Synthesis of *N*-Glycans and Azasugars to Target Disease

By

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Abstract

In this thesis two aspects of carbohydrate research will be discussed. First, the total synthesis of *N*-glycans found on allergens that are known to stimulate an allergic immune response and second, the synthesis of iminosugars in an attempt to extend the scope of the PGF-methodology.

Asthma affects 235 million people worldwide, with New Zealand ranking among the highest in the world. Although there is a good understanding of how allergens trigger the immune system on a “macroscopic” level, how an allergen’s molecular structure causes such an allergic response remains unknown. Upon close review of carbohydrates present on the allergens that are known to give an allergic T helper (Th 2) immune response, a common structure has been identified. The structure consists of a complex type *N*-glycan made up of a pentasaccharide core (Man₃(GlcNHAc)₂), with additional 1,3-linked α -L-fucose and 1,2-linked D-xylose cappings. As part of a structure relationship study this heptasaccharide and structural derivatives thereof have been synthesised. The synthesis of these *N*-glycans will allow a more detailed study of the role of these defined structures in triggering an allergic immune response.

The second part of this thesis focuses on the protecting group free (PGF) synthesis of iminosugars, which are potent glycosidase inhibitors and are currently used in the treatment of a variety of diseases. Synthetic strategies for the synthesis of iminosugars involve the use of protecting groups, which are necessary to block potential competing reactive centres within a molecule during the multistep synthesis. The disadvantage, however, is that the installation of protecting groups introduces additional steps to the total synthesis, which inevitably leads to lower yields and the generation of waste. Within our group, PGF methodologies have been

developed which allow for the synthesis of a variety of iminosugars. The work presented in this thesis extends the scope of this methodology for the synthesis of an important class of iminosugars, the 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidines. For the purpose of introducing an additional hydroxymethyl group, a ketose starting material was required, and therefore an efficient Vasella/reductive amination reaction using ketoses was developed. Additionally, iodocyclisation and carbamate annulation of the intermediate alkenylamines provided successful entry to the 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidines, exemplified by the efficient 6-step synthesis of 2,5-dideoxy-2,5-imino-L-iditol and 2,5-dideoxy-2,5-imino-D-mannitol (DMDP).

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List of abbreviations

Ac	acetyl	IR	infrared spectroscopy
AcOH	acetic acid	<i>J</i>	coupling constant
EtOAc	ethyl acetate	KLH	keyhole limpet hemocyanin
arom	aromatic	<i>m</i>	meta
aq.	aqueous	m	multiplet
bd	broad doublet	M	molar
bdd	broad double doublet	Man	mannose
Bn	benzyl	Me	methyl
Bu	butyl	MeCN	acetonitrile
Bz	benzoyl	MHz	mega hertz
Calcd	calculated	Min.	minute
cat.	catalytic	Mp	melting point
COSY	correlation NMR spectrometry	<i>m/z</i>	mass to charge ratio
C _q	quaternary carbon atom	NIS	<i>N</i> -iodosuccinimide
d	doublet	NMR	nuclear magnetic resonance
dd	double doublet	Obsd.	observed
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene	<i>o</i>	ortho
DMF	<i>N,N</i> -dimethylformamide	OMe	methoxy
DMAP	4-(dimethylamino)pyridine	<i>p</i>	para
dt	double triplet	PE	petroleum ether
equiv.	equivalent	Ph	phenyl
ER	endoplasmic reticulum	PMB	<i>p</i> -methoxybenzyl
ESI	electron spray ionization	ppm	parts per million
Et ₂ O	diethyl ether	<i>p</i> TsOH	<i>para</i> -toluenesulfonic acid
Et	ethyl	q	quartet
Fuc	Fucose	quant.	quantitative
GFP	green fluorescent protein	R _f	retardation factor
Glc	glucose	rt	room temperature
GlcNAc	<i>N</i> -acetyl-glucosamine	s	singlet
h	hour	t	triplet
HMBC	heteronuclear multiple quantum coherence	TBAI	tetrabutylammonium iodide
HRMS	High Resolution Mass Spectrometry	TBDMS	<i>tert</i> -butyldimethylsilyl
HSQC	heteronuclear single quantum coherence	TBDMSCl	<i>tert</i> -butyldimethylsilyl chloride
Hz	hertz	tert	tertiary
IL	interleukin	TES	Triethyl silane
Imid.	imidazole	TFA	trifluoroacetic acid

TFAA	trifluoroacetic anhydride
TfOH	trifluoromethane- sulfonic acid
Tf ₂ O	trifluoromethane- sulfonic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
Tol	toluene
Troc	2,2,2-trichloroethyl- carbamate
Xyl	xylose

Chapter 1: General introduction

Glycans are the most abundant and the most diverse biopolymers in nature.¹ Together with nucleic acids, proteins, and lipids, they are one of the most important types of natural organic products in living systems.² Carbohydrates are involved in many biological functions, from energy storage to cell signalling, cell-cell and cell-matrix interactions, as well as being structural components of cell walls.³ Although our understanding of the role of carbohydrates in these processes is still developing, recent progress has brought carbohydrates into focus as potential targets for treatment of a range of diseases.⁴

1.1 Glycobiology of glycoconjugates

Glycans, or polysaccharides, are unique in the complexity of their structures. The diverse nature of glycans comes from the monomeric carbohydrate building blocks they are made up from. Monosaccharides have multiple hydroxyls that can be glycosylated which allows for the formation of both branched and straight chain oligosaccharides.⁵ Unlike oligonucleotides, oligosaccharides do not encode biological information in their monomer sequences. Here, it is the three dimensional structure of oligosaccharides that is responsible for their functions. In biological systems, glycans are generally covalently bound to non-carbohydrate molecules such as lipids or proteins (where they are attached to serine, threonine, or asparagine residues).⁶ These glycans, or glycoconjugates, are found on red blood cells, in the cytoplasmic matrix and in subcellular organelles. The more complex glycans are mainly integrated into the lipid bilayer of the plasma membrane forming the glycocalyx (Figure 1).⁷ Cells and tissues use these glycans to communicate with the immune system, signalling if the tissue is healthy or injured. The recognition is based on a lock + key principle with carbohydrates forming ligands (keys) that can be recognised by a number of different molecules, such as lectins, enzymes, hormones and antibodies (lock).⁸ On the other

hand, tumour cells and viruses use the same recognition system to evade the immune system.^{9,10} In addition, microbes also use glycans to enable them to invade cells.¹¹

Figure 1. Representation of the plasma membrane with presence of several glycan moieties involved in cell adhesion and cell-cell interaction.

An example of extracellular glycan interactions is the ABO blood group system. The ABO classification is based on the presence or absence of the antigens, A and/or B, on red blood cells (Table 1). In addition, there are two antibodies anti-A and anti-B that always occur in the absence of the corresponding antigen. The minimal determinant saccharides in blood group antigens are depicted in table 1, here the core structure consists of an α -1,2-fucosylated galactose. Individuals with blood group A have an additional α -1,3-linked *N*-acetylgalactosamine (GalNHAc), whereas for those with

blood group B this is galactose. When no additional sugars are present this gives blood group O.⁵ Understanding these serological relationships has allowed for safe blood transfusions from one person to another.¹²

Phenotype of red blood cells	Minimal determinant saccharide (blood group antigen)	Antibodies found in plasma
A	<p style="text-align: center;">A</p>	Anti-B
B	<p style="text-align: center;">B</p>	Anti-A
AB	A and B	-
O	<p style="text-align: center;">H</p>	Anti-A and Anti-B

Table 1. The ABO blood group system.⁵

1.2 The biosynthesis of oligosaccharides

Two types of enzyme are involved in the biosynthesis of oligosaccharides: glycosyltransferases and glycosidases. Glycosyltransferases catalyse glycosylation reactions and ensure the stereo- and regio-specific build-up of oligosaccharides. Glycosidases, on the other hand, are involved in the complex process of glycoprotein modification and digestion.¹³ Glycosyltransferases use nucleoside diphosphate-sugars as activated-donors.¹⁴ An example is the uridine diphospho(UDP)-galactose donor **3** (Scheme 1). This is an important donor in the biosynthesis of complex type *N*-glycans.¹⁵ The nucleoside diphosphate-donor is synthesised, in two steps from galactose **1**. First, galacto kinase selectively phosphorylates the monosaccharide to galactosylphosphate **2**. Uridine diphospho(UDP)-galactosylpyrophosphorylase then installs the nucleoside to give activated donor **3**. Galactosyltransferase then glycosylates the GlcNHAc acceptor selectively to give saccharide **4**.

Scheme 1. Nucleosidediphosphate-sugars as donor substrates for glycosyltransferases in the synthesis of oligosaccharides.

Glycoside hydrolases (glycosidases) are enzymes which degrade poly- and oligosaccharides. Several types of these enzymes have been defined based on their modes of action.¹³ Exoglycosidases begin hydrolysing from the non-reducing end of a polysaccharide and endoglycosidases attack somewhere in the middle of the polysaccharide chain.^{13,16} Like glycosyltransferases, glycosidases are very specific and will only hydrolyse one specific linkage.¹³

1.2.1 The biosynthesis of *N*-glycans

Glycans can be linked to proteins via a nitrogen or an oxygen. Nitrogen bound glycans (*N*-glycans) are always attached the reducing end of a GlcNHAc residue to the side chain of an asparagine amino acid, whereas oxygen bound glycans (*O*-glycans) are generally bound to the hydroxyl group on the side chain of serine or threonine. Although structurally diverse, *N*-glycans follow the same biosynthetic pathway, whereas *O*-glycans do not have a general biosynthetic route.^{9,17}

The biosynthetic pathway for *N*-glycans in eukaryotes consists of three stages. The first stage is the dolichol cycle, second is transfer of the lipid bound glycans to protein-bound glycans, and the final stage is a series of “trimming” and “processing” events generating a diverse spectrum of glycans (Figure 2).¹⁰ The dolichol cycle takes place in the rough endoplasmic reticulum (ER) and involves the addition of carbohydrate portions to a dolichol, a long chain of polyprenols consisting in total of 80 to 100 carbon atoms.^{17,18} In the dolichol cycle an *N*-acetylglucosaminyl phosphate is attached to phosphodolichol (Figure 2). The resulting diphosphate then undergoes a series of addition reactions catalysed by membrane bound glycosyltransferases to form a Glc₃Man₉(GlcNHAc)₂-dolichol (**I**). Next, the glycan-dolichol is transported to the lumen of the ER where an enzyme, called oligosaccharyltransferase, transfers the lipid bound glycan to an asparagine side chain (**II**).¹⁸ The site where the glycan is attached to the polypeptide chain contains the amino acid sequence Asn-X-Ser/Thr, where X is any amino acid except proline. However, not all Asn-X-Ser/Thr sequences are glycosylated because other factors such as sterics also influence the recognition process.¹⁹ Finally,

a series of glycosidases “trim” the Asn-bound glycan after which it is transported to the Golgi apparatus where the *N*-glycan is further processed (III).^{5,10} Post processing, *N*-glycans can be classified into three types; high mannose type, complex type and hybrid type *N*-glycans. High mannose type are the simplest form of *N*-glycan, they contain only α -mannose residues bound to the mannose capping of the common core. In complex type *N*-glycans, the trimannose capping is elongated and has a varied number of GlcNHAc and GalGlcNHAc portions. The hybrid type *N*-glycans are a combination of the other two glycan types.⁵ Interestingly, plants use the same biosynthetic pathway up to transport to the Golgi apparatus, after which plant specific enzymes modify the glycan further to give rise to more plant specific glycoforms.¹⁷

Figure 2. A pictorial representation of the biosynthesis of *N*-glycans.¹⁷

As illustrated, carbohydrates participate in many biological processes.¹ It is therefore not surprising that they also play a major role in many diseases.²⁰⁻²² As our understanding of the structure–function relationships of complex glycans increases, so does our ability to treat various diseases. Although our knowledge of carbohydrate interactions is increasing, much remains to be understood and continued research into these glycans is needed.

This thesis will discuss the total synthesis of the carbohydrate portions of a complex type *N*-glycan, to study the structure–function relationship of allergens and their carbohydrate portions, and to understand their role in asthma and other allergic diseases. The second part of this thesis will discuss the protecting group free (PGF) synthesis of iminosugars, which are potential glycosidase inhibitors. Glycosidases are useful targets in the treatment of various diseases such as, diabetes,²⁰ cancer,²¹ viral and bacterial infection²⁰ and lysosomal processing disorders.²²

Chapter 2: Introduction to N-glycans

2.1 Asthma

Asthma is a chronic respiratory disease that affects over 235 million people worldwide.²³ Most of those affected live in western countries, however 80% of deaths caused by asthma occur in low to low-medium income countries.²³ In New Zealand, over 600,000 people are affected by asthma, which as a percentage of the population is among the highest in the world.²⁴

Asthma is a recurrent disease that requires daily medication. When an asthma attack occurs, the person suffering will experience shortness of breath, wheezing and tightness in the chest. There are several triggers that can cause an attack such as exercise, stress, and for some even cold air, though allergens that are inhaled are the most common cause of asthma attacks and are responsible for 70-80% of the cases in New Zealand.²⁵ Allergens can include dust mites, pollen, plant material, food, and animal hair and dander.

Despite there being a good understanding of the general causes of an asthmatic attack, little is known about how allergens stimulate the immune system at a molecular level. Accordingly, current asthmatic medications are non-specific and alleviate the symptoms only.²⁵ These medications are either anti-inflammatory (e.g corticosteroids) or bronchodilator drugs and help by relaxing the muscle bands around the airways thus opening the airways further. Inhaled corticosteroids are a very effective anti-inflammatory drug and can be used for patients of all ages and disease severity.^{23,25} They can be used daily on a long term basis as the human body will not build up immunity towards the drugs and, when used in standard doses, show minor side effects.²⁵ For an effective treatment with corticosteroids, regular usage is required. Bronchodilators can help relieve wheezing, coughing and tightness in the chest and, as they function instantaneously, are taken when needed. However, like corticosteroids, bronchodilators do not treat the underlying cause of asthma.

Accordingly, more specific medications that treat the cause of asthma rather than the symptoms are required.

2.2 Asthma and the Immune response

When an asthmatic attack occurs, an immune response known as a T helper (Th) 2 response is triggered. T helper cells are part of a class of white blood cells called lymphocytes and play an important role in the body's natural immune defence system. There are two T-cell lineages: the CD4⁺ T-cells (which contain the CD4 protein on their cell surface) and the CD8⁺ T-cells (those containing the CD8 protein on their cell surface). When stimulated by an allergen, the CD4⁺ T-cells mature and release a variety of cytokines such as interleukin (IL)4, IL5, IL10 and IL13. This gives rise to the recruitment of other cells such as B cells, mast cells, eosinophils and basophils at the site of inflammation.²⁶⁻²⁸ During an asthma attack, these cells build up in the bronchial tubes leading to inflammation, mucous formation and the tightening of the muscles around the bronchial tube. As a result the person suffering will feel tightness in the chest and will have difficulty breathing.²⁶⁻²⁸

Though much is known about the macroscopic triggers of the Th2 asthmatic response, little is known on the microscopic level about the causes of asthma. A review of the literature, however, reveals that the structural features of allergens known to give a Th2 immune response, such as pollen,²⁹⁻³¹ schistosomes,^{32,33} and food allergens,³⁴ are remarkably similar. In these allergens, core Man₃GlcNHAc₂ pentasaccharide (shown in black, Figure 3) is glycosylated with β -1,2-D-xylose (blue) and/or α -1,3-L-fucose (red) residue(s), whilst in humans, the core Man₃GlcNHAc₂ is instead capped with high mannose, lactosamine, and sialic acid residues.²⁹⁻³⁶ These *N*-glycans are asparagine (Asn) bound in an Asn-X-Ser/Thr sequence, with X being any amino acid except proline.³⁷ It is also interesting to note that *N*-glycans found on bacteria and viruses do not contain these additional xylose or fucose carbohydrate residues and also do not stimulate the Th2 immune response.³⁸⁻⁴⁰

Figure 3. Heptasaccharide found on different allergens. (A) pollen (B) schistosoma.

