to the fact that conformation C around this glycosidic linkage cannot be attained because of the cyclization. Therefore, the presence of the cycle poses a major constraint around the GlcNAcβ(1→2)Manα linkage and now the experimental NOEs of this compound can be described by conformer AA. In the absence of *J* coupling data, the assignment of diastereotopic protons heavily relies on the presence of a major conformation.[31] Thus, by considering the geometries (not the relative energies) deduced from the modeling studies, the diastereotopic methylene bridge protons could also be assigned. As mentioned above, cyclization of the trisaccharide highly restricts the GlcNAcβ(1→2)Manα linkage. Several NOEs were observed for the methylene bridge protons. Particularly important were those between the low-field proton with both Manα(1→3) H-1 and H-2. Again, and according to the modeling results, for all the found conformers, only the *pro-R* proton may show short distances with these two protons at the Man residue, thus providing the key for diastereotopic assignment. Additional NOEs are also observed between both methylene bridge protons with ManαOMe H-4, both GlcNAc and ManαOMe H-6s, and ManαOMe H-5. The pattern of the observed NOEs between both methylene bridge protons and the GlcNAc H-6s (strong *pro-R*-high field, weak *pro-R*-low field, strong *pro-S*-low field, weak *pro-S*-high field) seems to indicate that the low-field GlcNAc H-6 proton is indeed the *pro-S* proton. This assignment is also supported by observed intraresidue NOEs between the methylene GlcNAc H-6s with H-4 and H-5, that is medium/strong high field GlcNAc H-6–H-4, medium/strong low-field GlcNAc H-6–H-5, medium/weak high field GlcNAc H-6–H-5, weak low-field GlcNAc H-6–H-4. This is the expected NOE pattern for the usual gg/gt equilibrium around the GlcNAc C-5–C-6 bond.[34] The $J_{H5,\,H6S}$ and $J_{H5,\,H6R}$ of this residue are 2.0 and 4.9 Hz and therefore the experimental equilibrium is approximately gg:gt 60:40.[34]

The simultaneous existence of all the aforementioned NOEs can only be explained by the presence of mobility in this region of the molecule. Therefore, a major conformer AA may explain the conformational behavior of this trisaccharide, with local flexibility around the C-6-O-CH_2-O region of the macrocycle. Although only qualitative, the geometry of this conformer may explain the unusual chemical shift observed for H-1 and H-2 of the Manα(1→3) residue. These two atoms are in close proximity to O-4 ManαOMe (2.3 Å) and O-5 GlcNAc (2.5 Å). In addition, the relative deshielding of the *pro-R* methylene bridge proton with respect to the *pro-S* analogue could also be explained by its proximity to O-5 GlcNAc (2.4 Å) in the AA conformation.

The modeling results of the uncomplexed molecules are in good agreement with the experimental results, because the calculations (see above) predict an equilibrium in which conformer A is the major one for the GlcNAcβ(1→2)Man linkage, while an equilibrium between conformers A and D was predicted. The calculations for compound **III** predict a major conformer AA with variation around the angles defining the methylene orientation. These predictions are consistent with the experimental data, and the only discrepancy is the absence of conformer BA, which according to the calculations should be present to some extent.

Molecular modeling of the complexes between ConA and the trisaccharides:

All conformational families determined for the linear compound **I** were docked in the ConA binding site. All of the seven tested conformations could be accommodated in the binding site with no major changes in their geometries (Table 7), except for the Linear_BD conformation, which gave a much higher energy complex than the others. The complex with the lowest energy is ConA–Linear_BA. The preference of conformation B (the one that does not correspond to the *exo*-anomeric effect) for the βGlcNAc(1→2)Man linkage is marked, since this orientation is predicted to occur in the three lowest energy docking modes. This result is in agreement with previous calculations performed on the same complex by a different approach.[8]

Table 7. Low-energy conformations of linear trisaccharide **I** and cyclic trisaccharide **III** when docked in the binding site of ConA. Hb indicates the number of hydrogen bonds between the ligand and the protein. ΔE_{tot} (kcal mol^{-1}) is the difference between the energy of the complex and the energy of the noninteracting protein and trisaccharide, whereas ΔE_{int} is the interaction energy between protein and ligand.

Conformation	Φ_{1-2}	Ψ_{1-2}	Φ_{1-3}	Ψ_{1-3}	Hb	ΔE_{tot}	ΔE_{int}
ConA–Linear_BA	52.2	151.7	71.0	102.3	8	–37.9	–45.9
ConA–Linear_BC	51.5	150.5	161.7	166.1	11	–35.4	–43.7
ConA–Linear_BB	54.3	146.5	139.3	–69.8	8	–35.0	–43.2
ConA–Linear_AA	–55.8	–166.6	89.9	87.2	6	–31.5	–38.2
ConA–Linear_AB	–52.6	–158.2	83.9	–68.6	6	–31.1	–39.2
ConA–Linear_DC	–82.8	99.5	152.6	145.7	11	–26.4	–42.3
ConA–Cyclic_BA	48.8	148.9	70.7	88.9	9	–39.2	–45.6
ConA–Cyclic_BD	52.9	149.1	141.3	55.7	10	–36.3	–42.3
ConA–Cyclic_AA	–55.9	–154.7	100.5	90.1	7	–33.4	–41.2
ConA–Cyclic_BB	57.3	147.1	156.6	–20.2	10	–31.0	–40.9
ConA–Cyclic_CA	–62.1	–83.1	60.3	61.3	11	–27.5	–47.6
ConA–Cyclic_CD	–42.6	–156.8	128.4	71.6	8	–24.2	–38.3

The conformation of this linkage also affects the orientation of the central mannose in the binding site. When conformation B is adopted, the mannose is very deep in the binding site and all the hydrogen bonds have the characteristics observed in the ConA–αMan complex.[35] When conformation A is taken, the mannose is not so deeply docked, probably because of steric interactions of the GlcNAc with the protein surface, and some hydrogen bonds are weaker. The GlcNAc residue also establishes hydrogen bonds with the peptide, but only if linkage β(1→2) is not in conformation A. The third predicted conformation for the βGlcNAc(1→2)Man is termed D. In this intermediate case, the mannose is not very deep in the binding site, but many hydrogen bonds still exist, and furthermore, the GlcNAc presents some additional hydrogen bonds with the Thr^{226} side chain and Gly^{224} backbone. It has to be noted that this peculiar conformation is the one observed for the

βGlcNAc(1→2)Man disaccharide in the crystal structure of ConA–pentasaccharide complex.[36]

All of the 23 low-energy conformations of the cyclic trisaccharide **III** were considered for the docking study with ConA. After the central mannose residue was fitted in the binding site and the energy of the complexes was optimized with appropriate energy parameters,[37] 20 different conformers were stored in an energy window of 15 kcal mol^{-1}. The other conformers were rejected either because of their high energy or because their geometry converged close to one already considered. The conformers have been clustered in six families based on the conformation at the βGlcNAc(1→2)Man and αMan(1→3)Man linkages (Table 7). All conformations are displayed in Figure 6. When docked in the ConA binding site, the trisaccharide can in principle still adopt several different conformations. However, as for the linear saccharide, the B conformation of the βGlcNAc(1→2)Man linkage is energetically favored in the ConA complex state.