To demonstrate that specific xylose and/or fucose containing glycoproteins elicit an allergic immune response, various studies have been performed using isolated glycoproteins containing *N*-glycans with α -1,3-L-fucose and/or β -1,2-D-xylose residues. These studies illustrated that such glycoproteins caused an increase in the production of Th2 specific cytokines (such as IL4, IL5, IL10 and IL13),^{29,32,33,35,36,41} and a reduction in the Th1 cytokine IFN- γ .^{36,42} Production of immunoglobulin E (IgE)^{36,43,44} (a Th2 specific antibody) and IgE binding^{43,45-47} to a number of glycoproteins from different classes of allergen (*i.e.* cross reactivity between allergens) has also been observed. When the carbohydrate residues of the glycoprotein were cleaved, IgE antibody binding was inhibited^{30,48} and there was a significant decrease in the production of Th2 specific cytokines. These results suggest that the carbohydrate moiety of the glycoprotein allergen plays a vital role in producing the Th2 immune response.

2.3 Synthetic approach for the preparation of complex *N*-glycans

In nature, *N*-glycans exist as a heterogeneous mixture of glycoforms, which provide a wide range of functional diversity. Glycoproteins play an important role in controlling protein properties such as conformation, stability, susceptibility to proteases, and

circulatory lifetime. In addition, glycans are fundamental in cell-cell interaction and immune responses.^{49–51} It is therefore not surprising to find that *N*-glycans are immunogenic. To be better able to understand the effects that these individual *N*-glycans have on the immune response, there is a need to synthesise homogeneous glycoproteins. Due to the size and complex nature of these *N*-glycans, the only reliable way in which they can be obtained as a single glycoform is through chemical synthesis. Not surprisingly, the development of synthetic methodologies for the synthesis of homogeneous glycoproteins has become the objective of many synthetic chemists and many elegant strategies have been developed.^{52–55} These approaches are outlined below and focus on the key methodologies used to conjugate *N*-glycans to proteins or peptide chains and strategies for the synthesis of the complex *N*-glycans.

2.3.1 Conjugation strategies: Native chemical ligation

Native chemical ligation, a method developed by Kent and co-workers,⁵⁶ is a technique that allows for the direct synthesis of homogeneous native backbone proteins of a moderate size. In this methodology, thioester **4** and cysteine bearing peptide **5** undergo a chemoselective thiotransesterification to give the thioester-linked species **6** (Scheme 2). Spontaneous rearrangement of intermediate **6** by nucleophilic attack of the neighbouring primary amine then yields peptide **7**, which contains a native peptide linkage at the ligation site. Application of the native chemical ligation method to the total synthesis of a protein molecule was illustrated by the preparation of human interleukin 8 (IL-8). The ligation of a 33- and 39-residue gave the full length peptide (72 AA) with a native amino acid backbone. Kajihara and Dawson proposed the first synthetic derived asparagine bound *N*-glycan using the native chemical ligation methodology.⁵⁷ In addition Bertozzi and co-workers developed milder conditions for NCL which allowed for the synthesis of homogeneous *O*-linked glycans.⁵⁵ Several methods have been developed for the glycosylation of asparagine linked glycopeptides. Lansbury and co-workers were the first to report on a convergent method for the synthesis of small glycopeptides, via the conjugation of β -glucosamine with activated asparagine on a protected peptide of 4-5 amino acids (AA) long.⁵⁸ Overcoming problems such as intramolecular cyclisation through

intramolecular reaction of the activated amino acid and the development of a deprotecting strategy that did not cleave the newly formed bond were some of the challenges they had to overcome. The limitation of this methodology is that cysteine needs to be present in the peptide chain.

Scheme 2. *Native chemical ligation.*

2.3.2 Chemoenzymatic synthesis:

Another technique that has recently been explored for the synthesis of glycopeptides is a chemoenzymatic approach which uses glycosidases for transglycosylation. For a number of years, enzymes have been used to couple lactols to peptide backbones and a number of large *N*-glycans have been synthesised in this manner, albeit in low yield (ca. 5-20%)⁵⁹⁻⁶¹ due to competing enzyme hydrolysis of the newly formed product, as well as limitations of using only natural *N*-glycans as the donor substrates. Fuijta and co-workers were the first to show that oxazoline donors, mimics of the presumed oxazolinium ion intermediate formed in a retaining glycosylation mechanism, could be used as donor substrates for both Endo M and Endo A to give a significant increase in yield (77% Endo M, 44% Endo A).⁶² In 2005, Wang and co-workers took this concept further and used Endo-A and Endo-M for the introduction of more complex natural and unnatural glycans to small proteins (7-34 amino acids), thereby broadening the substrate availability as well as allowing for higher yielding syntheses of large *N*-glycans onto small proteins (Scheme 3).⁶³ In 2006, Fairbanks and co-workers further extended the scope of this methodology, by using proteins which were released of their natural glycans. Here the glycoprotein was treated with Endo A which cleaved the 1,4 GlcNHAc₂ glycosidic linkage to leave the singular GlcNHAc

residue attached to the peptide backbone, which was then used for the enzyme assisted glycosylation.⁵⁴

Chemoenzymatic synthesis of glycopeptides has provided a great tool for the synthesis of large well-defined glycoproteins but, even though some structural variations still result in product formation, the substrates which can be used are limited. For example chemoenzymatic introduction of an α -1-3-L-fucose bearing *N*-glycan is not accessible through this method, as it does not form a substrate for any of the glycosyltransferase enzymes.

Scheme 3. Wang and co-workers coupling of oxazoline donor to HIV-1 gp41 fragment.⁶³

2.3.3 Conjugation to a carrier protein

When studying the ability of small molecules (*e.g.* haptens) to modulate the Th2 response, haptens by themselves can not elicit an immune response. To be able to induce an immune response haptens need a carrier such as a protein that can be recognised by the immune system.⁶⁴ Once the body has generated antibodies to a hapten-carrier complex, the small-molecule hapten may also be able to bind to the antibody. Examples of such carrier proteins are Keyhole Limpet Hemocyanin (KLH) and Ovalbumin (OVA).⁶⁵

For the conjugation of carbohydrate portions to a carrier protein such as KLH several methods have been developed, allowing a carbohydrate to be covalently bound either directly or through a linker to the protein. KLH is particularly suitable as a carrier protein with the presence of a large number of lysine residues containing a primary amine on the side chain, that are available for binding. One of the ways to directly conjugate a carbohydrate portion to KLH is through a reductive amination with NaCNBH_3 as the reducing agent (Scheme 4).⁶⁶ Here, the primary amine of a lysine residue attacks lactol **8** in the aldehyde form resulting in the formation of imine intermediate **9**, subsequent nucleophilic attack of the hydride then results in glycoconjugate **10**. This process results in ring opening of the terminal sugar and covalently binds the glycan to the carrier protein. While this has been proven to be an effective method for the conjugation of carbohydrates to proteins, the downside is that the terminal sugar is reductively aminated and the structure altered from its natural form. In a structure-relationship study this is not ideal.

Scheme 4. *Conjugation of carbohydrates to a protein through reductive amination.*

Alternatively, a β -glycosylamines can be conjugated to a carrier protein through reductive amination with the addition of a linker (for instance glutaraldehyde). The β -glycosylamines itself can be obtained through the Katchetkov amination reaction (Scheme 5).⁶⁷ This involves treatment of a lactol with a large excess of ammonium bicarbonate in water. Here, nucleophilic attack of ammonia on GlcNHAc **11** in the aldehyde form results in the formation of imine intermediate **12**, ring closure through nucleophilic attack of the hydroxyl at the 5-position then gives the desired glycosylamine **13**. A large excess of the ammonium source is used not only to push the equilibrium to favour the anomeric amine, but also to prevent the formation of the dimer side product that can be formed as a result of the β -glycosylamine reacting with another molecule of lactol starting material. The disadvantage of this strategy is that

several milligrams of fully deprotected oligosaccharide are needed for the final conversion to the anomeric amine, before it can be coupled.

Scheme 5. *Katchetkov amination reaction to give β -glycosylamine.*

As illustrated above the reducing end of a sugar can readily be converted to an anomeric amine when subjected to an amine source. In a similar way *N*-alkylated β -glycosylamines can readily be obtained by subjecting a reducing sugar to the desired aliphatic amine to give the corresponding *N*-alkylated β -glycosylamine in excellent yield.^{68–70} With the abundance of lysine residues on KLH it is envisioned that a glycan (**14**) can be conjugated to KLH in a similar manner without the need of an additional reducing agent like sodium cyanoborohydrate (**15**, Scheme 6). This will allow small quantities of oligosaccharide to be conjugated to KLH directly without altering the three dimensional structure of the oligosaccharide.

Scheme 6. *The direct conjugation of carbohydrates to a protein.*

2.3.4 Glycan syntheses

Several routes have been developed for the synthesis of complex *N*-glycans with each using a variety of protecting group manipulations (e.g. acetylation, benzylation, de-silylation, hydrogenation and Birch reduction). Each applying key synthetic transformations to install the structural features present in the naturally occurring glycans, such as oxidation/reduction or S_N2 displacement for the inversion of stereo centers, and glycosylation reactions (e.g. intramolecular aglycon delivery (IAD) for β -mannose coupling, and thiol and imidate couplings). Two representative synthetic routes are summarised below.

In 2007, Nakano *et al.* synthesised complex-type glycans derived from parasitic helminths (Scheme 7).⁵² The key reactions in their synthesis were the use of IAD for β -mannosylation, de-silylation under high-pressure, and glycosylation in frozen solvent, is discussed in more detail below.

Scheme 7. Target glycans derived from parasitic helminths.

In glycosylation reactions D-mannose favours the formation of the α -anomer as this leads to the favoured trans relationship between the hydroxyl at the 2-position and the anomeric hydroxyl.⁷¹ Therefore to form the necessary β -mannose linkage, their route began with the subjection of PMB-ether **17** to the IAD process using alcohol **18** as the acceptor (Scheme 8). This led to the formation of hemiacetal **19**, which subsequently underwent a MeOTf promoted glycosylation to liberate dissacharide **20** as the β -mannoside exclusively (82% yield over 2 steps).

Scheme 8. *Presumed reaction pathway of PMB ether assisted IAD.*⁷²

Dissaccharide **20** was then subjected to a TMSOTf promoted glycosylation reaction with xylose imidate donor **28** or, for the synthesis of shorter fragments, acetylated (Scheme 9). Desilylation using HF/pyridine under high pressure then yielded xylose acceptor **21a** and acetylated acceptor **21b**, which underwent MeOTf promoted glycosylation with GlcMan donor **29** to yield the penta- and tetra-saccharide precursors **22a** and **22b**, respectively. *p*-TsOH·H₂O catalysed removal of the cyclohexylidene, followed by regioselective glycosylation of the 6-position with mannosyl chloride **30** (77-89% yield) gave hexa- and pentasaccharide **23a** and **23b**. Subsequent debenzoylation, peracetylation and conversion to the trichloroacetimidate (TCA) gave donor **24a** and **24b**. Attempts were then made to conjugate donor **24a** and **24b** to a 3,6-*bis*-fucosylated glucose acceptor **32c**, however several attempts to achieve this coupling resulted in no significant product formation, highlighting the severe steric congestion of the acceptor 4-hydroxy group. Glycosylation to a 3-*O*-fucosylated disaccharide acceptor also gave poor yields (<10%). To overcome this problem, a more divergent strategy was adopted and a less sterically hindered

fucosylated acceptor (**32a** or **32b**) was coupled to **24a/b** to give the corresponding complex oligosaccharides (**25a-d**) in good yield (70-81%). With saccharides **25a-d** in hand, acceptors **26a-d** were readily obtained under mild oxidation conditions using DDQ/ Mn(OAc)₃·2H₂O to remove the PMB protecting group. Fucosylation of acceptor **26b** and **26d** with thiofucoside donor **33** proceeded smoothly, resulting in **27b** and **27d**. For the fucosylation with the 6-*O*-fucosyl bearing acceptors **26a** and **26c** however, moderate yields were obtained and a large excess of donor (5 equiv.) had to be used. In addition, poor separation of acceptor and product made this an undesirable route. It was subsequently found that the glycosylation with methylthio glycoside **33** in *p*-xylene could be improved under 'frozen conditions' (whereby the reaction in *p*-xylene was cooled to 4°C to freeze the solvent and slow down the reaction) and this resulted in near full conversion of acceptors **26a** and **26c** into **27a** and **27c**, respectively. Oligosaccharides **25a**, **25c**, **26b**, **26d** and **27a-d** were then subjected to global deprotection in a uniform manner (i.e, sequential dephthaloylation, acetylation, *O*-deacetylation, and debenzylation) to provide the target structures **16a-h**.

Scheme 9. *i* TMSOTf, **28**, 68%; *ii* Ac₂O, Py, DMAP, 97%; *iii* HF·Py, 1GPa, 88-89%; *iv* **29**, MeOTf, DTBMP, 79-94%; *v* TsOH·H₂O, 86-91%; *vi* **30**, AgOTf, 77-89%; *vii* Pd(OH)₂, cyclohexene-EtOH-AcOH (2:1:1); *viii* Ac₂O, Py, 93-94% (2 steps); *ix* N₂H₄·AcOH; *x* DBU, Cl₃CCN, 74-75% (2 steps); *xi* TMSOTf, **31a** or **31b**; *xii* DDQ, Mn(OAc)₃·2H₂O, 64-81% (2 steps); *xiii* MeOTf, DTBMP, **32**, 80-92%; *xiv* (H₂NCH₂)₂; *xv* Ac₂O, Py; *xvi* NaOMe, MeOH; *xvii* Pd(OH)₂, aq MeOH, H₂, 68-88% (4 steps).

In 2006, Fairbanks and co-workers prepared a series of oxazoline donors,⁵⁴ which could be used subsequently in Endo-A and Endo-M mediated chemoenzymatic syntheses.⁷³ Key steps in this approach were the installation of the 1,4 β -D-Man-D-GlcpNAc linkage via epimerisation of glucose disaccharide substrates by sequential triflation and S_N2 nucleophilic substitution, according to the methodology of Schmidt and Boons,^{74,75} direct oxazoline formation from the *O*-*p*-methoxyphenyl protected *N*-acetyl glucosamine and efficient endohexosaminidase catalysed glycosylation of a synthetic β -D-GlcpNAcAsn glycosyl amino acid. Again, a specific example is outlined below.

Target oxazoline **33** was obtained in 5 steps from protected precursor **34** (Scheme 10), via sequential dephthaloylation, acetylation, catalytic hydrogenolysis, acetylation and treatment with acetyl bromide, BF₃·OEt₂, and zinc iodide. Hexasaccharide **34** itself was obtained by a [3+3] glycosylation methodology involving the TMSOTf-mediated glycosylation between the reducing trisaccharide **36** and mannose-capping residue **35**.

Scheme 10. *Retrosynthesis [3+3] coupling*

Preparation of the reducing end trisaccharide **36** commenced with the selective glycosylation of diol **38** on the 3-position using a TMSOTf promoted glycosylation with mannose imidate donor **37** to give **39** as the α -product selectively (Scheme 11). Protection of the 2-position as the levulinic ester then yielded glycosyl donor **39**. Glycosylation of glucosamine acceptor **40** with the thioglycoside **39** by activation with MeOTf yielded the β -trisaccharide **41** in good yield and again complete control of the anomeric stereochemistry due to neighbouring group participation. Removal of the levulinoyl ester was achieved with hydrazine acetate ($\text{H}_2\text{NNH}_2 \cdot \text{AcOH}$), triflation of the liberated 2-position and subsequent nucleophilic substitution by treatment with *n*- Bu_4NOAc in toluene under sonication yielded trisaccharide **42**. Finally, removal of the

4,6-benzylidene protecting group using aqueous acetic acid yielded the key diol acceptor **36**.

Scheme 11. *i* TMSOTf, 50%; *ii* Levulinic acid, DCC, DMAP, 99%; *iii* MeOTf, 77%;
iv H₂NNH₂·AcOH, 89% *v* (a) Tf₂O, Py, (b) *n*-Bu₄NOAc, 90% (2 steps) *vi* aq AcOH, 86%.

To prepare the required mannose capping residue (**35**), benzyl mannoside **43** was treated with trimethylorthobenzoate and *p*-TsOH in acetonitrile to yield a bis-orthoester, which was subsequently hydrolysed by exposure to aqueous trifluoroacetic acid (TFA) to give the 2,4-di-*O*-benzoate **44** (Scheme 12). Bisglycosylation of diol **44** by treatment with boron tri-fluoride diethyl etherate and an excess of trichloroacetimidate donor **45** then yielded trisaccharide **46** in 89% yield. Removal of the benzyl protecting group by catalytic hydrogenolysis followed by treatment with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) then gave donor **35**.

Scheme 12. *i* (a) PhC(OMe)_3 , TsOH , (b) 10% aq TFA, 46% (2 steps) *ii* $\text{BF}_3 \cdot \text{OEt}_2$, 89%; *iii* H_2 , Pd (10% on C), 76%; *iv* DBU, Cl_3CCN , 92%.