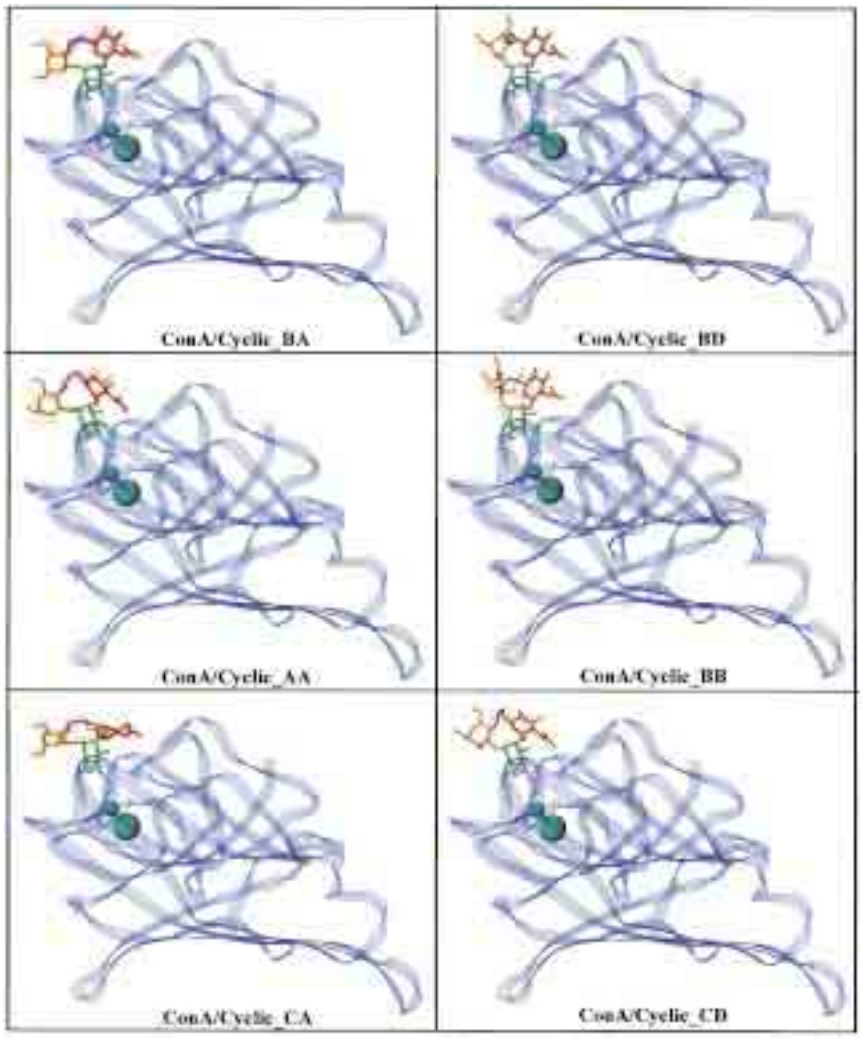

Figure 6. Graphical representation of the 20 conformers of cyclic trisaccharide **III** when interacting with ConA, distributed in six docking modes, based on the glycosidic linkage conformation. The protein is depicted by a ribbon-type representation.

Complexation studies of the saccharides with ConA:

Thermodynamic data obtained by isothermal microcalorimetry for complexation of the disaccharide αMan-(1→3)αManOMe, the trisaccharide **I** and **III** with ConA are listed in Table 8 together with literature data for related compounds. Although the entropy and enthalpy contributions of particular interactions vary between reporting authors, some clear tendencies can be identified. First, there is a limited variation of free energy of binding between mono-, di-, and trisaccharides, a phenomenon that is attributed to entropy/enthalpy compensation. Second, binding of αMan(1→3)Man-containing fragments has a more favorable enthalpy but a less favorable entropy contribution than its βGlcNAc(1→2)Man counterpart. The linear trisaccharide **I** has a slightly more favorable energy of binding than both disaccharide constituents do. Nevertheless, the thermodynamic characteristics of the linear oligosaccharide are close to those of the βGlcNAc(1→2)Man disaccharide.

Table 8. Thermodynamic data for the binding of trisaccharide **I** and **III** with ConA compared with literature data.

Compound	ΔG [kcal mol^{-1}]	ΔH [kcal mol^{-1}]	$T\Delta S$ [kcal mol^{-1}]	Ref.
Man	−4.4	−5.7	−1.3	[45]
αManOMe	−5.3	−6.6	−1.3	[46]
	−5.3	−6.8	−1.6	[45]
	−5.3	−8.2	−2.9	[47]
	−5.3	−6.8	−1.5	[48]
βGlcNAc(1→2)Man	−5.2	−5.3	−0.1	[46]
αMan(1→3)Man	−5.7	−10.2	−4.5	[47]
αMan(1→3)αManOMe	−5.7	−7.8	−2.0	[46]
	−6.2	−10.7	−4.5	[47]
	−6.0	−7.4	−1.4	[47]
	−5.9	−9.5	−3.6	Present work
βGlcNAc(1→2)αMan(1→3)αManOMe (**I**)	−6.1	−4.6	1.5	Present work
4→6, cyclic trisaccharide (**III**)	−5.8	−2.7	3.1	Present work

It was anticipated that the conformationally constrained trisaccharide **III** can adopt a conformation required for

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binding with the lectin ConA but will lose less conformational flexibility than the corresponding linear trisaccharide. Therefore, the entropic barrier associated with loss of flexibility of the cyclic oligosaccharide was expected to be smaller and should thus result in more favored binding. Indeed, the measured thermodynamic data revealed more favorable entropy of binding of compound **III**, which surprisingly is offset by a less favorable enthalpy term.

The conformational studies have shown that the introduction of the methylene acetal tether does not distort the conformation of compound **III**, but only reduces its conformational space as intended. Furthermore, the docking studies indicate that both compounds complex in similar secondary minimum energy conformations (BA) and have similar interactions with the protein surface (Figure 7).

Recently, Bundle et al.[38] reported complexation studies of preorganized branched trisaccharides with a monoclonal antibody. They found that the introduction of conformational constraints had little effect on the thermodynamic parameters of complexation and their data suggested that interresidue flexibility is not a major contributor to the weak association that characterizes oligosacharide–protein interactions. They also proposed that the absence of large impacts on ΔH and ΔS implies that oligosaccharides display a restricted range of conformations.

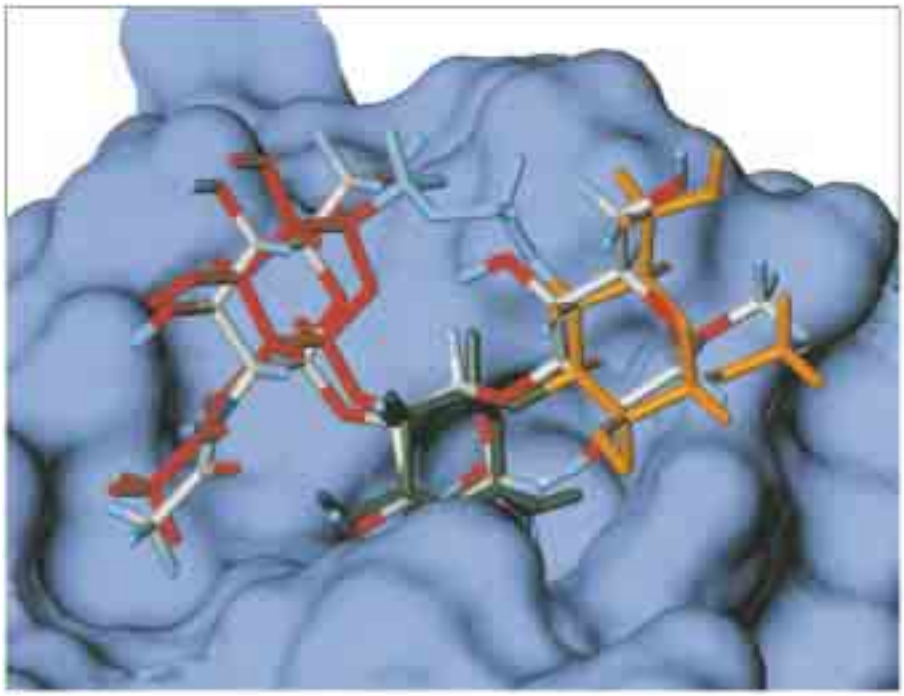

Figure 7. Comparison of the lowest energy complexes of ConA and both the linear trisaccharide **I** and the cyclic trisaccharide **III**. The solvent-accessible surface of the protein has been represented with the MOLCAD option in the SYBYL package. The linear trisaccharide **I** is colored by atom type whereas the cyclic trisaccharide **III** is colored by residue.