2.4 Target N-glycans

To determine how different *N*-glycans influence the Th2 immune response, the heptasaccharide (Figure 4), and structural versions thereof, will be synthesised, and screened for their ability to modulate the Th2 response. To provide an optimum Th2 immune response, the glycan will be conjugated to a carrier protein (KLH). To this end the focus of this Masters thesis is the synthesis of heptasaccharide **48a**, two hexasaccharide derivatives bearing either the β -D-xylose **48b** or the α -L-fucose **48c** capping residues, the inner core $\text{Man}_3\text{GlcNHAc}_2$ **48d**, and the xylose trimer **47** (Figure 4). KLH has been chosen as a protein carrier since it contains 300-600 lysines available for conjugation and is also highly antigenic, stimulating a strong CD4^+ T cell response.

Figure 4. Synthetic targets.

The proposed retrosynthetic analysis for the preparation of glycans **48a-d** is provided in Scheme 13. Here, the target glycans **48a-d** are obtained from their fully protected counterparts via reduction of the azide and subsequent acetylation and global deprotection. The protected glycans **49a-d** are in turn formed via a [5+2] or [5+1] coupling between TCA-donor **50** and fucosylated acceptor **51** or **52**, respectively. Though Nakano *et al.* reported poor yields (<10%) when attempting a similar [6+2] coupling when using a 3-*O*-fucosylated disaccharide acceptor, we anticipated that by using more electron donating protecting groups (e.g. benzyls, and silylanes) a better glycosylation yield could be obtained and thus a more convergent (and shorter) strategy could be achieved. Donors **50a** and **b**, are in turn obtained via a *bis*-glycosylation of **53** with **54a** or **54b**, respectively, and subsequent protecting group manipulations. Trisaccharide **54a** can be obtained by the coupling of xylose donor **56** with disaccharide **55**, itself formed via the glycosylation reaction between **52** and **57** with subsequent inversion of the stereochemistry at C2' via an oxidation/reduction protocol and protecting group manipulations. Building blocks **52**, **53**, **56**, and **57** are in turn derived from their parent saccharides in 3-6 steps. Target **47** can be obtained from fully protected saccharide **54a** via reduction of the azide and subsequent acetylation and global deprotection.

Scheme 13. *Retrosynthesis of target N-glycans.*

Chapter 3: Results and discussion

3.1 Synthesis of tetra and penta-saccharide precursors

3.1.1 Overview

A key aspect of this MSc thesis is the synthesis of the non-reducing end of the target *N*-glycans **58a** and **58b** and the coupling of this to the GlcNAc monomer **52** or dimer **51** to prepare the larger *N*-glycans (Scheme 14). Formation of **58a-b** could be achieved via the glycosylation of ManGlc dimer **55**, (previously prepared as part of the candidate's Bachelor of Science degree) with mannose imidate **53**, and xylose donor **56** when necessary. The focus of the first part of this report concerns the synthesis of key tetra- and pentasaccharide, **58a** and **58b**, respectively.

Scheme 14. Retrosynthesis of the key tetra- or pentasaccharide.

3.2 Synthesis of di- and trimer acceptor precursors

The first step in the strategy, was to prepare the ManGlc core required for the synthesis of the non-xylosated *N*-glycans (Scheme 15). To this end, it was envisioned

that ManGlc dimer **55** could be benzoylated at the 2-position of the mannose residue as this would provide a protecting group that could be efficiently removed during the global deprotection strategy at the end of the synthesis. Accordingly, ManGlc dimer **55** was first treated with benzoyl chloride (BzCl) and 4-dimethylaminopyridine (DMAP) in pyridine to give an overall concentration of dimer in pyridine of 5.0 mL/mmol. Following work-up and purification by gradient flash chromatography, this gave benzoylated dimer **54b** in a 72% yield. Though this result was acceptable, it was thought that the reaction yield could be improved if the reaction was performed at a higher concentration (2.5 mL/mmol) and, in addition, if the BzCl was added drop-wise to the solution at 0 °C. Indeed, using these conditions, benzoylated dimer **54b** was obtained in an excellent yield of 92%.

Scheme 15. *Benzoylation ManGlc Dimer **54b**.*

Having prepared the core building block required for the formation of the non-xylosylated *N*-glycans, we then turned our attention to finding a suitable xylose donor for the preparation of the xylosylated *N*-glycans. To this end, thio donor **61** was first prepared from D-xylose **59** in 2-steps (Scheme 16). This derivative was initially chosen due to its ease of synthesis, with an acetate being chosen at the 2-position to ensure β -linkage through neighbouring group participation.⁷⁶ Thus, D-xylose **15** was peracetylated using Ac₂O and NaOAc to give pyranoside **60**, from which the β -anomer could be selectively recrystallized using PE/EtOAc (1:2) in 30% yield.⁷⁷ Whilst the yield for **60** was only modest, this is in agreement with literature reported yields, and the crystallinity of **60** ensures a cheap and practical route to this building block.⁷⁷ Thus, xylose peracetate **60** was treated with tin tetrachloride (SnCl₄) and ethanethiol to give thioglycoside **61** in good yield (70%).⁷⁸

Scheme 16. *Synthesis of thioglycoside donor.*

Having successfully prepared thioglycoside **61**, attempts were then made to couple this to ManGlc acceptor **55** (Scheme 17). In glycosylation reactions, hydrolysis of the donor is a common problem in the presence of trace amounts of water. To minimise hydrolysis, acceptor **55** and xylose donor **61** were thus co-evaporated three times with toluene under an argon atmosphere prior to commencing the reaction. This is undertaken to azeotropically remove the last traces of water. In addition, the CH_2Cl_2 used in the reaction was freshly distilled over phosphorus pentoxide (P_2O_5) prior to use, and 4Å molecular sieves, activated by heating under a high vacuum, were added to the reaction before the promoter was added. Having undertaken these steps, *N*-iodosuccinimide (NIS) (1.5 equiv.) and TfOH (1%) were thus added to a solution of thioglycoside **61** and ManGlc acceptor **55** at $-50\text{ }^\circ\text{C}$. There was no appreciable change in the reaction, which was unusual since the formation of a red solution is indicative of an activated thio donor. Accordingly, the reaction was allowed to warm to room temperature and an additional portion of TfOH (2%) was added. Upon the addition of the additional TfOH, a colour change to dark red was indeed observed, however no product formation could be detected, as gauged by TLC analysis. The reaction was left for a further 24 h, but after work up, only donor, hydrolysed donor, and unreacted acceptor were isolated. This finding tended to suggest that although formation of the required oxocarbenium ion had occurred, (**62**, Figure 5), the required glycosylation reaction at the 2-position of mannose residue was slow, as evidenced by the formation of hydrolysed donor. Given the poor reactivity of the acceptor, a more reactive xylose donor was prepared.

Scheme 17. Attempted at the glycosylation reaction of ManGlc acceptor with thiodonor **17**.

Figure 5. Mechanism of thioglycoside donor activation with NIS and TfOH.

Trichloroacetimidates are valuable glycosyl donors that can be activated under mild conditions and have been successfully used for the preparation of a variety of complex carbohydrates.^{52,54,79,80} The most common type of imidate used is the trichloroacetimidate, however, more recently, an *N*-phenyl trifluoroacetimidate has been synthesised as this is less prone to imidate rearrangement.⁸¹ The disadvantage of using an *N*-phenyl trifluoroacetimidate, however, is that unlike the trichloroacetimidate, which is readily prepared using commercially available trichloroacetonitrile, trifluoroacetimidoyl chloride must first be synthesised. As we had an interest in preparing both xylose imidates to compare how these performed in glycosylation reactions with ManGlc acceptor **55**, trifluoroacetimidoyl chloride **67** was thus obtained in a one-pot procedure (Scheme 18). Here, triphenylphosphine, CCl₄, and triethylamine were added together followed by the drop-wise addition of trifluoroacetic acid (TFA) at 0°C. Aniline was then added drop-wise and the reaction initiated by gentle heating at which point white solids could be observed in the flask. Following completion of the reaction, dry hexanes were added and the resulting solids

filtered and washed. The mother liquor was then concentrated and the crude oil purified by vacuum distillation (52-54°C, 8 mbar) to give trifluoroacetimidoyl chloride **67** in 42% yield.⁸² Mechanistically, first chloride **63** forms via nucleophilic attack of the Ph_3P on the carbon of CCl_4 (Scheme 18). Second, nucleophilic attack of TFA on the phosphonium cation then forms intermediate **64**, aniline then reacts with the carbonyl carbon of **64** to form **65**. Imine formation followed by nucleophilic attack on another portion of phosphonium cation then forms imine **66**. Finally, displacement of $\text{Ph}_3\text{P}=\text{O}$ with chloride then results in trifluoroacetimidoyl chloride **67**.

Scheme 18. *Reaction mechanism for the formation of trifluoroacetimidoyl chloride **67**.*

Having successfully prepared, trifluoroacetimidoyl chloride **67**, both xylose imidate donors **69** and **70** were then prepared (Scheme 19). Starting from intermediate β -acetate **60**, the anomeric acetate was selectively removed using $\text{H}_2\text{NNH}_2 \cdot \text{H}_2\text{O}$ and glacial AcOH (1/1) to give lactol **68** in 60% yield. Treatment of lactol **68** with Cl_3CCN and DBU in CH_2Cl_2 then gave trichloroacetimidate **69** in a good (80%) yield.⁸³ In a similar manner, lactol **68** could be treated with *N*-phenyl trifluoroacetimidoyl chloride and caesium carbonate (Cs_2CO_3) to give the desired xylose imidate **70** in 89% yield.⁸⁴

Scheme 19. *Total synthesis xylose donors.*

With xylose donors **69** and **70** in place, attempts were then made to conjugate either donor to ManGlc acceptor **55** (Scheme 20). Again, all care was taken to ensure the reaction was free from traces of water and the donor and acceptor were co-distilled with toluene (3 x) prior to use. The first glycosylation reaction attempted involved the reaction of **55** with trichloroacetimidate **69** (Entry 1, Table 2). A sub-stoichiometric amount of TMSOTf (3%) was used and the reaction was kept at 0 °C for the duration of the reaction. After 75 min, TLC analysis of the reaction showed a higher running product ($R_f = 0.56$, 3/1, toluene/EtOAc, v/v), though as imidate donor **69** was still present, an additional portion of TMSOTf (3%) was added. This resulted in complete disappearance of the donor by TLC analysis and following work-up, the desired trisaccharide **54a** was obtained in 31% yield. Although an optimum yield was not obtained these results were encouraging, thus the reaction was repeated with donor **69** (entry 2, Table 2), this time using more activator (4%). Again the reaction was allowed to warm to room temperature and although formation of the desired product was observed via TLC analysis, product degradation was also observed, as evidenced by the presence of lower running products by TLC analysis which were subsequently determined to be trimer **54a** lacking the PMB ether and/or the benzyldiene protecting group. Nevertheless, following aqueous work-up and purification by gradient flash chromatography, the desired trimer **54a** was obtained in an improved (46%) yield. Although an improved yield was obtained this was not ideal with the degradation of product observed, and we therefore decided to explore the glycosylation of ManGlc

acceptor **55** with *N*-phenyl trifluoroacetimidate donor **70** to determine if this would lead to improved yields. Acceptor **55** was thus glycosylated with imidate **70** using 2% TMSOTf (Entry 3). TLC analysis, however, revealed the presence of unreacted donor and acceptor, and additional TMSOTf was added to the reaction mixture. Following work-up and purification, this approach, however, only gave a 20% yield of trisaccharide **54a**. With no significant improvement of yield we continue our glycosylation studies with the trichloro imidate donor **69**.

Scheme 20. *ManGlc dimer glycosidation.*

Entry	Donor	Equivalents	Conditions	Scale	Product yield	Additional activator
1	69	3.5	3% TMSOTf 0°C → rt	0.15 g acceptor	31%	3% TMSOTf
2	69	3.3	3.3% TMSOTf 0°C → rt	0.40 g acceptor	46%	4% TMSOTf
3	70	2.6	2.0% TMSOTf, 0°C	0.11 g acceptor	20%	2%, 1% TMSOTf
4	69	1.5 +2.5	2.5% TMSOTf, 0°C → rt	0.22 g acceptor	70%	2.5% TMSOTf
5	69	3.5	2.5% TMSOTf, 0°C → rt	1.15 g acceptor	72%	-

Table 2. *Attempt at forming xylose trimer 54a.*

With the best yield obtained with donor **69** so far, the manner in which the donor was added to the reaction vessel was explored (entry 4, Table 2), and in addition, the TMSOTf was freshly distilled prior to use to ensure the quality of this reagent. Indeed, by adding imidate **69** in two portions to the reaction vessel, an improved (70%) yield of trisaccharide **54a** was observed. (Table 2, entry 5). Encouraged by this result, freshly distilled TMSOTf (2.5%) was thus added to a solution of donor **69** and ManGlc acceptor **55** in CH₂Cl₂ and the reaction allowed to warm to room temperature (entry 5, Table 1). Indeed, this strategy resulted in a good (72%) yield of the desired trisaccharide **54a**, for which the identity was confirmed by HRMS [calcd. for [C₆₁H₇₆Cl₃NNaO₂₀Si]⁺: 1298.3688, obsd.: 1298.3687]. To confirm that the desired β -anomer was formed, a combination of 1D (¹H and ¹³C) and 2D (HH-COSY, HSQC and HMBC) NMR spectroscopic analyses were performed. In the ¹H NMR, a coupling of $J_{1'',2''} = 8.1$ Hz was observed for the xylose anomeric proton, which is consistent with the formation of the β -linkage. A correlation between C-1'' and H-2' in the HMBC confirmed linkage to the 2-position of the mannose residue (Figure 6).

Figure 6. Correlation between C-1'' and H-2' in the HMBC spectrum.

3.3 Synthesis of the di and trimer acceptor

Having successfully obtained trisaccharide **54a** and disaccharide **54b**, synthesis of diol acceptor, suitable for coupling with the mannose imidates to form the mannan capping residues, could then be explored. This involved removal of the *para*-methoxyphenyl ether and selective ring opening of the benzylidene to give the free 6'-OH.

First, investigations into the synthesis of the non-xylosed *N*-glycans was explored (Scheme 21). Accordingly, ManGlc dimer **54b** was treated with 5,6-chloro-2,3-cyano-1,4-benzoquinone (DDQ) in CH₂Cl₂/H₂O to give benzylidene protected dimer **71** in 77% yield (*R*_f = 0.44 Pe/EtOAc, 2/1 v/v). Here, it should be noted that careful monitoring of the reaction by TLC was required as prolonged reaction times resulted in the loss of a benzyl protecting group, as evidence by the formation of a lower running product on TLC (*R*_f = 0.31, Pe/EtOAc, 2/1 v/v) and LRMS [calcd. for [C₄₂H₅₂Cl₃NNaO₁₃Si]⁺: 934.2, obsd.: 934.2]. Selective ring opening of the 4',6'-benzylidene to the free 6'-OH position proved more difficult and several attempts were made to achieve this transformation. The use of BH₃.THF and Cu(I)Cl in CH₂Cl₂ gave only starting material despite prolonged reaction times and the use of additional portions of the reagents, while the use of BH₃.THF and Bu₂BOTf.THF in CH₂Cl₂ resulted in cleavage of the benzylidene and TBDMS protecting groups. A change to triethylsilane (Et₃SiH) and dichlorophenyl borane (PhBCl₂) however, gave the corresponding diol dimer **72** in good (81%) yield. HMBC analysis was used to confirm that the desired product had formed with a correlation between C-4' and the CH₂ of the benzyl group observed (Figure 7).⁸⁵ The regioselectivity of this reductive ring opening arises from coordination of PhBCl₂ to the less sterically hindered 6-hydroxyl, followed by reduction to give the desired 4-*O*-Bn.⁸⁵

Diol acceptor **74** was then obtained using a similar strategy as that for acceptor **72** (Scheme 22). Thus, xylose trimer **8** was treated with DDQ in CH₂Cl₂/H₂O to give the selectively deprotected trimer **73** in 62% yield (Scheme 22). Again, the reaction should be carefully monitored to avoid additional debenzylolation under extended reaction times. Selective ring-opening of the benzyldine group in **73** was then attempted. Trimer **73** was thus subjected to Et₃SiH and PhBCl₂ in CH₂Cl₂ and the reaction stirred at -78°C for 2 hr. Following work-up and purification by gradient flash chromatography, a disappointing (50%) yield of diol **74** (*R_f* = 0.46, 2/1, toluene/EtOAc, v/v), was obtained. In addition to the desired product, a side-product was also isolated (~ 35% yield), which was later identified as the deacetylated product (*R_f* = 0.14, 2/1, toluene/EtOAc, v/v) by NMR, with the absence of one of the acetates and (lower molecular weight MS, calcd. for [C₅₁H₆₈Cl₃NNaO₁₈Si]⁺: 1138.3163, obsd.: 1138.3167). During the course of the reaction, this by-product was initially mistaken for the desired product by TLC analysis and the reaction was thus left to stir for a longer time than required, thus accounting in part for the low yield of **74**. In addition, only a small *R_f* difference between starting material (*R_f* = 0.49, 2/1, toluene/EtOAc, v/v) and product (*R_f* = 0.46, 2/1, toluene/EtOAc, v/v) also meant that care needed to be taken when analysing the reaction by TLC so as to not mistake the desired product for starting material. With this knowledge, the reductive ring-opening of the benzyldine was thus repeated though this time, the reaction was quenched after 40 min, and following aqueous work-up and purification, trisaccharide **74** was obtained in a much improved 82% yield. As with dissaccharide **72**, conformation that the 6'-position contained the hydroxyl was achieved via 1D and 2D NMR analysis.

Scheme 22. Preparation of trisaccharide acceptor **74**.

3.4 Bis-glycosylation with Mannose imidate donor

The next step in the reaction sequence involved installation of the mannose capping residues. First, attempts were made to couple mannose imidate **53** to the recently prepared disaccharide **72** to form the core mannose-capped tetrasaccharide **58b** (Scheme 23). Synthesis of the mannose imidate **53** itself can be achieved in seven steps starting with D-mannose.^{79,86} This building block was previously prepared by other members of the candidate's research group and was thus readily available in the laboratory.

Scheme 23. Coupling of the mannose imidate donor to acceptor **72**.

The first attempt at forming tetrasaccharide **58b** involved the coupling of acceptor **72** with five equivalents of imidate donor **53** using TMSOTf (2%, 8 μ l of a 0.1M stock solution) as the activating agent. The reaction was started at -40 °C then allowed to warm to room temperature. Under these conditions, however, no product was observed by TLC analysis and only a lower running spot (corresponding to hydrolysed donor) was noted. The reaction was then cooled to -45 °C and two additional portions of TMSOTf (1.5% after 1 h and 2% after 3 h) were then added and the reaction allowed to warm to 0 °C in an attempt to generate the desired tetrasaccharide. Unfortunately, there was no significant product formation by TLC analysis, so an additional 2% TMSOTf was added (at 0 °C). This resulted in complete disappearance of the disaccharide acceptor **72** on TLC and a major product running higher than the diol acceptor by TLC analysis was observed. Following an aqueous work-up, ^1H NMR of the crude reaction mixture indicated that the desired tetrasaccharide had been formed, however, the complexity of the spectra and the presence of more than one product made it difficult to determine this with certainty. Attempts were then made to purify the complex product mixture by silica gel gradient flash chromatography, however, this proved problematic. Accordingly, size exclusion chromatography (Sephadex[®] LH-20, bead size 25-100 μ) was attempted as it was thought that, in addition to the desired *bis*-mannosylated product **58b**, mono-mannosylated products (*e.g.* **75a** and **75b**, Scheme 24), were formed. Fortunately, size-exclusion chromatography did allow for partial purification of tetrasaccharide in modest (*ca.* 45%) yield; (HRMS (ESI) m/z calcd. for $[\text{C}_{117}\text{H}_{124}\text{Cl}_3\text{NNaO}_{25}\text{Si}]^+$: 2098.7189, *odsd.*: 2098.7188). The two side products, however, eluted together (R_f = 0.50, $\text{CH}_2\text{Cl}_2/\text{EtOAc}$, 20/1 v/v) suggesting that they were of similar size, with high resolution mass spectrometry confirming the presence of mono-glycosylated products (HRMS (ESI) m/z calcd. for $[\text{C}_{83}\text{H}_{92}\text{Cl}_3\text{NNaO}_{19}\text{Si}]^+$: 1532.4991, *odsd.*: 1532.4993). Another attempt was then made at synthesising tetramer **58b**, this time taking into account the previous results which indicated that activation of the imidate donor **53** required > 5% of TMSOTf, with the reaction occurring at 0 °C. In addition, to encourage the reaction to go to completion, an additional portion of donor (0.5 equiv.) was added after TLC analysis indicated complete disappearance of the imidate donor and the

presence of a mono-glycosylated product. Indeed, using this approach, only minor traces of the trimer were observed and the desired tetrasaccharide **75** could be obtained in excellent (91%) yield with following purification by silica gel gradient flash chromatography.

Scheme 24. Bisglycosylation to form tetrasaccharide **58b**.

Though a benzoyl participating group at the C-2 position of imidate donor **53** is expected to give the desired α -product, this needed to be confirmed. Here, ^1H NMR could not provide conclusive proof as to the stereochemistry of the newly formed glycosylated linkages as both α - and β -mannoses give a small coupling constant for the anomeric proton ($J_{1,2} = >2$ Hz). Pedersen and co-workers, however, have shown that the $^1J_{\text{C1,H1}}$ coupling constant can be used to determine the anomeric configuration.⁸⁷ For β -anomers, a $J_{\text{C1,H1}}$ of ca. 160 Hz is observed, whereas the α -

anomers have a $^1J_{C1,H1}$ of *ca.* 170 Hz.⁸⁷ Detailed analysis of 1D and 2D NMR spectra of tetrasaccharide **58b** thus enabled all ^1H and ^{13}C resonances of the molecule to be assigned and a ^{13}C - ^1H coupled HSQC provided the coupling constants for the [1→3] and [1→6] mannose residues (Figure 8). For 1→3 linked mannose, a coupling of 173.0 Hz was observed, while for the 1→6 linked residue, a coupling of 173.1 Hz was seen, thus confirming that the desired α -linkage was obtained for both mannose capping residues.

Figure 8. Coupled HSQC proving the desired α -linkage was formed.

Having successfully prepared tetrasaccharide **58b**, it was thus expected that similar *bis*-glycosylation conditions could be used for the synthesis of target pentasaccharide **58a** (Scheme 25). Accordingly, five equivalents of mannose donor were added to a solution of acceptor **74** in CH_2Cl_2 at 0 °C and to this TMSOTf (5.5%) was added. Multiple products were observed by TLC analysis, though there was no trace of both acceptor **74** or mannose donor **53**. The reaction was then quenched with sat. aq. NaHCO_3 and the products separated by gradient flash chromatography, followed by

size exclusion chromatography, to reveal the presence of both the desired pentasaccharide **58a** as well as the mono-glycosylated product **76**. Tetrasaccharide **76** was identified to be the 1→6 mannosylated product by NMR spectroscopy with a coupling between the H-1''' and the C-6'' observed, in the HMBC which is not surprising given the greater reactivity of the less sterically hindered primary 6-hydroxyl compared to the secondary 3-hydroxyl. Having illustrated that pentasaccharide **58a** can be formed, the reaction was then repeated, again using 5 equivalents of imidate donor **53**, though this time an additional 0.5 equivalents of mannose donor **53** was added after 1.5 h to ensure that the reaction had gone to completion. No further conversion was observed after stirring the solution for an additional 2 h, so the reaction was quenched and, following an aqueous work-up, purified by silica gel gradient flash chromatography then size exclusion chromatography to give pentasaccharide **58a** in 66% yield. In addition, a 24% yield of the mono-glycosylated product **76** was obtained, indicating the difficulties of undertaking a *bis*-glycosylation on trisaccharide **74**. Applying the same coupling conditions to mono-mannosylated product **76** then gave the desired pentasaccharide **58a** in excellent (93%) yield. Taken together, this gives a combined yield of 88% for pentasaccharide **58a** (Scheme 25). NMR spectroscopy revealed the desired α -linkage was also obtained for product **58a** [α -Man (1→3) $J_{C1,H1}$ = 171.9 Hz, α -Man (1→6) $J_{C1,H1}$ = 172.7 Hz].

Scheme 25. Synthesis of pentasaccharide **76**.

3.5 Synthesis of the trichloro-imidate donor

The next step in the total synthesis was the assembly of suitable donors from tetrasaccharide **58b** or pentasaccharide **58a** to be used for the subsequent glycosylation reactions with the reducing end mono- or di-saccharides. To meet this objective, the anomeric silane from tetrasaccharide **58b** and pentasaccharide **58a** needed to be removed and a suitable leaving group then introduced at the anomeric position.

With this objective in mind, lactol **77** was thus prepared in 99% yield via the desilylation of tetrasaccharide **58b** using 70% HF in pyridine (Scheme 26).⁸⁸ To achieve this transformation, the protected tetrasaccharide **58b** was transferred to a falcon tube, dissolved in THF (freshly distilled over sodium wire), and a solution of 70% HF in pyridine added to the reaction vessel. The reaction was then left to stand until clean conversion was observed by TLC analysis ($R_f = 0.31$, 1/1, Pe/EtOAc, v/v). Using this approach, an excellent (99%) yield of the desired lactol was obtained following work up with sat. aq. NaHCO₃, and purification by gradient flash chromatography. A solution of lactol **77** in CH₂Cl₂ was then subjected to trichloroacetonitrile and DBU and the reaction stirred at room temperature for 75 min at which point complete disappearance of starting material was observed and a higher running product was formed by TLC analysis ($R_f = 0.22$, 2/1, PE/EtOAc, v/v). Given that the transformation into trichloracetimidate **50b** appeared to occur smoothly by TLC analysis, it was thus surprising to find that, after work-up and purification by gradient flash chromatography, only a 54% yield of the desired tetrasaccharide imidate **50b** was isolated. Analysis of other material that eluted from the column in more polar solvents, however, saw recovery of significant amounts of impure hydrolysed donor. Imidates are reactive intermediates which can be hydrolysed under mildly acidic conditions, and thus it was believed that the silica column had catalysed hydrolysis of imidate **50b** during purification. To overcome this problem, 1% triethylamine solution can be used to neutralize the column prior to the subjecting the crude reaction to gradient flash chromatography, however, as sufficient

material was obtained for the subsequent coupling reaction, attempts were first made to forge forward with the synthesis rather than spending time on optimising the yield for the imidate formation.

Scheme 26. Formation of trichloroimidate donor **50b**.

With the trichloroacetimidate donor **50b** in hand, an attempt was made to conjugate this with FucGlc acceptor **51** using TMSOTf as an activator (Scheme 28). To this end, a solution of imidate **50b** and FucGlc acceptor **51** were co-distilled with toluene (3 x) then dissolved in CH₂Cl₂ and the solution cooled to -40°C prior to the addition of TMSOTf (5%, via the addition of 6 µL of a 0.1 M stock solution in CH₂Cl₂). Shortly after addition of the activator, TLC analysis showed two higher running products (R_f = 0.59, R_f = 0.49 6/1, toluene/EtOAc, v/v) and remaining imidate donor. An additional portion of TMSOTf (25%, 30 µL at 0 °C) was then added which resulted in the complete disappearance of the donor. The reaction was then quenched, via the addition of triethylamine, the solvent removed under reduced pressure, and the crude reaction purified by gradient flash chromatography (PE/EtOAc, 5/1→4/1 v/v) to

provide pure samples of the two higher running products, as well as unreacted acceptor and hydrolysed donor. NMR and mass spectrometry analysis then allowed the higher running product to be identified as the desired hexasaccharide **49c** [HRMS (ESI) m/z calcd. for $[C_{157}H_{167}Cl_3N_4NaO_{33}Si]^+$: 2792.0240, odstd.: 2792.0244]. Coupled HSQC was used to confirm that the desired β -linkage was obtained ($J_{C1'',H1''} = 158.0$ Hz) as it was impossible to conclusively determine the configuration by 1H NMR due to the complexity of the 1H spectrum. HRMS of the lower running product revealed an identical molecular weight to that of the desired imidate donor **50b**, however, the 1H NMR spectra did not match that of the imidate with no distinct NH signal at 8.5 ppm and was therefore believed to be the rearranged imidate. Rearranged imidates are not uncommon when using trichloroacetimidates donors for difficult glycosylation reactions,⁸¹ and their formation can be readily explained, when a imidate is activated (**78**, Scheme 27) the imidate group leaves as an amide (**80**) during the course of the glycosylation reaction and can then subsequently react with donor starting material (**79**) to result in the formation of a rearranged imidate (**81**).

Scheme 27. *Mechanism of rearranged imidate formation.*

Overall, 15% of the desired fucose containing hexasaccharide **49c**, and 19% of the rearranged imidate, were isolated. Even though only a moderate yield of hexasaccharide **49c** was obtained, this was nonetheless promising as Nakano *et al.* reported no significant product formation (<10%) when using a similar acceptor.⁵²

Scheme 28. *First attempt at the [4+2] coupling.*

Given the generation of the rearranged imidate during the glycosylation of tetrasaccharide **50b** with the FucGlcNAc donor **51**, it was decided that an *N*-phenyl trifluoroacetimidate donor should be used. The more electron-withdrawing phenyl and trifluoromethyl groups reduce the nucleophilicity of the formed amide via delocalisation of the electron pair of the nitrogen and by reducing the electron density on the nitrogen, the amide becomes less nucleophilic, thus reducing the chance of rearranged imidate being formed. In addition to the electronic effect, the phenyl group creates more steric bulk, which also disfavours imidate rearrangement. Having previously synthesised the necessary trifluoroacetamidoyl chloride **67** for the synthesis of the xylose *N*-phenyl trifluoroacetimidate donor, this was added to a solution of lactol **77** in acetone and the solution cooled to 0 °C before caesium carbonate (Cs₂CO₃) was added and the solution stirred at room temperature for 2 hr until there was no more starting material, as determined by TLC analysis. (0). The reaction mixture was then filtered over celite to remove remaining Cs₂CO₃ and the solvent removed under reduced pressure, and imidate donor **82** was subsequently obtained in a good (81%) yield following purification by gradient flash

chromatography. Here, it should also be noted that minor amounts of an unidentified side-product ($R_f = 0.61$, 6/1, toluene/EtOAc, v/v; HRMS (ESI) m/z *odsd.*: 1998.6487) was also formed after prolonged reaction times, though this could be circumvented by careful monitoring of the reaction. To reduce the acid catalysed hydrolysis of the target imidate during silica gel gradient flash chromatography, as was previously observed for the analogous trichloroacetimate, the column was pre-neutralised by passing one column volume of 1% triethylamine in CH_2Cl_2 through the column prior to use.

Scheme 29. Formation of trifluoro imidate **82**.

With the *N*-phenyl trifluoroacetimidate donor **82** now in hand, another attempt was made to form hexasaccharide **49c** (Scheme 30). To this end, imidate **82** and acceptor **51** were subjected to a TMSOTf-promoted coupling reaction in CH_2Cl_2 with the reaction being activated with a sub-stoichiometric amount of TMSOTf (5%, 7.5 μl of a 0.1 M stock solution) and the resulting mixture left to react for 18 h at room temperature. At this point, TLC analysis indicated no product formation and the continued presence of donor imidate, so additional portions of TMSOTf (totalling greater than 1 *equiv.* over 3 h) were added. Unfortunately, this did not result in activation of the donor and the reaction was discontinued. When the solvent was changed to toluene and TfOH was used as activator, however, hexasaccharide **49c** was obtained in an improved 31% yield. In addition to the desired hexasaccharide **49c**, trehalose octamer **83** was also formed (Scheme 30), which results from the hydrolysed donor reacting with imidate donor **82**, indicating the poor reactivity of

FucGlc acceptor **51**. Although a relatively low yield was obtained for the target pentasaccharide **49c**, this was deemed sufficient considering the difficult nature of the coupling reaction due to the steric congestion of the 3-OH on the FucGlc acceptor. In addition, formation of donor trehalose suggested that an alternative donor would not give a significant increase in product formation and therefore no further optimisation was attempted.