The data presented in this paper show that loss of flexibility contributes significantly to the entropy of binding. Recently, Quiocho and co-workers[39] made a similar observation for the binding of linear and cyclic oligosaccharides to the maltodextrin-binding protein of *Escherichia coli*.

It is important to speculate about the origin of loss of enthalpy of binding since it may provide opportunities to design high-affinity carbohydrate ligands.[40] The NMR studies demonstrated that in solution both compounds populate the BA binding conformer less than 5%. Thus, upon binding a considerable unfavorable enthalpy term is introduced for this conformational change and this term may well be larger for the conformationally constrained compound explaining the less favorable enthalpy of binding. We attempted to confirm the predicted BA bound conformation of the saccharides by measuring TR-NOE's. However, despite major efforts, no TR-NOE's could be measured and the failure of these experiments is probably due to the time scale of complex dissociation and/or to the presence of a paramagnetic Mn^{2+} ion close to the sugar-binding site.

It is important to note that the calculations do not take into account enthalpy effects arising from water rearrangement and the assumption was made that the bulk solvent should have the same enthalpic effect on the complexes involving the linear and cyclic trisaccharides. However, we may speculate that the desolvation enthalpy of the cyclic compound is significantly higher than for the linear compound.

Conclusion

The synthesis of a cyclic conformationally constrained trisaccharide is desribed, the design of which is based on mimicking an internal hydrogen bond which is present in a docking mode of a ConA–saccharide complex.

NMR spectroscopic and molecular modeling studies have shown that the cyclic compound **III** is indeed considerably less flexible than the linear compound **I**; however, both compounds adopt mainly the conformation AA. It was anticipated that the entropy barrier of complexation of compound **III** with ConA should be smaller because less conformational flexibility is lost during binding. Indeed, the complexation has a more favorable entropy term but surprisingly this term is offset by a smaller enthalpy contribution, and the linear and cyclic compounds have similar binding constants. Molecular modeling studies have been performed to explain the loss of enthalpy of binding, and it was predicted that both compounds are complexed in the local minimum energy conformation BA. Figure 7 displays a comparison of the lowest energy complexes of the two compounds; both have very similar interactions with the protein, and thus the small enthalpy term of complexation of the cyclic compound cannot be explained by fewer interactions with the protein.

The NMR studies have shown that in solution the BA conformation for both compounds is less than 5% populated.

Thus, a considerable amount of energy will be lost for this conformational change and it may be possible that this energy term is considerably more unfavorable for the cyclic compound. On the other hand, the observed differences of enthalpy for **III** and **I** may arise from differences in solvent reorganization.

The data presented in this paper suggest that loss of flexibility during complexation accounts for a significant entropic penalty. Further thermodynamic measurements and NMR experiments will be conducted to validate the models proposed in the present study, and these results will be important for the future design of putative high-affinity carbohydrate ligands. Cocrystallization experiments with ConA and the ligand are under way, and these experiments will confirm whether the ligands are bound in the predicted BA conformation. Other conformationally constrained saccharides that adopt the BA conformation will be prepared to study the general applicability of the observed binding data.

Experimental Section

Nomenclature: Schematic representations of the cyclic trisaccharide **I** and **III** are given in Figure 1. The three carbohydrate residues are labeled from unprimed to primed and double-primed from the ManOMe residue, by analogy to the linear compound. The relative orientation of a pair of contiguous residues about each glycosidic linkage is described by a set of two torsional angles: Φ_{1-3} = O-5′-C-1′-O-1′-C-3 and Ψ_{1-3} = C-1′-O-1′-C-3-C-4 for the αMan(1→3)Man linkage and Φ_{1-2} = O-5″-C-1″-O-1″-C-2′ and Ψ_{1-2} = C-1″-O-1″-C-2′-C-3′ for the βGlcNAc (1→2)Man linkage. The orientation of the methylene acetal bridge is given by a set of five torsion angles: ω_1 = O-5″-C-5″-C-6″-O-6″, ω_2 = C-5″-C-6″-O-6″-C-7″, ω_3 = C-6″-O-6″-C-7″-O-4, ω_4 = O-6″-C-7″-O-4-C-4 and ω_5 = C-7″-O-4-C-4-C-5 (Figure 3). The sign of the torsion angles is given as proposed by the Commission on Nomenclature.[41]

Equipment: ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC300 spectrometer equipped with a B-ACS60 autochanger, and an Aspect 3000 off-line editing computer. The ^{1}H 2D-COSY[45] spectra were recorded on a Bruker AMX400 spectrometer and an Aspect station I off-line editing computer. Chemical shifts (δ) were measured with tetramethylsilane as internal standard. Fast atom bombardment (FAB) mass spectra were recorded by means of a VG Zabspec spectrometer with *m*-nitrobenzyl alcohol as matrix.

All calculations were performed on Silicon Graphics workstations.

General methods and materials: All chemicals were purchased from Aldrich, Fluka, and Lancaster. 4 Å Molecular sieves were purchased from Avocado, activated at 300 °C for 5 h, and stored at 180 °C. Hydrogen and nitrogen (White Spot) were supplied by British Oxygen Company.

All reaction solvents were distilled prior to use: dichloromethane and 1,2-dichloroethane were distilled from P_2O_5, acetonitrile, pyridine, and diethyl ether from CaH_2, tetrahydrofuran (THF) from $LiAlH_4$, and methanol from magnesium activated with iodine.

Chromatography was performed with Merck 7734 silica gel 60, and flash chromatography with Crosfield ES70X microspheroidal silica gel. TLC analyses were carried out on silica gel plates (Merck 1.05554 Kieselgel 60 F_{254}). Compounds on TLC were visualized by UV light and/or by dipping in H_2SO_4/MeOH (1:10 *v*/*v*/*v*) followed by subsequent charring at 140 °C.

Methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranoside (3): A mixture of ethyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-α-D-mannopyranoside (**1**) (6.06 g, 11.31 mmol), methyl 2-*O*-benzyl-4,6-di-*O*-benzylidene-α-D-mannopyranoside (**2**) (3.66 g, 9.84 mmol), and 4 Å powdered molecular sieves (10 g) was stirred for 1 h in CH_2Cl_2 (120 mL). Subsequently, a solution of *N*-iodosuccinimide (120 mL, 0.1 M in ether/CH_2Cl_2, 1:1 *v*/*v*) and TMSOTf (0.232 mL, 1.2 mmol) were added. The mixture was stirred at room temperature for 1 h, and then neutralized with triethylamine. The solution was filtered through Celite®, washed with MeOH/CH_2Cl_2, 5:95 *v*/*v*/*v*, and the combined filtrates were concentrated to dryness. The residue was dissolved in CH_2Cl_2 (100 mL), and the solution was washed with $Na_2S_2O_3$ (1 M, 2 × 100 mL) and water (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the crude product by column chromatography on silica gel (CH_2Cl_2/acetone, 99:1 *v*/*v*) afforded the desired disaccharide (**3**) as a colorless oil (8.0 g, 96% yield); R_f (acetone/CH_2Cl_2, 3:97 *v*/*v*) = 0.70; $[\alpha]^{22}_{(kap)D(/kap)}$ = +12.1 (CH_2Cl_2, *c* = 13.13 mg mL^{-1}); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.52–7.15 (m, 25H, arom H), 5.63 (s, 1H, PhC*H*-), 5.62 (m, 1H, H-2′), 5.32 (d, 1H, $J_{1',2'}$ = 2.0 Hz, H-1′), 4.89 (d, 1H, J_{gem} = 11.0 Hz, -C*H*$_2$- benzyl), 4.67 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.77–4.60 (4d, 4H, J_{gem} = 12.0 Hz, -C*H*$_2$- benzyl), 4.51–4.41 (3d, 3H, -C*H*$_2$- benzyl), 4.25 (m, 2H, H-3, H-5′), 3.98 (dd, 1H, $J_{3',2'}$ = 3.5 Hz, $J_{3',4'}$ = 9.0 Hz, H-3′), 3.92–3.65 (m, 8H, H-4, H-4′, H-5, H-2, H-6, H-6′), 3.30 (s, 3H, -OC*H*$_3$), 2.10 (s, 3H, -C*H*$_3$ acetyl); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.1, (C=O), 138.7, 138.4, 137.9, 137.4 (Cq benzyl + Cq benzylidene), 128.6–126.1 (arom C), 101.1 (PhC*H*-), 100.6, 98.9 (C-1, C-1′), 79.1, 78.0, 77.4, 74.3, 73.2, 72.2, 68.2, 63.9 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 75.1, 73.7, 73.5, 71.6 (-*C*H$_2$- benzyl), 69.1, 68.8 (C-6, C-6′), 54.9 (-OCH$_3$), 21.1 (-*C*H$_3$ acetyl); MS (FAB): *m*/*z* (%): 885 (4) [*M*+K]$^+$, 869 (100) [*M*+Na]$^+$; HRMS (FAB): calcd $C_{50}H_{54}O_{12}Na$ [*M*+Na]$^+$ 869.35; found 869.35.

Methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside (4): 4 Å molecular sieves (7 g) and sodium cyanoborohydride (4.8 g, 76.47 mmol) was added to a solution of compound **3** (6.47 g, 7.65 mmol) in dry THF (100 mL). Subsequently, a solution of HCl in ether (1 M, 99 mL) was slowly added over a period of 20 min to the mixture, which was stirred for 3 h under argon. The reaction mixture was neutralized by the addition of triethylamine, and washed with saturated $NaHCO_3$ (2 × 100 mL) and water (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography with acetone/CH_2Cl_2 (3:97 *v*/*v*) as the eluent, and the desired disaccharide (**4**) was isolated as a colorless oil (6.03 g, 93% yield); R_f (acetone/CH_2Cl_2, 2:98 *v*/*v*) = 0.10; $[\alpha]^{22}_{(kap)D(/kap)}$ = +27.5 (CH_2Cl_2, *c* = 29.26 mg mL^{-1}); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.12 (m, 25H, arom H), 5.50 (m, 1H, H-2′), 5.37 (s, 1H, H-1′), 4.89 (d, 1H, J_{gem} = 11.0 Hz, -C*H*$_2$- benzyl), 4.75–4.45 (m, 11H, J_{gem} = 11.4 Hz, -C*H*$_2$- benzyl, H-1), 4.11 (dd, 1H, $J_{4,3}$ = 9.2 Hz, H-4), 3.98 (m, 3H, H-3′, H-5, H-5′), 3.74 (m, 7H, H-2, H-3, H-6, H-4′, H-6′), 3.33 (s, 3H, -OC*H*$_3$), 2.10 (s, 3H, -C*H*$_3$ acetyl); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.4, (C=O), 138.6, 138.0, 137.9 (Cq benzyl), 128.4-127.6 (arom C), 98.8, 98.7 (C-1, C-1′), 77.9, 77.5, 74.5, 71.9, 71.0, 68.8, 68.6 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 74.9, 73.7, 72.6, 71.8, 70.8, 69.4 (-*C*H$_2$- benzyl, C-6, C-6′), 54.9 (-OCH$_3$), 21.1 (-*C*H$_3$ acetyl); MS (FAB): *m*/*z* (%): 887 (4) [*M*+K]$^+$, 871 (100) [*M*+Na]$^+$; HRMS (FAB): calcd $C_{50}H_{56}O_{12}Na$ [*M*+Na]$^+$ 871.37; found 871.37.

Methyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-4-*O*-methylthiomethyl-α-D-mannopyranoside (5): Dimethyl sulfide (5.2 mL, 70.3 mmol) was added to a cooled solution (0 °C) of **4** (5.96 g, 7.03 mmol) in dry acetonitrile (80 mL). Benzoyl peroxide (6.8 g, 28.12 mmol) was added in portions over a period of 45 min under the exclusion of light, and the mixture was stirred for 18 h and gradually allowed to reach room temperature. TLC analysis (acetone/CH_2Cl_2, 3:97 *v*/*v*) showed complete conversion of the starting material into the product. The solution was diluted with ethyl acetate, and washed with NaOH (1 M, 2 × 100 mL) and brine (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. Purification by column chromatography on silica gel (petroleum ether 40–60/CH_2Cl_2, 1:1 *v*/*v*, followed by CH_2Cl_2 and acetone/CH_2Cl_2, 1:99 *v*/*v*) gave the compound **5** as a pale yellow foam (5.55 g, 87% yield); R_f (acetone/CH_2Cl_2, 3:97 *v*/*v*) = 0.62; $[\alpha]^{22}_{(kap)D(/kap)}$ = +37.8 (CH_2Cl_2, *c* = 25.73 mg mL^{-1}); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.39–7.15 (m, 25H, arom H), 5.35 (dd, 1H, $J_{2',1'}$ = 1.8 Hz, $J_{2',3'}$ = 3.3 Hz, H-2′), 5.10 (d, 1H, H-1′), 4.89 (d, 1H, J_{gem} = 11.0 Hz, -C*H*$_2$- benzyl), 4.78 (d, 1H, J_{gem} = 11.4 Hz, -C*H*$_2$- benzyl), 4.72 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.67 (d, 1H, -C*H*$_2$- benzyl), 4.64–4.43 (m, 9H, -C*H*$_2$SCH$_3$, -C*H*$_2$- benzyl), 4.06 (dd, 1H, $J_{3,2}$ = 3.3 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 3.97 (dd, 1H, $J_{3',4'}$ = 9.2 Hz, H-3′), 3.88 (m, 2H, H-4, H-5′), 3.77 (m, 4H, H-2, H-4′, H-6a, H-6a′), 3.68 (m, 3H, H-5, H-6b, H-6b′), 3.30 (s, 3H, -OC*H*$_3$), 2.22 (s, 3H, -C*H*$_3$ acetyl), 2.02 (s, 3H, -SC*H*$_3$); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.3, (C=O), 138.7, 138.4, 138.1, 137.9 (Cq benzyl), 128.5–127.6 (arom C), 99.8, 99.3 (C-1, C-1′), 77.9, 77.3, 74.5, 74.4, 72.2, 71.4, 69.1 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′), 75.0, 73.5, 73.4, 73.0, 72.1, 72.0, 69.5, 69.3 (-*C*H$_2$- benzyl, -*C*H$_2$SCH$_3$, C-6, C-6′), 54.9 (-OCH$_3$), 21.2 (-*C*H$_3$ acetyl), 14.8 (-SCH$_3$); MS

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(FAB): *m/z* (%): 948 (8) $[M+K]^+$, 932 (100) $[M+Na]^+$; HRMS(FAB): calcd $C_{52}H_{60}O_{12}SNa$ $[M+Na]^+$ 931.37; found 931.37.