Scheme 30. *Improved coupling reaction.*

After the establishment of a suitable method for the preparation of tetrasaccharide donor **82** and subsequent conditions for the glycosylation reaction with the FucGlc donor **51**, it was envisioned that the core (non-xylosylated or fucosylated) pentasaccharide **50d** could be synthesised using similar reaction conditions (Scheme 31). Thus, trifluoro imidate **82** and azide acceptor **52** were subjected to a TfOH-mediated glycosylation, which resulted in the formation of two higher running products by TLC analysis ($R_f \alpha = 0.47$, $R_f \beta = 0.53$, 6/1, toluene/EtOAc, v/v). Separation of the two products was achieved by careful silica gel gradient flash chromatography

to reveal the formation of an α/β mixture of **49d** (1/1) in a combined yield of 63%. Formation of the α -product was unexpected as the Troc protecting group had been used because of its ability to favour formation of the β -anomer due to neighbouring group participation.⁸⁹ In each instance, the anomeric configuration of the newly formed glycosidic bond was determined by the coupling constant from a coupled HSQC, whereby the α -anomer had a $^1J_{C1',H1'} = 176.5$ Hz, where the β -anomer had a $^1J_{C1',H1'} = 160.0$ Hz. Additionally, a small coupling constant of 3.4 Hz was observed in the 1H spectrum of the α -product, which further supports the formation of the newly formed α -linkage.

Scheme 31. Synthesis of core pentasaccharide **49d**.

Having demonstrated that the *N*-phenyl trifluoroacetimidate is a suitable donor for the glycosylation reaction of the non-xylosylated carbohydrate motifs, the glycosylation properties of pentasaccharide **58a** containing a similar imidate donor were then explored (Scheme 32). To this end, pentamer imidate **85** was obtained in two steps from pentasaccharide **58a**, the first step of which involved anomeric

desylation (mediated by HF/Pyridine) to give lactol **84** in excellent (99%) yield according to protocols previously discussed. The trifluoroimidate leaving group was introduced at the anomeric centre via treatment of lactol **84** with trifluoroacetimidoyl chloride **67** and Cs_2CO_3 in acetone at 0 °C. Purification of the crude product, obtained as a yellow oil after work-up and removal of the solvents under reduced pressure gave target donor **85** in good (80%) yield. Though this yield was good, it should also be noted that, in a similar manner to the previously prepared *N*-phenyl trifluoroacetimidate, prolonged reaction times resulted in the formation of an unknown side product ($R_f = 0.61$, 6/1, toluene/EtOAc, v/v), and thus the reaction (**84** → **85**) had to be monitored carefully by TLC. It should also be noted that a pre-neutrilsed silica column was used during the purification step.

Scheme 32. Pentasaccharide donor **85** formation.

The next step in the synthetic strategy was the synthesis of xylose hexasaccharide **49b** via a [5+1] coupling reaction between donor **85** and azide acceptor **52** (Scheme 33). To this end, trifluoro imidate **85** and azide acceptor **52** were subjected to a TfOH

mediated glycosylation reaction in toluene which resulted in the formation of a higher running product by TLC analysis ($R_f = 0.34$, 6/1, toluene/EtOAc, v/v). NMR analysis of this product revealed that although the target hexassacharide had been formed in 70% overall yield, this consisted of a 1:1 α : β mixture with an coupling of 3.4 Hz observed in the ^1H spectrum and a coupling of $^1J_{\text{C1',H1'}} = 171.6$ Hz in the coupled HSQC. Despite several attempts to separate the anomers by silica gel gradient flash chromatography, this could not be achieved at this stage of the synthesis and the hexasaccharide **50b** was thus used in the subsequent reaction as an α / β mixture.

Scheme 33. *Synthesis of xylose bearing hexa saccharide 50b.*

The last remaining target was heptasaccharide **49a**, which was accessible via TfOH-mediated [5+2] glycosylation between donor **85** and FucGlc acceptor **51** (Scheme 33). Again, traces of water were azeotropically removed from donor and acceptor with toluene (3 x) prior to use. In the first attempted glycosylation reaction, the imidate donor was activated at -40 °C using TfOH (1% as 0.1 M stock solution). After 45 min TLC analysis revealed some hydrolysed donor, suggesting activation of the donor, and

also the presence of remaining donor. Accordingly, additional portions of TfOH were added, however after work-up and purification, no product was isolated. Having observed better results when performing imidate couplings at 0°C and bearing in mind that a more stable imidate was used in this reaction, the coupling reaction was repeated though this time activation was achieved using 2% TfOH at 0 °C. These conditions resulted in complete disappearance of donor **85** and the starting material after 3 hr, and the formation of what initially appeared to be two products (R_f = 0.32 and 0.43, Pe/EtOAc, 2/1, v/v). Following work-up and gradient column chromatography (toluene/EtOAc, 2% → 6%) the acceptor could be separated from the two products, however, NMR analysis of these products revealed the presence of three products, of which the major was identified to be the desired heptasaccharide **49a**. Following careful silica gel gradient flash chromatography, the top product (R_f = 0.43) was identified as heptasaccharide **49a**, though unfortunately this contained a small amount of an unidentified side product which couldn't be separated from the target product despite several attempts. Separation of a second by-product, however, was achieved, and this product was tentatively assigned using HRMS (ESI) (HRMS (ESI) m/z $[C_{115}H_{118}Cl_3NNaO_{30}]^+$ calcd.: 2120.6696 odsd.: 2120.6777) to be trichloroethoxyoxazoline **86** (Scheme 34), which can be formed by participation of the troc protecting group. In 2005, Imamura *et al.* reported on the formation of an oxazoline by-product for some of their reactions and using TLC analysis, observed that the oxazoline was an intermediate in all of their coupling reaction.⁹⁰ Imamura also showed that the isolated the oxazoline could be coupled in absence of an activator to give the desired product in good yield (85%). It was therefore believed that a prolonged reaction time at a higher temperature and the use of more activator would reduce the formation of oxazoline side product. Accordingly, the coupling reaction was repeated, though this time additional TfOH (5%) was added at 0°C, after which time the reaction was allowed to warm to room temperature. Additional TfOH was added during the course of the reaction (5% after 3 h and 2.5% after 7 h) and the reaction was left to react for a total of 12 h. This resulted in the formation of heptasaccharide **49a** in addition some oxazoline was still formed though, given the

difficulty of this [5+2] glycosylation, it was deemed that enough of the desired heptasaccharide had been obtained.

Scheme 34. *Trifluoroimide coupling.*

3.6 Deprotection strategy

Given the lengthy synthesis of the protected oligosaccharides and the low yields obtained for some of the glycosylation reactions, a short and convergent deprotection strategy was desired so that sufficient quantities of the target *N*-glycans could be obtained. Here, the protected amines needed to be converted to their acetamide counterparts, then the aromatic, ester, and anomeric silane protecting groups needed to be removed. In addition, it is known that the α -L-fucose glycosidic bond is susceptible to hydrolysis under acidic conditions⁹¹ and thus strongly acidic conditions for the deprotection steps needed to be avoided. With the objective of preparing a variety of truncated substrates that could be coupled to KLH for biological assessment, the deprotection methodology was first developed on the small di- and tri-saccharide motifs, and then later extended to the more complex and larger oligosaccharides.

3.6.1 Conversion of the Troc- and azide-protecting amines to acetamides.

The 2,2,2-trichloroethoxycarbonyl (Troc) group can be reductively removed by activated zinc under mildly acidic conditions (Scheme 36). One acid commonly used for this purpose is acetic acid^{66,92} and accordingly, this was the first strategy used for the attempted conversion of the fully protected reducing end GlcGlc disaccharide **87** into its *N*-acylated counterpart **89** (Scheme 36). Here, oxidative insertion of the zinc into the carbon chloride bond of **87** followed by elimination results in the formation of amine **88** and 1,1-dichloroethene and CO₂ (g), subsequent acetylation then results in **89**. Troc-protected dimer **87** was thus dissolved in THF and to this activated zinc (2.5 equiv.) and acetic acid (20 equiv.) added. When no product formation was observed by TLC analysis after 8 h, additional portions of acetic acid (70 equiv.) and zinc (20 equiv.) were added. Unfortunately, despite prolonged reactions times (48 h), none of the desired amine intermediate **88** was formed.

Scheme 35. Zinc reduction of the 2,2,2-trichloroethoxycarbonyl.

Grozinger and co-workers have shown that the Troc protecting group could be removed under mildly basic conditions using ammonium acetate (pH 7.2), or mildly acidic conditions using disodium hydrogen phosphate (Na_2HPO_4 , pH 6.5) or potassium dihydrogen phosphate (KH_2PO_4 , pH 4.5), to give the desired amine products.⁹³ Mildly basic conditions were preferred due to the potential liability of the α -L-fucose glycosidic bond and these conditions were thus attempted first. To this end, Troc protected dimer **87** was dissolved in THF and activated zinc and a solution of 1 M ammonium acetate in H_2O was added. Though some product had been observed after 48 h, there was still starting material present, as evidenced by TLC analysis, and all zinc had oxidised. Accordingly, additional portions of activated zinc and acid were added, but unfortunately, the reaction did not go to completion. Repeating the reaction with 1 M disodium hydrogen phosphate gave similar results, however, the use of 1 M potassium dihydrogen phosphate gave clean conversion (by TLC analysis) of Troc-protected dimer **87** into the corresponding amine. The reaction mixture was diluted with EtOAc, filtered over celite to remove the unreacted zinc, then concentrated *in vacuo*. Acetylation of the crude reaction mixture then gave acetamide **89** in 64% overall yield following purification by gradient flash chromatography (Scheme 36). Along with the desired product, a side product containing acyl-groups at the 3', 4' and 6'-positions of the terminal glucose residue was also formed. As no baseline material was observed during the zinc reduction, it was believed that this side-product was formed during the work-up of the reaction. As both the PMB ether and benzylidene group are acid labile protecting groups, it was believed that the crude reaction mixture had become acidic when concentrated *in vacuo* and that this led to the formation of the triol which was subsequently acetylated. To overcome this problem, the crude reaction mixture can be co-concentrated with pyridine.

Scheme 36. Reductive removal of the Troc protecting group.

Having successfully converted the Troc-protected disaccharide **87** to the *N*-acylated derivative **89** using zinc and disodium hydrogen phosphate for the reduction step, similar methodology was thus used for the conversion of trisaccharide **54a** to the acetamide **90** (Scheme 37). Here, trisaccharide **54a** was subject to 30 equivalents of zinc in a 5:1 solution of THF:1 M Na₂HPO₄ in H₂O. After 24 h, TLC analysis revealed the formation of two products that could both be stained by ninhydrin on a TLC plate, thus suggesting the presence of an amine functional group in each product. Though initially confusing, the formation of the two products by TLC was later attributed to protonation/deprotonation of the product on silica, as is frequently observed when working with free amines. The crude reaction mixture was then diluted with EtOAc, filtered over celite, and pyridine added before concentrating so as to ensure the reaction did not become too acidic which would lead to a compromise in yield. The resultant oil was then subjected to pyridine:Ac₂O (2:1) for 24 h. after which EtOAc was added and the organic layer was washed with with sat. aq. NaHCO₃ to remove remaining Ac₂O. The solvent was then removed *in vacuo* and the residue purified by silica gel gradient flash chromatography to give acetamide **90** in a good (87%) yield.

Scheme 37. *Conversion of the Troc protected amine to the N-acetate.*

Having established good conditions for the conversion of the Troc-protected amine to the acetamido functionality, attention was focused on converting the azide-protected amine to its acetamido counterpart for the more complex oligosaccharides. When considering methodology to achieve this transformation, it was noted that Block and co-workers successfully developed a strategy which enabled the Troc and azide functionalities of one molecule to both be converted to their corresponding amines by way of a zinc reduction with AcOH/Ac₂O in THF.⁹⁴ As illustrated above, the

methodology that allowed for the removal Troc group from disaccharide **87** was similar to that used by Block, and thus it was envisioned that these may also lead to the conversion of an azide into a protected amine. To explore this hypothesis and avoid experimentation on the more complex, and more difficult to synthesise, oligosaccharides, azide **91** (prepared by the candidate during his bachelor's degree programme)⁹⁵ was used as a model substrate. Accordingly, azide **91** was dissolved in THF and activated zinc was added, followed by 1 M aq. Na₂HPO₄. Gratifyingly, this resulted in the smooth transformation into amine **92** (Scheme 38). Acetylation of the crude reaction mixture under standard conditions then gave acetamide **93** (85% yield), thus illustrating the potential of this methodology for the dual reduction of both a Troc-protected amine and an azide.

Scheme 38. Zinc-mediated azide reduction.

With the successful conversion of azide **91** to its *N*-acylated counterpart using activated zinc and 1 M aq. Na₂HPO₄ and the use of similar methodology for the deprotection of Troc-protecting groups (*e.g.* **54a** → **90**, Scheme 37, pg 56), attempts were then made to convert fucose hexasaccharide **49c** to its *N*-acylated counterpart **94**. To this end, hexasaccharide **49c** was dissolved in THF and activated zinc (30 equiv.) and 1 M Na₂HPO₄ was added. Further equivalents of zinc and acid were then added after 3 h and again at 5 h and 7 h, at which point no further product formation was observed by TLC. The reaction was then filtered, the solvent reduced *in vacuo*, and the residue resubmitted to the reduction conditions (60 equiv. Zn, THF/ 1 M Na₂HPO₄, 5/1 v/v). This resulted in complete disappearance of the starting material and the subsequent subjection of the crude amide to standard acetylation conditions then gave hexasaccharide in 53% yield following purification by silica gel gradient

flash chromatography. As longer reaction times were needed, a variety of by-products were also formed which could not be resolved by NMR and HRMS. Accordingly, the reduction was repeated, though this time fucose hexasaccharide **49c** was reacted with activated zinc (250 equiv.) as in previous reactions additional portions of zinc had to be added each time and rather than using 1 M Na₂HPO₄, 1 M KH₂PO₄ was used, as this reagent gave a faster conversion in the reaction of dimer **87** to **89** (Scheme 35, pg 54). The reaction was stirred for 2 h at room temperature, at which point TLC analysis revealed the complete disappearance of starting material, the solution was then filtered, the solvent removed *in vacuo* and the resulting crude oil subjected to pyridine/Ac₂O (2/1). Using these conditions, acetamide **94** was obtained in 64% yield with still some of the unknown side product formed. Although this was not a big improvement in yield, the shortened reaction time meant that KH₂PO₄ was preferred choice of acid.

Scheme 39. Acetamide formation.

Using the optimised reduction conditions, pentasaccharide **49d** was then reacted with activated zinc in THF/1M KH_2PO_4 (5/1, v/v), which resulted in the complete disappearance of the starting material and the formation of one product by TLC analysis after 1.5 h (Scheme 40). Acetylation of the crude (pyridine/ Ac_2O , 2/1) then gave penta acetamide **95**, albeit in a disappointing 29% yield. In addition to the desired *N*-acylated amine, a mixture of products was obtained though these could not be identified by NMR and/or HRMS analysis. The formation of side products can potentially be attributed to acidification of the crude upon concentration *in vacuo* as pyridine was not added at this stage. Although the yield of **95** was disappointing, sufficient material was obtained and due to the interests of time and the amount of starting materials available, a decision was made to forge ahead with the synthesis without further optimisation of this reaction.

Scheme 40. Zinc reduction of Troc and azide to the acetamide of the core penta saccharide.

The next oligosaccharide to be converted to its *N*-acylated counterpart, was hexasaccharide **49b**. As described previously (see Scheme 33, page 51), the [5+1] coupling resulted in an inseparable mixture of the α - and β - xylosylated hexasaccharide **49b**. Given that conversion of the Troc and azide functionalities in **49b** to an *N*-acetylated amine would result in a more polar product, it was anticipated that better separation could be achieved after this transformation. To this end, the anomeric mixture **49b** was converted to the corresponding acetamide by subjecting xylose hexasaccharide **49b** to the zinc-mediated reduction with activated zinc in THF/1 M KH_2PO_4 (5/1, v/v) (Scheme 41). This resulted in the formation of two products by TLC analysis. Acetylation of the crude product mixture under standard conditions then gave an α - and β -mixture of **96** in 48% yield following silica gel gradient flash chromatography, and a higher running by-product, which unfortunately could not be identified. Having obtained **96**, separation of the desired β -anomer could now be achieved via careful silica gel chromatography using a long single gradient column (PE/EtOAc, 1/1 v/v).

Scheme 41. Zinc-promoted reduction of the amine protecting groups.

The final substrate to undergo the zinc-mediated reduction was heptasaccharide **49a** (Scheme 42). Using the standard conditions developed, [zinc in THF/1M KH_2PO_4 (5/1, v/v)], three products were observed by TLC analysis after 7 h, and the reaction was then diluted with EtOAc, filtered over celite, and concentrated in the presence of pyridine. Reaction of the resultant crude oil with pyridine/ Ac_2O (2/1) then gave heptasaccharide **97** together with a side product (product/byproduct, 4.3/1).

Scheme 42. Zinc reduction of target hepta saccharide **49a**.