Methyl 2,6-di-*O*-benzyl-3-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside-*p*-methoxyphenyl 3″,4″-di-*O*-benzyl-2″-deoxy-2″-phthalimido-β-D-glucopyranoside-4,6″-methylidene acetal (7): A mixture of **5** (5.1 g, 5.62 mmol), *p*-methoxyphenyl 3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**6**) (2.90 g, 4.88 mmol) and powdered molecular sieves (8 g) was stirred for 1 h in a mixture of THF and 1,2-dichloroethane (1:1 *v/v*, 100 mL) under argon. The solution was cooled to 0 °C. *N*-iodosuccinimide (1.35 g, 6 mmol) was dissolved in a mixture of THF and 1,2-dichloroethane (60 mL, 1:1 *v/v*). Trifluoromethanesulfonic acid (54 μL, 0.6 mmol) was then added and the solution stirred for further 30 s. The resulting mixture (0.1M, 58.7 mL) was added quickly to the initial solution which was then stirred for 30 min at 0 °C. TLC analysis indicated the conversion of the starting material into a major product. The reaction mixture was neutralized by the addition of triethylamine and filtered through Celite. The resulting solution was diluted with CH_2Cl_2 (200 mL)and washed with $Na_2S_2O_3$ (2 × 100 mL), $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the crude product by column chromatography on silica gel (CH_2Cl_2/acetone, 98:2 *v/v*) afforded the trisaccharide 7 as a colorless oil (5.39 g, 76% yield); R_f (acetone/CH_2Cl_2, 4:96 *v/v*) = 0.66; $[\alpha]^{22}_{(kap)D(/kap)} = +44.4$ (CH_2Cl_2, $c = 26.8$ mg mL^{-1}); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.65$ (br s, 4H, arom H -Pht), 7.38–6.85 (m, 35H, arom H), 6.80, 6.65 (2 m, 4H, arom H -MP), 5.59 (d, 1H, $J_{1'',2''} = 8.1$ Hz, H-1″), 5.56 (dd, 1H, $J_{2',1'} = 1.8$ Hz, $J_{2',3'} = 3.3$ Hz, H-2′), 5.21 (d, 1H, H-1′), 5.02 (d, 1H, $J_{gem} = 6.3$ Hz, -OCH_2O-), 4.94–4.42 (m, 16H, H-1, -OCH_2O-, -CH_2- benzyl), 4.40 (m, 1H, H-2″), 4.02 (dd, 1H, $J_{3,2} = 2.6$ Hz, $J_{3,4} = 9.6$ Hz, H-3), 4.38–3.53 (m, 15H, H-2, H-4, H-5, H-6, H-3′, H-4′, H-5′, H-6′, H-3″, H-4″, H-5″, H-6″), 3.62 (s, 3H, -OCH_3 -MP), 3.25 (s, 3H, -OCH_3), 2.10 (s, 3H, -CH_3 acetyl); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 170.0$ (C=O), 151.0 (Cq -MP), 138.0 (Cq benzyl), 133.7, 123.3 (arom C -Pht), 131.6 (Cq -Pht), 128.5–127.4 (arom C), 118.6, 114.4 (arom C -MP), 100.1, 98.8, 97.7 (C-1, C-1′, C-1″), 97.8 (-OCH$_2$O-), 79.6, 79.1, 77.8, 77.5, 74.3, 74.1, 72.2 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-3″, C-4″, C-5″), 74.8, 73.4, 73.0, 72.1, 71.9, 69.8, 69.2, 67.5 (-CH_2- benzyl, C-6, C-6′), 64.1 (C-6″), 55.9, 55.7, 54.8 (-OCH_3 -MP, -OCH_3, C-2″), 21.1 (-CH_3 acetyl); MS (FAB): *m/z* (%): 1478 (100) $[M+Na]^+$; HRMS (FAB): calcd $C_{86}H_{89}NO_{20}Na$ $[M+Na]^+$ 1478.59; found 1478.59.

Methyl 2,6-di-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside-3″,4″-di-*O*-benzyl-2″-deoxy-2″-phthalimido-β-D-gluco-pyranose-4,6″-methylidene acetal (9): Potassium *tert*-butoxide (0.412 g, 3.67 mmol) was added to a solution of trisaccharide **7** (5.34 g, 3.67 mmol) in methanol (50 mL). The resulting mixture was stirred at room temperature under argon. After 4 h, TLC analysis (acetone/CH_2Cl_2, 3:97 *v/v*) showed the complete conversion of the starting material. The reaction mixture was neutralized with Dowex-50WX8-[H$^+$] and filtered, and the filtrate concentrated to dryness in vacuo. The crude compound **8** was used without further purification. Compound **8** was dissolved in a mixture of toluene/acetonitrile/water (60 mL, 1:4:1 *v/v/v*), and cerium ammonium nitrate (6.04 g, 11.01 mmol) was added. The mixture was stirred for 4 h at room temperature under the exclusion of light, after which time the solution was diluted with ethyl acetate. The organic layer was washed with saturated $NaHCO_3$ (2 × 75 mL) and brine (75 mL), dried ($MgSO_4$), and filtered, and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel with acetone/CH_2Cl_2 (4:96 *v/v*) as the eluent. The trisaccharide **9** was obtained as a pale yellow oil (2.16 g, 45% yield); R_f (acetone/CH_2Cl_2, 5:95 *v/v*) = 0.20; $[\alpha]^{22}_D = +29.8$ (CH_2Cl_2, $c = 20.8$ mg mL^{-1}); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.80–7.60$ (4H, m, arom H -Pht), 7.41–6.88 (m, 35H, arom H), 5.32 (d, 1H, $J_{1',2'} = 1.1$ Hz, H-1′), 5.25 (d, 1H, $J_{1'',2''} = 8.5$ Hz, H-1″), 4.85 (2d, 2H, $J_{gem} = 11.0$ Hz, -CH_2- benzyl), 4.70 (d, 1H, $J_{1,2} = 1.4$ Hz, H-1), 4.81–4.39 (m, 15H, -OCH_2O-, -CH_2- benzyl, H-3″), 4.27 (br s, 1H, H-2′), 4.13 (dd, 1H, $J_{2'',3''} = 10.7$ Hz, H-2″), 4.09 (m, 1H, H-3), 4.01–3.54 (m, 14H, H-2, H-4, H-5, H-6, H-3′, H-4′, H-5′, H-6′, H-4″, H-5″, H-6″), 3.31 (s, 3H, -OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 168.2$ (C=O), 138.7, 138.4, 138.3, 138.2, 138.0 (Cq benzyl), 133.7, 123.2 (arom C -Pht), 131.8 (Cq -Pht), 128.5–126.6 (arom C), 100.7, 98.6, 92.9 (C-1, C-1′, C-1″), 97.6 (-OCH$_2$O-), 79.7, 79.3, 79.2, 78.0, 76.3, 75.7, 74.6, 74.5, 71.7, 71.5, 68.3 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-3″, C-4″, C-5″), 74.8, 74.6, 73.4, 71.9, 69.5, 69.3 (-CH_2- benzyl, C-6, C-6′, C-6″), 57.7, 54.9 (-OCH_3, C-2″); MS (FAB): *m/z* (%): 1346 (3) $[M+K]^+$, 1330 (100) $[M+Na]^+$; MS (FAB): calcd $C_{77}H_{81}NO_{18}Na$ $[M+Na]^+$ 1330.54; found 1330.53.

Methyl 2,6-di-*O*-benzyl-3-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside-trichloroacetimidate 3″,4″-di-*O*-benzyl-2″-deoxy-2″-phthalimido-β-D-glucopyranoside-4,6″-methylidene acetal (10): - Trichloroacetonitrile (0.782 mL, 7.80 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (19.5 μL, 0.13 mmol) were added to a solution of **9** (1.7 g, 1.3 mmol) in CH_2Cl_2 (30 mL) and the reaction mixture was stirred under argon. After 20 min the TLC analysis (CH_2Cl_2/acetone, 95:5 *v/v*) showed almost complete conversion of the starting material into the product. The mixture was concentrated in vacuo and the oily residue was applied onto a column of silica gel and eluted with acetone/CH_2Cl_2 (1:99 *v/v*). Concentration of the appropriate fractions gave **10** as a white foam (1.22 g, 65% yield); R_f (acetone/CH_2Cl_2, 5:95 *v/v*) = 0.50; $[\alpha]^{22}_D = +53.3$ (CH_2Cl_2, $c = 1$ 9.6 mg mL^{-1}); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 8.41$ (s, 1H, C=N*H*), 7.65 (br s, 4H, arom H -Pht), 7.40–6.80 (m, 35H, arom H), 6.37 (d, 1H, $J_{1'',2''} = 8.5$ Hz, H-1″), 5.15 (d, 1H, $J_{1',2'} = 1.1$ Hz, H-1′), 4.87 (d, 1H, $J_{gem} = 11.4$ Hz, -CH_2- benzyl), 4.85 (d, 1H, $J_{gem} = 11.0$ Hz, -CH_2- benzyl), 4.82–4.35 (m, 17H, -OCH_2O-, -CH_2- benzyl, H-1, H-2″, H-3″), 4.15 (br s, 1H, H-2′), 4.03 (dd, 1H, $J_{3,2} = 3.3$ Hz, $J_{3,4} = 9.2$ Hz, H-3), 3.98–3.52 (m, 14H, H-2, H-4, H-5, H-6, H-3′, H-4′, H-5′, H-6′, H-4″, H-5″, H-6″), 3.31 (s, 3H, -OCH_3), 3.10 (br s, 1H, -O*H*); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 168.0$ (C=O), 160.9 (Cq imidate), 138.7, 137.9 (Cq benzyl), 133.8, 123.3 (arom C -Pht), 131.5 (Cq -Pht), 128.5–127.4 (arom C), 102.1, 98.3, 94.0 (C-1, C-1′, C-1″), 97.6 (-OCH$_2$O-), 80.2, 79.5, 78.9, 77.6, 75.7, 74.8, 74.6, 71.3, 68.4 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-3″, C-4″, C-5″), 74.9, 74.7, 73.5, 73.2, 72.1, 69.7, 69.5, 67.1 (-CH_2- benzyl, C-6, C-6′, C-6″), 54.8 (-OCH_3, C-2″); MS (FAB): *m/z* (%) 1473 (73) $[M+Na+3\times{}^{35}Cl]^+$, 1476 (100), $[M+Na+2\times{}^{35}Cl+1\times{}^{37}Cl]^+$, 1479 (32) $[M+Na+1\times{}^{35}Cl+2\times{}^{37}Cl]^+$; HRMS (FAB): calcd $C_{79}H_{81}N_2O_{18}{}^{35}Cl_3Na$ $[M+Na]^+$ 1473.44; found 1473.44; calcd $C_{79}H_{81}N_2O_{18}{}^{35}Cl_2{}^{37}ClNa$ $[M+Na]^+$ 1475.44; found 1475.44; calcd $C_{79}H_{81}N_2O_{18}{}^{35}Cl{}^{37}Cl_2Na$ $[M+Na]^+$ 1477.44; found 1477.44.

Methyl (3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside-4,6″-methylidene acetal (11): A mixture of **10** (1.22 g, 0.84 mmol) and 4 Å powdered molecular sieves (1 g) was stirred for 30 min in CH_2Cl_2 (50 mL) under argon. The solution was cooled to −20 °C and trimethylsilyl trifluoromethanesulfonate (8 μL, 0.042 mmol) was added and the reaction mixture was stirred for 10 min. The reaction mixture was quenched by the addition of triethylamine and filtered through Celite; the filtrate was diluted with CH_2Cl_2, and washed with $NaHCO_3$ (2 × 50 mL) and water (50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to dryness. Purification of the residue by column chromatography on silica gel (CH_2Cl_2/acetone, 98:2 *v/v*) afforded the cyclic trisaccharide **11** as a colorless oil (891.8 mg, 82% yield); R_f (CH_2Cl_2/acetone, 96:4 *v/v*) = 0.78; $[\alpha]^{22}_D = +42.1$ (CH_2Cl_2, $c = 20.53$ mg mL^{-1}); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.52$ (br s, 4H, arom H-Pht), 7.41–6.80 (m, 35H, arom H), 5.75 (2d, 2H, $J_{1'',2''} = 7.7$ Hz, H-1″, H-1′), 5.06 (d, 1H, $J_{gem} = 4.0$ Hz, -OCH_2O-), 4.88 (d, 1H, $J_{gem} = 11.0$ Hz, -CH_2- benzyl), 4.67 (d, 1H, $J_{1,2} = 1.5$ Hz, H-1), 4.83–4.23 (m, 15H, -OCH_2O-, -CH_2- benzyl, H-2″, H-2′), 4.15 (d, 1H, $J_{gem} = 10.7$ Hz, -CH_2- benzyl), 4.11 (dd, 1H, $J_{3,2} = 2.9$ Hz, H-3), 3.99 (2dd, 2H, $J_{4,3} = 9.9$ Hz, H-4, H-4″), 3.82–3.44 (m, 13H, H-2, H-5, H-6, H-3′, H-4′, H-5′, H-6′, H-3″, H-5″, H-6″), 3.25 (s, 3H, -OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 167.9$ (C=O), 139.1, 138.4, 138.1, 137.9, 137.7 (Cq benzyl), 133.4, 123.1 (arom C -Pht), 131.6 (Cq -Pht), 128.5–126.8 (arom C), 100.0, 99.0, 94.5 (C-1, C-1′, C-1″), 96.9 (-OCH$_2$O-), 79.2, 78.9, 77.2, 76.8, 76.0, 75.3, 74.5, 72.0, 71.8, 71.3 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-3″, C-4″, C-5″), 74.9, 74.3, 73.5, 73.2, 72.5, 72.3, 69.1, 68.8, 66.2 (-CH_2- benzyl, C-6, C-6′, C-6″), 54.7, 53.8 (C-2″, (-OCH_3); MS (FAB): *m/z* (%): 1328 (8) $[M+K]^+$, 1312 (100) $[M+Na]^+$; HRMS (FAB): calcd $C_{77}H_{79}NO_{17}Na$ $[M+Na]^+$ 1312.52; found 1312.52.