3.6.2 Global deprotection

Having successfully prepared the *N*-aceylated oligosaccharides, the final step in the reaction sequence was global deprotection of each of the specific substrates. Here, it was envisioned that the aromatic protecting groups could be removed by a dissolving metal reduction and that by subsequently adding methanol to the reaction flask, sodium methoxide would be formed *in situ*, which would lead to the removal of the ester protecting groups (acetate or benzoate). Removal of the anomeric silane was

then thought to occur via the use of HF/pyridine (as previously discussed, *e.g.* see Scheme 26, pg 44).

To this end, acetamide **89** was dissolved in THF and liquid ammonia was then added (via the use of a cold trap), followed by the addition of sodium until the blue colour of the solution remained (Scheme 43). The solution was then stirred for 45 min, at which point methanol was added and the solution allowed to warm to room temperature while stirring. To neutralise the reaction, DOWEX[®] H⁺ resin was added, and the solution was then filtered and concentrated under reduced pressure. To our surprise, analysis of the resulting oil by ¹H NMR spectroscopy revealed that the fully deprotected disaccharide **43** had been formed. Here, the acidic DOWEX[®] H⁺ had unexpectedly cleaved the anomeric silane and as a result, the fully deprotected product was obtained in a highly efficient manner. Following purification by reverse phase (HyperSep C18 column), disaccharide **43** was obtained in an excellent (99%) yield.

Scheme 43. *One pot three step deprotection.*

With the global deprotection strategy in place, an attempt was then made at deprotecting xylose acetamide **90** (Scheme 44). The benzyl protecting groups of Xylose acetamide **90** were thus reductively cleaved, followed by the addition of methanol to the reaction vessel and then neutralisation with DOWEX[®] H⁺. This time, removal of the anomeric silane was incomplete and a 1/1 mixture of silane protected trisaccharide **100** and deprotected trimer **99** was observed. Separation could readily be achieved by reverse phase chromatography (HyperSep C8 column) with fully deprotected trimer **99** eluting first in H₂O (52% yield), followed by silane protected

trimer **100** eluting in 50% methanol (48% yield). Removal of the *tert*-butyldimethylsilane was then achieved by dissolving the silane-protected trimer in MeOH (0.3 mL) to which approximately 20 kernels of DOWEX® H⁺ resin were added.⁹⁶ The flask was then left to stand for two days, and following purification by (HyperSep C8 column), deprotected trimer **99** was obtained in an excellent (99%) yield. When combined with the 52% yield initially obtained for the synthesis of **99**, this gives a quantitative yield for the global deprotection strategy.

Scheme 44. Deprotection of trisaccharide **90**.

Acetamide **94** was then subjected to the global deprotection strategy using the general methodology previously highlighted (Scheme 43), though multiple additions of sodium were required to maintain the blue colour of the solution during the reduction (Scheme 45). Surprisingly, NMR analysis of the crude reaction mixture revealed multiple products, some of which contained aromatic groups, thus indicating incomplete reduction of the starting material. The crude reaction mixture was then resubmitted, though this time a flow of argon was applied over the reaction vessel to ensure that the reaction remained free from H₂O. Following work-up, purification of the crude residue by C8 reverse phase chromatography revealed the formation of two major products, though these were not separable. Further purification of the products using a long C18 column however, enabled the products to be separated under the conditions tried. HRMS indicated that the product that eluted first was the

defucosylated pentamer **102** (35% yield), and that the second product to elute from the column was the desired fucose hexasaccharide **101** (37% yield). The formation of defucosylated product can be explained by the prolonged exposure to DOWEX® H⁺.

Scheme 45. Deprotection of fucose hexasaccharide **94**.

The next oligosaccharide to be deprotected was pentasaccharide **95**. To this end, **95** was dissolved in THF and liquid ammonia added, followed by sodium (Scheme 46). The reaction was kept blue for 1.5 hour via the addition of sodium, when required, after which time methanol was added and the reaction left to stand for 15 min. The remaining ammonia was removed under vacuum, then the remaining solution was neutralised with DOWEX® H⁺ and concentrated. Purification of the crude residue by reverse phase (HyperSep C8 column) and Biorad P2 size exclusion gel then gave fully deprotected pentamer **102** in a good yield (70%).

Scheme 46. *Global deprotection of the pentasaccharide.*

The next oligosaccharide to be deprotected was xylose hexasaccharide **96** (Scheme 47). Therefore **96** was subjected to dissolving metal conditions for 1.5 hours, then quenched with MeOH and the resulting solution allowed to warm up to room temperature and the excess ammonia to evaporate. After 1.5 hours the solution was neutralised with DOWEX® H⁺ and left for an additional 30 min, then filtered and concentrated. NMR analysis of the crude product revealed what appeared to be the presence of silane remaining. Therefore the crude material was resubjected to DOWEX® H⁺ for an additional 30 min to remove the remaining silane. Purification of the crude residue by reverse phase Biorad P2 size exclusion and gel (HyperSep C8 column) then gave fully deprotected hexasaccharide **103** in a 65% yield.

Scheme 47. *Global deprotection of xylose hexasaccharide*

The final product to be fully deprotected was heptasaccharide **97** (Scheme 48). Here NMR also suggested the presence of two products with the same mass as the fully protected heptasaccharide. Acetamide **97** was thus subjected to the global deprotection strategy using Birch-reduction, followed by quenching with MeOH and left to warm up to room temperature to remove remaining ammonia. The solution was then neutralised with DOWEX® H⁺, as for hexasaccharide **61** (see Scheme 45, pg 64) loss of the α-L-fucose was observed, the reaction was left for 10 min followed by filtration and concentration *in vacuo*. NMR analysis of the crude revealed some silane still present confirmed by HRMS [(ESI) *m/z* calcd. for [C₅₁H₉₀N₂NaO₃₄Si]⁺: 1325.5036, odsd.: 1325.5057] and therefore resubjected to DOWEX® H⁺ in MeOH for 20 min. Purification by Biorad P2 size exclusion an reverse phase (HyperSep C18 column) then gave heptasaccharide **104** in 65% yield.

Scheme 48. *One pot global deprotection of heptasacchatride 97.*

3.7 Conclusion

In summary, the five target structures have been synthesised, in a total of 20 steps in the longest linear sequence. Although some of the yields were moderate, this was nevertheless a notable achievement in itself. It was shown that the trifluoroimide was a good leaving group in the [4+1], [4+2], [5+1] and [5+2] coupling reactions. This approach allowed for the formation of the target structures in a more convergent manner than has been previously described in the literature for these type of structures. In addition, a highly efficient global deprotection strategy was developed whereby the target structures were fully deprotected, in only two steps, from their fully protected counterparts. Key in the deprotection was a dual reduction for the conversion of both the Troc- and azide-protected amines to the free amine in a one pot procedure. Second, a one pot global deprotection strategy for the removal of all aromatic protecting groups, ester protecting groups and the anomeric silane was found.

3.8 Future direction

Future direction of this work could investigate whether the target structures do indeed stimulate the immune system towards the Th 2 immune response, as hypothesized. In order to do this, the glycans must be conjugated to a carrier protein. If these structures show good immunological activity further research into whether more truncated versions of the full structure also induce the allergy specific response would be warranted. Figure 9 shows potential targets for further study.

Figure 9. Potential targets for future study.

Chapter 4: Introduction to azasugars

4.1 Azasugars: an overview

Since the isolation of nojirimycin (**105**, Figure 10) in 1966,⁹⁷ there has been much interest in iminosugars (or azasugars) due to their broad biological activity.^{98,99} Azasugars are structural analogues of normal sugars and contain a nitrogen atom in the ring rather than an oxygen atom. Nojirimycin, a six-membered azasugar (or piperidine), was first isolated from *Streptomyces roseochromogenes* and later from *Streptomyces nojiriensis*.¹⁰⁰ It is a potent inhibitor of α - and β -glucosidases and has anti-microbial activity. It took another ten years, however, before the first five-membered azasugar (or pyrrolidine) was isolated, this being 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP, **106**) was isolated from the leaves of *Derris elliptica*.¹⁰¹ DMDP has shown good inhibitory activity towards several glycosidases including α - and β -glycosidases. In addition to the five- and six-membered azasugars, there are three more classes of naturally occurring azasugars: the pyrrolizidines (*e.g.* casuarine **107**, isolated from *Casuarina equisetifolia* L.),¹⁰² the indolizidines (*e.g.* swainsonine **108**, isolated from *Swainsona canescens*),¹⁰³ and the nortropanes (*e.g.* calystegine A₃ **109**, isolated from *Calystegium sepium*).¹⁰⁴ All have interesting and varied glycosidase activities.⁹⁸

Figure 10. Examples of the different classes of azasugars.

4.1.1 Azasugars as glycosidase inhibitors

Glycosyl hydrolases are an important class of enzymes that play a role in many processes in the human body, and, not surprisingly, are therefore implicated in a variety of diseases^{105,106} including cancer,²¹ Gaucher's disease,²² viral and bacterial infection and diabetes.²⁰ Glycosidases catalyse the hydrolysis of glycosidic bonds (**I**) to release smaller carbohydrate motifs (**III**) (Figure 11). Key in this hydrolysis is the formation of an oxocarbenium ion containing transition state (**II**). At physiological pH, azasugars are protonated and thus have an ability to mimic this positively charged oxonium ion intermediate and bind to the active site of the glycosidase.

Figure 11. Cartoon representing the enzymatic hydrolysis of carbohydrates.

The therapeutic potential of azasugars is primarily due to their ability to mimic oxocarbenium ion transition states of glycosidase reactions.¹³ Glyset (**110**, Scheme 49) and Zavesca (**111**, Scheme 49), which are deoxy-derivatives of nojirimycin, are used for the treatment of non-insulin-dependent diabetes and function by inhibiting α -glycosidase, thus slowing down the breakdown of starch into glucose during the digestion process.²⁰ The naturally occurring 3,4-dihydroxy-2,5-dihydroxymethylpyrrolidine (**106**, DMDP) and its analogues have also shown an ability to selectively inhibit glycosidases. This inhibitory activity has been attributed to a close conformational resemblance of **106** to the presumed half-chair conformation of the oxocarbenium transition state formed in certain glycosyl hydrolases (Figure 12).^{13,107} In particular, DMDP shows strong inhibition against α - and β -glucosidases, and weak inhibition for α -L-fucosidase. In contrast, polyhydroxy-pyrrolidine **112** (Scheme 49) shows strong inhibition of α -L-fucosidase and only mild inhibition towards α - and β -glucosidases. It is this specific biological activity of each isomer that has led to much interest in the synthesis and testing of pyrrolidines over recent years.⁹⁹

Scheme 49. *Examples of potent azasugars.*

Figure 12. Comparison of DMDP with the oxocarbenium transition state generated during the hydrolysis of α - and β -glycosides.¹⁰⁸⁻¹¹⁰

4.2 Previous syntheses of azasugars

Given the therapeutic potential of azasugars, it is not surprising that a number of routes have been developed for their synthesis. Each strategy includes various key synthetic transformations such as radical reactions, aldol reactions, Michael additions, Wittig reactions, reductive amination, substitution reactions, ring-closing metathesis (RCM), and allylic alkylation. Details about these approaches can be found in a number of recent reviews on the subject matter.¹¹¹⁻¹¹⁴ Of particular focus of this MSc thesis is the synthesis of 2,5-dihydroxymethyl-pyrrolidines via a protecting-group-free (PGF) approach. To place this work into context, a survey of some recent syntheses of related compounds is discussed below.

4.2.1 The synthesis of pyrrolidines using protecting groups

In 2006, Trost *et al.* illustrated the potential of a palladium catalysed asymmetric allylic alkylation for the synthesis of a variety of iminosugars including 2,5-dihydroxymethyl-3,4-dihydropyrrolidine (+)-DMDP **106**, (-)-bulgecinine **113** and (+)-broussonetine G **114** (Scheme 50).¹¹⁵ In this work a process known as dynamic kinetic asymmetric transformation (DYKAT) was used to convert a racemic compound into a single enantiomer. Here, the starting material exists in an equilibrium and only

one enantiomer reacts. This means that the racemic starting material can be converted into a single chiral product and, in theory, 100% conversion can be achieved.

Scheme 50. *The synthetic targets of Trost et al.*¹¹⁵

To achieve their target syntheses, Trost *et al.* used DYKAT to prepare the key synthetic precursor **121** (Scheme 51). In this work, a palladium catalysed DYKAT reaction of phthalimide **115** and butadiene monoxide (**116**), mediated by the addition of chiral ligand **122** was performed. This led to the generation of enantio-pure alcohol **117** in excellent yield and enantioselectivity (99% yield, 94% ee). Removal of the phthalimide protecting group and subsequent cyclisation with triphosgene then afforded oxazolidinone **118**, which was subjected to a second palladium catalysed DYKAT reaction to yield diene **119**, again in excellent yield and enantioselectivity. Diene **119** was then protected with a benzyl group to give **120**, with this protection being necessary in order to achieve the subsequent ring closing metathesis (RCM). RCM of **120** was then achieved using Grubbs' second-generation catalyst to form the key intermediate dihydropyrrole **121**.

Scheme 51. **i** 0.4 mol-% $[(\eta^3\text{-C}_3\text{H}_5)\text{PdCl}]_2$, 1.2% (R,R)-**122**, 1% Na_2CO_3 , CH_2Cl_2 , room temp., 12 h, 99% (94% ee); **ii** ethylenediamine, EtOH; **iii** triphosgene, NaHCO_3 , 71% (2 steps); **iv** 1% $[\text{Pd}_2\text{dba}_3]$, 3% (R,R)-11, 1% DBU, CH_2Cl_2 , room temp., 8 h, 91% (dr = 93:7); **v** NaH, BnBr, TBAI; 84% **vi** 3x 0.4 mol-% Grubbs II cat., CH_2Cl_2 , 91%.

From key intermediate **121**, (+)-DMDP was synthesised in 5-steps and in an overall yield of 22% (Scheme 52). Here, intermediate **121** was hydrolysed, followed by selective protection of the secondary amine as the benzylcarbamate to give 2,5-dihydropyrrole **123**. Epoxidation of **123** with methyltrioxorhenium (MTO) then gave **124** as a single diastereomer due to hydroxyl direction, which was subsequently hydrolysed under acidic conditions to give the *trans*-diol, followed by palladium-catalyzed hydrogenolysis of the CBz to afford (+)-DMDP **106**.

Scheme 52. **i** NaOH, EtOH/ H_2O 3:1, reflux; **ii** $\text{BnOCOC}_2\text{H}_5$, NaHCO_3 , Na_2CO_3 , H_2O , 0 °C, 99% (2 steps); **iii** 0.4 mol-% MTO, urea- H_2O_2 , CH_2Cl_2 , 40 °C, 91%; **iv** TFA, THF, H_2O , 65 °C, 72%; **v** Pd/C, H_2 , HCl, MeOH, 96%.

(-)-Bulgecinine **113** was also readily obtained from common intermediate **121** (Scheme 53). For this synthesis, selective hydroboration-oxidation of **121** with 9-borabicyclo(3.3.1)nonane (9-BBN), sodium hydroxide and hydrogen peroxide gave **125** as a single product due to the steric influence of the 6-*O*-benzyl. Sequential protection of the secondary hydroxyl with a benzyl group gave carbamate **126**, which was hydrolysed, and the resulting amine subsequently protected to afford benzyl carbamate **127**. The synthesis was completed by way of a TEMPO oxidation of the primary alcohol to the carboxylic acid followed using global deprotection by palladium-catalyzed hydrogenolysis to give **113** in a total of 12-steps and 18% overall yield.

Scheme 53. **i** 9-BBN, NaOH, H₂O₂, 75%; **ii** NaH, BnBr, TBAI, THF 92%; **iii** NaOH, EtOH/H₂O 3:1, reflux; **iv** BnOCOCl, NaHCO₃, Na₂CO₃, H₂O, 0 °C, 85% (2 steps); **v** TEMPO, NaOCl, KBr 75%; **vi** Pd/C, H₂, MeOH 95% (2 steps).