Methyl (3,4-di-*O*-benzyl-2-acetimido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-2,6-di-*O*-benzyl-α-D-mannopyranoside-4,6″-methylidene acetal (12): Hydrazine monohydrate (1.78 mL, 36.69 mmol) was added to a solution of **11** (945.8 mg, 0.733 mmol) in EtOH (10 mL) and the reaction mixture was heated under reflux for 18 h. TLC analysis with ninhydrin showed complete conversion of the starting material into an amino-containing intermediate (R_f (acetone/CH_2Cl_2, 4:96 *v/v*) = 0.10). The solvent was removed in vacuo and the residue coevaporated with toluene. The amine thus obtained was subsequently dissolved in a mixture of pyridine (10 mL) and acetic

anhydride (10 mL) and the solution was stirred at room temperature for 4 h. The mixture was then concentrated and coevaporated with toluene (3×20 mL), ethanol (2×20 mL) and CH_2Cl_2 (2×20 mL). Purification of the crude product by column chromatography on silica gel (CH_2Cl_2/acetone, 97:3 *v*/*v*) gave the title compound **12** (563.4 mg, 64% yield) as a colorless oil; R_f (acetone/CH_2Cl_2, 4:96 *v*/*v*) = 0.23; $[\alpha]_D^{24} = +31.2$ (CH_2Cl_2, $c = 16.93$ mg mL^{-1}); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.45-7.11$ (m, 35H, arom H), 5.43 (d, 1H, $J_{NH,\,2''} = 6.3$ Hz, N*H*), 5.39 (s, 1H, H-1′), 5.22 (d, 1H, $J_{1'',\,2''} = 8.8$ Hz, H-1″), 4.91, 4.90, 4.85 (3d, 3H, $J_{gem} = 11.0$ Hz, -C*H*$_2$- benzyl), 4.79–4.48 (m, 15H, -OC*H*$_2$O-, -C*H*$_2$- benzyl, H-1, H-3″), 4.22 (s, 1H, H-2′), 4.08–3.97 (m, 2H, H-3, H-4), 3.88–3.48 (m, 13H, H-2, H-5, H-6, H-3′, H-4′, H-5′, H-6′, H-4″, H-5″, H-6″), 3.27 (s, 3H, -OC*H*$_3$), 2.91–2.80 (m, 1H, H-2″); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 171.0$ (C=O), 139.3, 138.5, 138.3, 138.1, 137.8 (Cq benzyl), 129.0–127.5 (arom C), 102.9, 98.6, 96.3 (C-1, C-1′, C-1″), 97.2 (-OC*H*$_2$O-), 79.0, 78.2, 78.0, 77.5, 76.8, 76.3, 75.9, 74.3, 72.6, 72.1, 70.5 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-3″, C-4″, C-5″), 75.1, 74.8, 74.5, 73.6, 72.6, 69.3, 69.1, 66.1 (-C*H*$_2$- benzyl, C-6, C-6′, C-6″), 56.2, 54.8 (C-2″, -OC*H*$_3$), 23.5 (-C*H*$_3$ acetyl); MS (FAB): *m*/*z* (%): 1240 (5) $[M+K]^+$, 1224 (100) $[M+Na]^+$; HRMS (FAB): calcd $C_{71}H_{79}NO_{16}Na$ $[M+Na]^+$ 1224.53; found 1224.53.

Methyl (2-acetimido-2-deoxy-β-D-glucopyranosyl)-(1→2)-(α-D-mannopyranosyl)-(1→3)-α-D-mannopyranoside-4,6″-methylidene acetal (III): Compound **12** (536.6 mg, 0.447 mmol) in ethanol (10 mL) was hydrogenated (H_2, 54 mg $Pd(OAc)_2$) for 18 h. The mixture was filtered though Celite and concentrated. The cyclic trisaccharide **III** was obtained as a white solid (230 mg, 90% yield); R_f (MeOH/CH_2Cl_2, 20:80 *v*/*v*) = 0.05; $[\alpha]_D^{22} = +34.7$ (MeOH, $c = 3.4$ mg mL^{-1}); ^{1}H NMR (400 MHz, D_2O): $\delta =$ 5.68 (d, 1H, $J_{1',\,2'} = 1.3$ Hz, H-1′), 5.15, 5.02 (2d, 2H, $J_{gem} = 5.1$ Hz, -OC*H*$_2$O-), 4.95 (d, 1H, $J_{1'',\,2''} = 8.4$ Hz, H-1″), 4.83 (d, 1H, $J_{1,\,2} = 1.5$ Hz, H-1), 4.37 (dd, 1H, $J_{2',\,3'} = 3.9$ Hz, H-2′), 4.20–3.92 (m, 8H, H-2, H-3, H-6a, H-3′, H-6a′, H-2″, H-6a″, H-6b″), 3.90–3.56 (m, 9H, H-4, H-5, H-6b, H-4′, H-5′, H-6b′, H-3″, H-4″, H-5″), 3.49 (s, 3H, -OC*H*$_3$), 2.13 (s, 3H, -C*H*$_3$ acetyl); ^{13}C NMR (100 MHz, D_2O): $\delta = 177.5$ (C=O), 103.1 ($J_{C1,\,H1} = 173.5$ Hz, C-1), 102.0 ($J_{C1',\,H1'} = 173.3$ Hz, C-1′), 100.4 (-OC*H*$_2$O-), 99.6 ($J_{C1'',\,H1''} = 166.4$ Hz, C-1″), 78.7, 77.7, 76.9, 76.4, 75.7, 75.0, 74.4, 72.5, 71.8, 71.6, 69.3 (C-2, C-3, C-4, C-5, C-2′, C-3′, C-4′, C-5′, C-3″, C-4″, C-5″), 70.4 (C-6″), 63.4, 62.7 (C-6, C-6′), 57.3 (-OC*H*$_3$), 56.6 (C-2″), 24.8 (-C*H*$_3$ acetyl); MS (FAB): *m*/*z* (%): 616 (7) $[M+2\times Na]^+$, 594 (100) $[M+Na]^+$; MS (FAB): calcd $C_{22}H_{37}NO_{16}Na$ $[M+Na]^+$ 594.20; found 594.20.

Microcalorimetry: Isothermal titration calorimetry (ITC) experiments to measure the binding of saccharides to concanavalin A were done at 25 °C by means of a Microcal MCS titration microcalorimeter following standard instrumental procedures[42] with a 250 μL injection syringe and 400 rpm stirring. Concanavalin A (Sigma) was used without further purification and was dissolved in a buffer (0.1 M Tris, 0.5 M NaCl, 1 mM $MnCl_2$, 1 mM $CaCl_2$, pH 7.0) and degassed gently immediately before use. Saccharide ligands were dissolved in the same buffer. Protein concentrations in the ITC cell were determined from UV absorbance measurements at 280 nm with the molar extinction coefficient $\varepsilon_{280} = 33\,000$ (ConA). A typical binding experiment involved 25×10 μL injections of ligand solution (typically around 10 mM concentration) into the ITC cell (ca. 1.3 mL active volume) containing protein (0.1–0.5 mM). Control experiments were performed under identical conditions by injection of ligand into buffer alone (to correct for heats of ligand dilution) and injection of buffer into the protein mix (to correct for heats of dilution of the protein). Integrated heat effects, after correction for heats of dilution, were analyzed by nonlinear regression in terms of a simple single-site binding model using the standard Microcal ORIGIN software package. For each thermal titration curve this yields estimates of the apparent number of binding sites (N) on the protein, the binding constant (K, M^{-1}) and the enthalpy of binding (ΔH, kcal mol^{-1}). In cases of weak ligand binding the titration curve is too gradual to allow unambiguous estimation of N, and in such cases the stoichiometry was fixed at $N = 1$ for regression fits. Other thermodynamic quantities were calculated with the standard expression $\Delta G^\circ = -RT\ln K = \Delta H^\circ - T\Delta S^\circ$.

Molecular modeling:

Starting models for the oligosaccharides and for ConA: The trisaccharide βGlcNAc(1→2)αMan(1→3)Man was constructed with the monosaccharides obtained from a database of three-dimensional structures.[43] All subsequent calculations were performed by SYBYL software. Cyclization was performed through a systematic search around the four torsion angles of the glycosidic linkage, while a short distance was imposed between the atom O-4 and O-6″. The solution with the best energy was taken as a starting point for the cyclic compound. The coordinates of concanavalin A were taken from the 2.0 Å resolution crystal structure of the ConA–mannose complex.[35] Four conserved water molecules, which are involved in the coordination of the structural cations, were incorporated into the model. All hydrogen atoms were added and their position optimized with the Tripos force field.[44]

Systematic conformational search for the trisaccharides: For the linear trisaccharide, a four-dimensional systematic search was performed by rotating the Φ and Ψ angles of the two glycosidic linkages by 10° steps. The SEARCH procedure of the SYBYL software was used for this purpose together with energy parameters appropriate for carbohydrates.[37] In order to avoid limitation of conformational space due to steric conflicts in the rigid-residue approach, the hydroxyl hydrogens were omitted and the hydroxymethyl groups at C-6 were replaced by methyl groups. In order to limit the computation time needed, all conformations with penetration of van der Waals spheres larger than 40% were rejected prior to any energy calculations.

A systematic search of the possible conformations of the 13-membered ring has been performed on the cyclic trisaccharide. In the 13-membered ring of this cyclic trisaccharide, nine torsion angles could be rotated, one of which was used as a ring-closure bond. An eight-dimensional systematic conformational search was performed by rotating the Φ and Ψ torsion angles of the two glycosidic linkages as well as the torsion angles of the methylene acetal bridge by 10° steps, except for ω_3, which corresponds to the ring-closure bond.

In order to establish the correct stereochemistry at the ring-closure point and to relieve small steric conflicts, each of the resulting conformations of the cyclic trisaccharide was submitted to several steps of energy minimization. The hydroxyl atoms were restored, and the charges and atom types specially defined for carbohydrate parameterization[37] were used. The first cycles included constraints on the torsion angles. The final optimization was run with a termination gradient of 0.05 rms with no constraints. The structures obtained after the systematic search were checked for the puckering of the pyranose rings and chirality of carbon atoms.

A family analysis method[8] was used to identify groups of conformations. In this approach, a family algorithm was used which concludes that an object belongs to a group if there is only a single-step change to at least one of the objects in the group. For this purpose, the torsion angles of both the βGlcNAc(1→2)Man and the αMan(1→3)Man glycosidic linkage were used as selection criteria and the step limit was fixed at 30°.

Docking in the binding site of ConA: The lowest energy conformation of each family of compound **I** and each of the low-energy conformations of compound **III**, in an energy window of 10 kcal mol^{-1}, were used as starting structures to be docked in the binding site of ConA. This was performed by superimposing the central mannose unit of the cyclic trisaccharide onto the sugar ring in the ConA–mannose complex.[35] The hydroxyl groups were oriented in order to create the appropriate hydrogen network between the mannose residue and the amino acids of the binding site. Each of these ConA–trisaccharide complexes was optimized by means of the appropriate energy parameters.[37] Two shells of amino acids were considered for the optimization cycle. A 10 Å shell around the binding site (33 amino acids as well as the water molecules and cations) was taken into account for the energy calculations. A 4 Å shell containing the 15 amino acids closest to the carbohydrate (Tyr^{12}, Asn^{14}, Thr^{97}, Gly^{98}, Leu^{99}, Tyr^{100}, Ser^{168}, Ala^{207}, Asp^{208}, Gly^{224}, Ser^{225}, Thr^{226}, Gly^{227}, Arg^{228}, and Leu^{229}) was defined as the hot region to be optimized. In the first optimization cycles, only the cyclic trisaccharide and the side chains of these 15 amino acids were allowed to optimize. In the second step, the backbone of these amino acids was also optimized.

Conformational analysis by NMR spectroscopy: NMR experiments were recorded on a Bruker DRX500 spectrometer, with an approximately 5 mg mL^{-1} solution of the trisaccharides in D_2O at 299 K. Chemical shifts are reported in ppm, with the residual HDO signal ($\delta = 4.72$) and external TMS ($\delta = 0$) as references. The double quantum filtered COSY spectrum was recorded with a data matrix of 256×1 k to digitize a spectral width of 2000 Hz. 16 scans were used with a relaxation delay of 1 s. The 2D-TOCSY experiment was performed with a data matrix of 256×2 k to digitize a spectral width of 2000 Hz; 16 scans were used per increment with a relaxation delay of 2 s, and MLEV 17 with 100 ms isotropic mixing time. The one-bond proton–carbon correlation experiment was collected by means of the gradient-enhanced HMQC sequence. A data matrix of 256 ×

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1 k was used to digitize a spectral width of 2000 Hz in F2 and 10 000 Hz in F1. 4 scans were used per increment with a relaxation delay of 1 s and a delay corresponding to a J value of 145 Hz. ^{13}C decoupling was achieved by the WALTZ scheme.

The 2D-HMQC-TOCSY experiment was conducted with 80 ms mixing time (MLEV 17). The same conditions as for the HMQC were employed. HMBC experiments were performed with the gradient-enhanced sequence with a data matrix of 256×2 k to digitize a spectral width of $2000 \times 15\,000$ Hz. Eight scans were acquired per increment with a delay of 65 ms for evolution of long-range couplings. NOESY experiments were recorded with mixing times of 100, 200, 300, and 400 ms. ROESY experiments used mixing times of 100, 200, 300, and 400 ms. The rf carrier frequency was set at $\delta = 6.0$, and the spin locking field was 3.0 kHz. Good linearity of the build-up curves was observed up to 250 ms (NOESY) and 300 ms (ROESY). Estimated errors are smaller than 10%. Assuming that the motion of two interacting protons can be described by a monoexponential autocorrelation function, the corresponding cross-relaxation rates are given in Equations (1) and (2).

$$\sigma_{NOESY} = (k_2/10r^6)[6J(2\omega) - J(0)] \quad (1)$$

$$\sigma_{ROESY} = (k_2/10r^6)[2J(0) + 3J(\omega)] \quad (2)$$

Cross-relaxation rates were estimated from the build-up courses by extrapolation at zero mixing time.[31, 32] Effective correlation times and, thus, interproton distances, for selected proton pairs may be obtained from $\sigma_{NOESY}/\sigma_{ROESY}$ ratios, since they only depend on τ_c. Developing the spectral density functions, $J(n\omega)$, as a function of the correlation time, τ_c, and of the spectrometer frequency, ω_0, we obtain Equation (3), which is a quartic

$$\sigma_{ROESY}/\sigma_{NOESY} = (5 + 22\omega_0^2\tau_c^2 + 8\omega_0^4\tau_c^4)/(5 + \omega_0^2\tau_c^2 - 4\omega_0^2\tau_c^4) \quad (3)$$

equation in τ_c, which can be easily solved. It should be stressed that the assumption made here does not require equal mobility between different proton pairs, as in a rigid molecule. Therefore, it is possible to obtain internuclear distances r (geometrical parameter) and local correlation times (dynamic parameter) for any pair of protons.[33] This approach reduces the intrinsic error resulting from the use of an internal reference (as in the isolated-spin-pair approximation).

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