In 2006, Fernández and co-workers reported on an improved syntheses of DMDP **106** and 2,5-dideoxy-2,5-imino-D-glucitol DGDP **128** by applying an effective intramolecular benzyl protection/delivery system (Scheme 54).¹¹⁶ The synthesis started by subjecting D-fructose-derived acetonide **129** to α,α' -dibromoxylene (**130**) to afford 3-*O*-(2-bromomethyl)benzyl ether **131** in excellent (91%) yield. Selective acid-catalyzed hydrolysis of the 4,5-*O*-isopropylidene group then provided diol **132**, which was activated by treatment with NaH in *N,N*-dimethylformamide (DMF) to give alcohol **133** containing a fused eight-membered ring between the C-3 and C-4 positions. Conversion of **133** into the iodide and subsequent treatment with sodium

azide then gave azide **134** with overall retention of stereochemistry. Removal of the acid labile protecting groups in **134** and catalytic hydrogenation then resulted in a one-pot reductive amination-cyclisation to yield DMDP **106** in a total of seven-steps and an impressive 61% overall yield. Formation of azide **135** with inverted stereochemistry at the 5-position, formed through azide substitution of the triflate derived from **133**, led to the preparation of DGDP **128** in 6-steps and an overall yield of 55%.

Scheme 54. **i** NaH, DMF 91%; **ii** 60% aq. AcOH, 45 °C, 87%; **iii** NaH, DMF 93%; **iv** I₂, PPh₃, imidazole, toluene 80 °C, 98%; **v** NaN₃, DMF, 80 °C 91%; **vi** Tf₂O, Py, CH₂Cl₂, rt; **vii** NaN₃, DMF, rt, 83% (2 steps); **viii** Pd/C, H₂, 4 bars, HCl, MeOH-H₂O 2:1, 95% for **106**, 96% for **128**.

4.2.2 The protect-group-free (PGF) synthesis of pyrrolidines:

To date, two different approaches have been used for the synthesis of pyrrolidines without the need of protecting groups. In 2005, Lindström *et al.* devised an extremely efficient PGF asymmetric synthesis by applying a double epoxidation approach to give azasugars **136** (Scheme 55).¹¹⁷ Later, in 2009, the group of Stocker and Timmer developed a PGF I₂-mediated carbamate annulation approach for the synthesis of a variety of pyrrolidines.¹¹⁸

The asymmetric approach developed by Landström *et al.* provided pyrrolidine **136** in only 4-steps from cheap, commercially available 1,6-dibromodiene **137**, and in an overall yield of 60% (Scheme 55). In this route, subjection of **137** to a modified Sharpless asymmetric dihydroxylation gave diol **138** in good yield and excellent enantioselectivity. The allylic position was then hydrolysed to yield triol **139**. Formation of epoxide **140** proved more difficult and several attempts were made to obtain the desired product, when triol **139** was subjected to catalytic dinuclear peroxotungstate, epoxide **140** was obtained in excellent yield and selectivity (99%, 92% ds). Finally, displacement of the bromide with ammonia by subjecting **140** to 10% ammonia in water resulted in spontaneous cyclisation and gave pyrrolidine **136** in 88% yield. Remarkably, all reactions were performed in water and showed excellent selectivity.

Scheme 55. **i** AD-mix α , NaHCO_3 , $\text{Me-SO}_2\text{NH}_2$, $\text{H}_2\text{O}/t\text{-BuOH}$ (1:1), 0 °C, 70% (97% ee); **ii** H_2O , 50 °C, 98%; **iii** H_2O_2 , cat. $\text{K}_2[\text{W}_2\text{O}_3(\text{O}_2)_4(\text{H}_2\text{O})_2]$, H_2O , rt, 99%, (92% ds); **iv** 10% $\text{NH}_3/\text{H}_2\text{O}$, 4 h, 88%.

In the PGF synthesis of hydroxyl-pyrrolidines, the group of Stocker and Timmer developed two new reaction methodologies, the first being a reductive amination using ammonia as the nitrogen source, and the second involving an I_2 -mediated carbamate annulation.^{111,118-121} An illustrative example of this approach is given in scheme 57, whereby 1,4-dideoxy-1,4-imino-L-lyxitol **141** was prepared in five steps with an overall yield of 55%.¹¹⁸ Here, D-ribose **142** was subjected to Fisher glycosylation, followed by selective iodination of the primary hydroxyl to yield methyl iodoglycoside **143** in 67% over 2 steps. Applying the developed Vasella reductive amination gave alkenylamine **144** in an excellent (91%) yield, and carbamate **145** was then obtained via a halocyclization/carbonylation with iodine and NaHCO_3 again in great stereoselectivity and excellent yield (93%). Finally, hydrolyses of **145** with NaOH in ethanol gave 1,4-dideoxy-1,4-imino-L-lyxitol **141**.

Scheme 56. **i** *AcCl, MeOH, 24h*; **ii** *PPh₃, I₂, imidazole, THF, reflux, 2 h, 67% (two steps)*; **iii** *Zn, NH₄OAc, NH₃, NaCNBH₃, EtOH, reflux 18 h, 91%*; **iv** *I₂, NaHCO₃, H₂O, rt, 18 h, 93%*; **v** *NaOH, EtOH, reflux, 2 h, 97%*.

The exact mechanism of the key I₂-mediated carbamate annulation has not yet been determined, however three possible pathways have been proposed. It is known that the primary iodine is a key intermediate *en route* to the formation of the carbamate,¹²² and from this species, the target carbamate can be formed via an aziridine intermediate and subsequent ring opening by CO₂ (route I, Scheme 57), via displacement of the iodide to give a carbamate which then undergoes ring closure (route II), or by the formation of carbamic acid (III). Results to date indicate that the aziridine is not an intermediate in the reaction as subjection of an aziridine to the I₂-intermediate annulation conditions does not yield the corresponding carbamate.¹²² Investigation into the other two pathways is currently on-going.

Scheme 57. *Three possible mechanisms for the formation of the carbamate product.*

4.2.3 The PGF synthesis of piperidines

In addition to the synthesis of pyrrolidines, the Stocker-Timmer PGF methodology also has been used for the synthesis of piperidines.^{123,124} Key in this work was the optimisation of a carbamate annulation that favors the formation of six-membered carbamate containing piperidine over the five-membered counterpart.

The PGF synthesis of 1-deoxygalactonojirimycin (DGJ) **146**, currently in clinical trials for the treatment of fabry's disease, is discussed below (Scheme 58). When commercially available methyl α -D-galactopyranoside **147** was treated with triphenylphosphine, imidazole and iodine, only a modest yield of **149** (49%) was obtained in addition 45% of methyl 3,6-anhydro- α -D-galactopyranoside was formed preventing purification by crystallisation. Accordingly, another route was developed for the formation of **149**, starting from acetonide **148** via iodination, then removal of the acetonites followed by methylation of the anomeric hydroxyl. This route was preferred when multigram quantities were required as all intermediates could be

purified by crystallisation or distillation. Glycoside **149** was then readily converted to the desired alkenylamine **150** with the developed Vasella reductive amination in good yield (85%). When alkenylamine **150** was subjected to the carbamate annulation along with the desired carbamate **151a**, the trans product **151b** and furan **151c** were also obtained. The best ratio (**151a**:**151b**:**151c**, 3:1:1) was obtained when a large excess of iodine was used and the reaction was performed at 50°C. Hydrolyses of carbamate **151a** with NaOH in refluxing ethanol then gave DGJ **146**, providing the first example of an iodine mediated-carbamate annulation used in the formation of piperidines.

Scheme 58. **i** I_2 , PPh_3 , imidazole, THF, reflux; 49%; **ii** I_2 , PPh_3 , imidazole, THF, reflux; 85%; **iii** MeOH, HCl aq, rt, 92%; **iv** Zn, $NaCNBH_3$, NH_3 , NH_4OAc , EtOH, reflux, 85%; **v** I_2 , $NaHCO_3$, H_2O , 50°C; **vi** NaOH, EtOH, reflux, 40% from **150**.

4.3 PGF synthesis of 2,5-dimethylhydroxy-pyrrolidines

The objective of this work is the PGF synthesis of 2,5-dimethylhydroxy-pyrrolidines. These compounds have important biological activities making them attractive synthetic targets, and moreover, provide an interesting chiral scaffold by which to explore the scope and limitation of the PGF reductive amination and carbamate annulation strategy previously developed for the synthesis of other azasugars.

4.3.1 Why protecting-group-free synthesis?

As illustrated, many elegant syntheses have been developed for the synthesis of pyrrolidines both with or without the use of protecting groups. Protecting groups are often incorporated into a synthesis to 'mask' competing reactive centres around a molecule, however the down-side of this approach is that the use of protecting groups inevitably leads to additional steps in the total synthesis. In addition, the introduction and removal of protecting groups leads to a loss of material (as quantitative recovery is not possible), and the generation of more waste.¹²⁵ Another disadvantage of protecting groups is that organic solvents are typically required for the reaction, work-up and purification, again this also increases cost and waste. Thus, while many elegant syntheses incorporating the use of protecting groups have been reported over the years, there has been a growing interest in the development of PGF-methodology.¹²⁵⁻¹²⁷ The development of a PGF synthetic strategy, however, requires the careful planning of reaction sequences to enable chemoselective reactions to occur when more than one potentially reactive functional group is present.¹²⁵

4.3.2 Retrosynthetic plan for the synthesis of 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidines

The retrosynthetic plan for the PGF-synthesis of 2,5-dimethylhydroxypyrrolidines is presented (Scheme 59). Here, it is envisioned that the target pyrrolidine **152** can be obtained via the hydrolysis of carbamate **153** itself accessible by iodine-mediated carbamate annulation of alkenylamine **154**. Whilst the stereochemistry of the newly formed chiral centre (C5) cannot be conclusively defined at this stage it is envisioned

that this will be *cis* to C4.¹²⁰ Amine **154**, in turn, is accessible through Vasella reductive amination of known methyl iodoglycoside **155** using ammonia as the nucleophile. The facial selectivity of the reductive amination will determine the stereochemistry at the C2 of the target pyrrolidine and it is anticipated that selectivity could be enhanced by the chirality at C3 and/or the use of chelating reagents. The required iodoglycoside **155** can readily be obtained from D-fructose **156** in 2-steps using known methodology.¹²⁸

Scheme 59. *Proposed retrosynthesis of 2,5-dihydroxymethyl-pyrrolidines.*

As illustrated in the retrosynthetic analysis, the two key synthetic transformations required to prepare the target 2,5-dimethylhydroxypyrrolidine **152** are the reductive amination and the I₂-mediated carbamate annulation. Though both methodologies have been previously used during the synthesis of less complex pyrrolidines,^{120,121} there are a number of factors that should be considered when attempting to apply these methodologies to the synthesis of 2,5-substituted pyrrolidines and will be discussed below.

4.3.3 Reductive amination as a key step in the synthesis of 2,5-dimethylhydroxy-3,4-dihydroxypyrrolidines

The Vasella reductive amination developed by the Stocker/Timmer group allows for the synthesis of linear alkenyl amines from methyl iodoglycosides without the need for protecting groups. The reaction consists of two parts, the Vasella reaction to give the linear alkenal, followed by a reductive amination (with ammonia as the amine source) to give the primary amine (Scheme 60). Starting from methyl iodoglycoside **143**, the original application of this methodology can be illustrated. Here, the Vasella proceeds by oxidative insertion of zinc into the iodine-carbon bond to form intermediate **157**, which undergoes reductive ring-opening to form the aldehyde product **158**. Applying similar conditions, Madsen and co-workers were able to form ketone **160** from benzyl protected methyl iodoglycoside **159**.¹²⁸ It is envisioned that when applying similar conditions to known D-fructose derived methyl iodoglycoside **155**, the unsaturated ketone intermediate **161** can be formed. Both the aldehyde **158** and ketone **161** can then be reduced via the agency of NaCNBH₃ and NH₃ to give the corresponding primary amines, **144** and **154**, respectively.

Scheme 60. The Vasella reductive amination.

In the reductive amination of aldehydes with ammonia, dimer formation is often observed (Scheme 61). Here, aldehyde **162** reacts with ammonia to form imine **163** that is subsequently reduced to give primary amine **164**. The more reactive primary amine **164** can then undergo another reductive amination sequence to give **165** which is again reduced to give the undesired secondary (**166**) or tertiary (**167**) amine product. To avoid dimer formation, protected amines are traditionally used, which are subsequently removed in a later step. In 2004, Miriyala *et al.* had shown that a variety of aromatic and aliphatic ketones could be reductively aminated to the corresponding amine using a large excess of ammonia in ethanol as the nitrogen source. When applying this methodology to aldehydes only dimer products were observed.¹²⁹ In 2009, Dangerfield *et al.*, however, were able to optimise the metal hydride/ammonia-mediated reductive amination to give the primary amines selectively with no or minimal formation of the secondary amine by-product.¹¹⁹ This involved the use of a large excess of ammonia, a pH of approximately 12 and an aldehyde concentration of 50 mM in sat. NH_4OAc in ethanol.

Scheme 61. Reductive amination with ammonia.

While there is much precedent to suggest that the D-fructose derived methyl iodoglycoside **155** can be converted to the desired secondary amine without the need to use a protecting group, one key difference between this reductive amination and that used by the group of Stocker/Timmer to produce primary amines is the

generation of a chiral centre during the reduction of ketone **161** to amine **154**. Two potential models to rationalise the stereoselective outcome of the reductive amination are the Cram chelating model,¹³⁰ and the Felkin Anh model (Figure 13).¹³¹ In the Cram chelating model, the Zn^{2+} coordinates to the α -hydroxyl and imine, forming a five-membered ring which fixes the conformation. Nucleophilic attack then takes place from the least hindered side (here from the top face) resulting in the formation of the *syn*-product. When applying the Felkin Anh model to the imine intermediate again the amine **154a** is favoured. Though these models predict for α -hydroxyketones to be reduced *anti* to the chiral hydroxyl group, the presence of multiple free hydroxyls in **155** (all of which could be involved in a chelation controlled reduction), makes it difficult to predict the stereochemistry at C2 at the onset of the synthesis.

Figure 13. Cram-chelate and Felkin Anh model in the prediction of the stereochemical outcome of the reductive amination.

4.3.4 Carbamate annulation as a key step in the synthesis of 2,5-dimethylhydroxypyrrolidines

There are a number of factors to consider when applying the I₂-mediated carbamate annulation to the synthesis of 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidines. First, there is the stereochemistry of the annulation and in particular, how the presence of a substituent at C2 influences this. The second point to consider is the regiochemistry of the annulation and whether *O*- or *N*- cyclisation will be observed.

To explain the preferred '*cis*' stereochemistry of the annulation, a model has been proposed whereby the allylic hydroxyl exerts stereocontrol over the reaction.¹²⁰ As mentioned previously (Scheme 56, pg 79), the formation of the intermediate iodide sets the stereochemistry of the newly formed bond, thus a model has been proposed whereby the iodine promoted cyclisation goes through a five-membered transition structure that allows the nitrogen to attack the I₂-ethylene complex following a S_N2 trajectory (Figure 14). In this envelope conformation, the hydroxyl substituent on the ring, depicted in blue, can be positioned in the plane of the double bond (**A**, O-in-plane), or the hydrogen in the plane (**B**, H-in-plane). With the hydroxyl in the plane of the double bond there is minimal overlap between the electron-withdrawing σ_{C-O}^* and reacting $\pi_{C=C}$ orbitals. Whereas with H-in-plane conformer the electrons of the double bond $\pi_{C=C}$ overlap with the hydroxyl σ_{C-O}^* . This destabilises the I₂-ethylene complex resulting in a higher energy transition state. As a result, in the absence of a substituent at the C2 position the only product observed is the '*cis*' product resulting from transition state **A**.

Figure 14. Proposed transition structure in the formation of the iodo-amine intermediate

When the scope of this methodology was extended for the synthesis of aminoiminohexitols,¹³² however, sometimes minor amounts of the *trans* product were also observed (Scheme 62). As illustrated, treating *syn*- α -aminonitrile with I_2 and $NaHCO_3$ resulted in the smooth formation of the carbamate product with the *cis*-carbamate as the only product observed (**I**, Scheme 62). When the *anti*- α -aminonitrile was cyclised, a slight loss in stereo-control was observed (6:1 *cis:trans*)(**II**). When applying the transition structure model to the cyclisation of α -aminonitriles, the influence that the dipole moment of the nitrile group has on the electron density of the amino nucleophile needs to be taken into consideration (Figure 15). For the *syn*- α -aminonitriles, the transition structure with 'O-in-plane' results in an angle of nitrile dipole and the attacking lone pair of the electrons on the amine of approximately 90° (transition state **C**). Therefore the nitrile substituent does not greatly affect the electron density of the amine, and thus, the transition state with 'O-in-plane' (**C**) still dominates and exclusive formation of the *cis*-carbamate is observed. For the *anti*- α -

aminonitrile however, the transition structure with 'O-in-plane' (**D**) places the dipole of the nitrile approximately 180° to the electrons of the amine. This time the nitrile does affect the lone pair of the attacking amine, withdrawing the electron density of the amine through overlap of $n-\sigma^*_{\text{CCN}}$ with the amine electron pair raising the energy of the 'O-in-plane' transition state. This brings the energy of transition state with 'O-in-plane' closer to that of H-in-plane, thus giving a 6:1 ratio of *cis:trans*.

Scheme 62. Carbamate formation of *syn*- and *anti*- α -aminonitrile

Figure 15. Transition state leading to the formation of carbamate

As illustrated, though formation of the 4,5-cis carbamate is favoured, the presence of a substituent at the 2-position can influence the stereochemical outcome. The theory to date indicates that this is due to the electronic nature of the substituent at the C-2. Thus, it will be interesting to see how the presence of a hydroxymethyl group, which is required for the synthesis of 2,5-dimethylhydroxypyrrolidines, will influence the annulation. In addition to stereochemical concern, the regioselectivity of the annulation *en route* to the synthesis of target 2,5-disubstituted pyrrolidine needs to be considered. As was illustrated in the synthesis of DGJ (Scheme 58, pg 81), competition between *O*- versus *N*-cyclisation resulted in the formation of the 5 and 6 membered rings, and similar outcome may also be observed upon attempted I₂-mediated cyclisation of alkynalamine **154**, with either pyrrolidine **167** ($\rightarrow$ carbamate **168**) or pyran **169** being formed (Scheme 63). Whilst it is expected that *N*-cyclisation to form the 5-membered ring would be favoured according to Baldwin's rules,¹³³ this outcome is not guaranteed and reaction conditions may need to be optimised to form the desired product. My finding on this will be reported in the following section.

Scheme 63. Potential cyclisation products of the iodine promoted carbamate annulation.

Chapter 5: Results and discussion: Azasugars

5.1 Synthesis of D-fructose derived methyl iodoglycoside

In order to explore the PGF synthesis of 2,5-dihydroxymethylpyrrolidines, an efficient synthesis of the precursor methyl iodoglycoside **155** was first required. To this end, D-fructose **156** was subjected to a Fischer glycosylation with methanol under kinetic conditions (Scheme 65).¹³⁴ Here, a short reaction time was essential to minimise formation of the pyranose product and D-fructose was thus treated with sulfuric acid (0.9 equiv.) in methanol at room temperature for 45 min. This resulted in complete disappearance of starting material and the formation of two new products, as gauged by TLC analysis ($R_f \alpha/\beta = 0.68$ and 0.54 6/4/1, EtOAc/isopropanol/H₂O). To neutralise the reaction, DOWEX® OH⁻ was then added,¹³⁴ however after concentration, NMR analysis revealed a complex mixture of lower running products, none of which appeared to be the desired methylglycoside. This was surprising given the apparent clean conversion of the reaction as previously observed via TLC analysis. Accordingly, it was believed that the reaction was not fully neutralised upon work-up. To ensure the quality of the DOWEX® OH⁻ resin used, the resin was activated overnight with 1M NaOH, however, when the reaction was repeated and again quenched with DOWEX® OH⁻, a similar result was observed. The choice of base for the neutralisation step was then altered and gratifyingly, it was found that when the reaction was quenched with triethylamine or co-concentrated with pyridine, the desired furanose methyl glycoside **170** was obtained in a α/β mixture of 1:1, with only a minor amount of pyranose **171** being the only other product observed (10/1). This product mixture was then used without further purification.

Scheme 64. *Fischer glycosylation to give methyl- α/β -D-fructofuranocide.*

In 2006, Madson and co-workers illustrated that the 6-position of methyl glycoside **170** could be selectively iodinated while the primary hydroxyl at the 1-position remained unreacted (Scheme 65).¹²⁸ This selectivity arises from the steric congestion of the neopentyl position, making the hydroxyl at the 1 position less reactive. Accordingly, methyl glycoside **170** was subjected to 1.5 equivalents of iodine (1.5 equiv.), triphenylphosphine (2.0 equiv.) and imidazole (2.0 equiv.) in refluxing THF for 2 h. TLC analysis revealed the formation of a single higher running product ($R_f = 0.75$ CHCl₃/MeOH, 5/1 v/v), which was unexpected when compared to the literature ($R_f \alpha = 0.67$ and $R_f \beta = 0.57$, CHCl₃/MeOH, 5/1 v/v). In addition, NMR analysis (¹H and ¹³C) did not reveal any of the desired product. With the presence of multiple free hydroxyls on methyl glycoside **170**, it was thus thought that over iodination may have occurred and the reaction was therefore repeated, this time applying a shorter reaction time (1 h). Unfortunately, this also did not result in the formation of the desired product and only a higher running product (by TLC analysis) was observed. Madson and co-workers had reported that the iodine (in a solution of THF) was added drop-wise to the reaction mixture.¹²⁸ Accordingly, this exact protocol was followed and a single product, with R_f similar to that reported, was observed by TLC analysis. Following work-up and purification by silica gel gradient flash chromatography, methyl iodoglycoside **155** was thus obtained in 65% yield over 2-steps

Scheme 65. *Selective iodination of methyl glycoside **170**.*

5.2 Synthesis of the alkenylamine

Having established a good method to form the desired methyl iodoglycoside **155**, attempts at the Vasella reduction, and the Vasella reductive amination were then made. In the initial Vasella reductive amination work presented by Dangerfield *et al.* (whereby an aldehyde was formed as a key intermediate),¹¹⁸ it was found that isolation of the aldehyde product was problematic and therefore formation of the primary amine was performed *in situ*.¹¹⁸ In the work presented herein, the fragmentation reaction of methyl iodoglycoside **155** with zinc under the Vasella conditions results in the formation of a potentially more stable ketone, and thus it was thought the ketone could indeed be isolated. Accordingly, methyl iodoglycoside **155** was subjected to zinc in ethanol and a catalytic amount of acetic acid under reflux conditions (Scheme 66), and after two hours, complete disappearance of starting material was observed by TLC analysis (**I**). Though this result was initially encouraging, both high resolution mass spectrometry and NMR analysis did not reveal any of the desired product. In the formation of benzyl protected ketone **160** from protected methyl iodoglycoside **159**, Madsen and co-workers performed the reaction at 40 °C to yield ketone **161** in 74% yield (**II**).¹²⁸ To investigate how the temperature effects the reaction methyl iodoglycoside **155** was subjected to similar conditions starting at 40 °C, however, no conversion was observed until the temperature was raised to 78 °C (reflux). Again, none of the desired ketone product was observed and only a white insoluble solid was isolated. With no product detected from the Vasella reaction, the Vasella reaction with *in situ* reductive amination was then attempted.

Scheme 66. Formation of the ketone product from the fragmentation reaction of methyl iodoglycoside **155**. I no product; II, work presented by Madson and co-workers.

5.2.1 Vasella reductive amination with Ph₂CHNH₂

In the work presented by Dangerfield *et al.*, diphenylmethylaniline (Ph₂CHNH₂) was successfully used in the Vasella reductive amination to give the secondary amine. To investigate if the secondary amine could be formed *in situ* from the unprotected iodide glycoside **155**, Ph₂CHNH₂ was therefore chosen as the amine source (**I**, Scheme 67). To this end, methyl iodoglycoside **155** was subjected to zinc, acetic acid, Ph₂CHNH₂ (1.2 equiv), and NaCNBH₃ (as the reducing agent) to yield secondary amines **172a** and **172b** as a 1:1 mixture of diastereoisomers. Whilst it was difficult to determine the yield for this reaction due problems in removing the excess Ph₂CHNH₂, **172a** and **172b** (R_f = 0.88 and 0.80 [(CH₂Cl₂/EtOH/MeOH/30% aqueous NH₃, 5/2/2/1, v/v/v/v)/CH₂Cl₂ 1/1 v/v]) were the only products observed and the transformation appeared to occur smoothly. The only slightly disappointing aspect of this Vasella reduction amination, however, was the poor diastereoselectivity and it was hoped that some facial selectivity would be observed during the reductive amination. That said, these results are in accordance with work presented by Madson and co-workers who applied the reductive amination to the benzyl protected ketone **160** with NaCNBH₃ as the reducing agent to form amine **173a** and **173b** (1:1, *R:S*)(**II**, Scheme 67). In addition, Madson and co-workers illustrated that the use of

triacetoxyborohydride or L-selectride did not result in an improvement of the stereoselectivity and mainly reduction of the ketone was observed. When indium trichloride was used as a Lewis acid (**III**, Scheme 67), a slight improvement in stereoselectivity was observed (3:2 *R:S*).¹²⁸ This modest improvement in diastereoselectivity, however, was not deemed sufficient to warrant investigation into similar indium-mediated reductive aminations of methyl iodoglycoside **155** at this stage of the synthesis.

Scheme 67. *The Vasella reductive amination.*

Having successfully prepared alkenes **172a** and **172b**, several attempts were then made to remove the excess amine (reverse phase HP20 column chromatography and silica gel gradient flash chromatography), however, this proved futile with the Ph_2CHNH_2 co-eluting with the desired product. Whilst the amount of Ph_2CHNH_2 used in the reaction could possibly be reduced to aid in this separation, it has been

previously observed that reductions in the amount of amine nucleophile used results in poorer reaction yields,¹³⁵ and it was thus decided that the excess amine could be removed after removal of the diphenylmethyl protecting group. Removal of the diphenylmethyl protecting group can be achieved under acidic conditions in the presence of a reducing agent to quench the liberated carbocation. Accordingly, a mixture of **172a-b** and Ph₂CHNH₂ were subjected to triethylsilane (Et₃SiH) in refluxing trifluoroacetic acid (TFA).¹¹⁸ This resulted in the formation of the desired alkenylamine **154a-b** in an initial 45% yield (following work-up and purification by flash silica gel column chromatography) together with a side-product (**Fout! Verwijzingsbron niet gevonden.**). Upon examination of the ¹H NMR spectrum of this side-product, the presence of an internal alkene was observed, with signals at 6.01 ppm and 5.71 ppm, each integrating for one proton. In addition, high resolution mass spectrometry gave a mass of 148.0897 [M+H⁺]. Taken together, these results suggest that alkene **174** was formed as a by-product during deprotection of amine **172a** and **172b**.

Scheme 68. *Deprotection of diphenylmethyl protecting group.*

Formation of **174** can be explained by protonation of the hydroxyl at the 4-position of **154a-b** under the acidic conditions used, with subsequent S_N2' substitution by nucleophilic attack of an hydroxyl at the allylic position **I** (**175**, Scheme 69). Alternatively, **174** can be formed via a S_N1 mechanism, whereby water leaves forming the allylic cation **176** which then undergoes nucleophilic attack by a hydroxyl to give alkene **174**. When the reaction time was shortened to 3 hours, instead of overnight,

amines **154a** ($R_f = 0.30$) and **154b** ($R_f = 0.30$) could be obtained in an improved yield of 70% over 2-steps and formation of **174** was significantly reduced (ca. 5%).

Scheme 69. *Proposed reaction mechanism of (3R)-2-Amino-hex-4-ene-1,3,6-triol*

5.2.2 Vasella reductive amination

Having successfully illustrated that the desired amines **154a** and **154b** could indeed be prepared, a protecting group free approach for the synthesis of these substrates was then explored before attempts were made to separate the two diastereomers. In 2009, the Stocker/Timmer group showed that aldehydes (*e.g.* **177**), (**I**, Scheme 70) and acetals could be reductively aminated to give the corresponding primary amines **178** with ammonia as the nitrogen source.¹¹⁹ Similar methodology was also applied in combination with the Vasella reaction to form the unsaturated primary amine product **144** from methyl iodoglycoside **143** (**II**, Scheme 70).¹²¹ Accordingly, it was envisioned that applying these conditions to methyl iodoglycoside **155** could lead to the

formation of amine **154a** and/or **154b** without the need for a temporary amine protecting group.

Scheme 70. *Examples of the reductive amination and the Vasella reductive amination developed by the Stocker/Timmer group.*

As described in the introduction to this chapter (see Chapter 4, pg 85), it is important to use a large excess of ammonia in the Vasella reductive amination, to perform the reaction at a pH of ~ 12 , and to use a low concentration (20 mL sat. NH_4OAc per mmol substrate) to prevent dimer formation. In addition, NaCNBH_3 was chosen as the reducing agent as it shows good chemoselectivity in reductive aminations,¹³⁶ reducing the intermediate imine to the desired amine without reducing the carbonyl starting material (Scheme 71). Accordingly, these conditions were then applied to methyl iodoglycoside **155**, however, unfortunately, it appeared as if none of the desired amine product was formed as observed by NMR analysis. Due to the large excess of salts used during the reaction, it was difficult to conclude with certainty, what products had been formed by NMR analysis of the crude reaction mixture. To remove the excess of ammonium salts, the crude product was thus passed through a DOWEX[®] H^+ resin. The resin would bind the amine product and allow for the excess salts to be removed by washing the column with water. The desired product could be eluted via 30% aq. ammonia.¹¹⁹ Applying the crude reaction mixture to this purification procedure, however, resulted in no observable material following elution of the

column with 15% → 30% aq ammonia. This suggests that no amine-containing product was formed.

Scheme 71. *Attempts at the reductive amination of the D-fructose derived methyl iodide **155***

A different purification procedure was then attempted in order to gain insight into what had happened during the treatment of methyl iodoglycoside **155** to the Vasella reductive amination conditions. To identify any potential non-aminated product (which would otherwise be eluted from the DOWEX® H⁺ resin along with the water-soluble salts) a different ‘desalting’ purification procedure was used (Scheme 72). Thus, following treatment of **155** to the aforementioned Vasella reductive amination conditions, 1M aq. HCl was then added to the reaction vessel to convert the NH₄OAc to NH₄Cl and acetic acid, which was then removed under reduced pressure. The NH₄Cl was then converted to NaCl by basifying with 1M NaOH (1 equiv. towards NH₄OAc) and the remaining NaCl salt, which is less soluble in ethanol, this allows for a large excess of the salt to be removed by filtration. Though this ‘desalting’ strategy proved useful for removing much of the excess NH₄OAc used during the course of the reaction, unfortunately, the complexity of the resulting ¹H and ¹³C NMR spectra provided no further insight into what was formed during the reductive amination of **155**, only that a mixture of products was obtained of which no major product(s) could be identified. In 1971, Manning *at. al* applied the reductive amination to a selection of ketones in presence of ammonium acetate and NaCNBH₃ at room temperature.¹³⁶ Since then this method has been successfully applied to a variety of ketone substrates.^{137–139} To investigate how temperature influences the reductive amination,

the Vasella reaction was performed (reflux 2 h, in presence of activated zinc and AcOH), then cooled to rt and the crude reaction mixture saturated with NH₄OAc followed by the addition of NaCNBH₃ and left for 42 hours. Unfortunately also this did not result in the desired product.

Scheme 72. Removal of ammonium acetate by desalting

5.3 The carbamate annulation

Although the synthesis of alkenylamines **154a** and **154b** could not be achieved without the use of a temporary amine protecting group, due to the interests of time, efforts were made to forge ahead with the synthesis to reach the target dihydroxylpyrrolidines. To this end, alkenylamines **154a** and **154b** were subjected to the I₂-mediated carbamate annulation conditions to determine if the desired furanose (as compared to the pyranose) chiral skeleton would be formed, and whether the carbamate annulation would indeed be *cis*-selective, as has previously been suggested.¹²⁰

As separation of the alkenylamines **154a** and **154b** proved to be difficult by gradient flash chromatography, the diastereomeric mixture of **154a** and **154b** were subjected to iodine (1.5 equiv) in sat. aq. NaHCO₃ (Scheme 73). This resulted in complete consumption of the starting material as gauged by TLC analysis, however, the carbamate products could not be visualised on TLC by staining (ninhydrin, permanganate, molybdenum, iodine in silica or 10% H₂SO₄ in EtOH). Given that a mixture of **154a** and **154b** was used and an additional chiral centre is formed in the reaction, four carbamate isomers can potentially be formed. Upon work-up (by

removal of the solvent under reduced pressure), analysis of the crude reaction mixture by ^1H NMR, revealed three products, one major and two minor, in a 6:2:1 ratio as determined by the integration of corresponding ^1H NMR signals. As discussed in Chapter 4 pg 90 in the cyclisation both five- and six-membered rings can form, with the five-membered ring as the favoured product according to the Baldwin's rules. To determine if a five- or six-membered ring is formed, HMBC long range correlation spectroscopy can be used, for five-membered rings a correlation between the 2-and 5-position can be expected whereas in six-membered rings a correlation between the 1 and 5 position can be observed. Indeed upon close examination of the HMBC NMR spectrum, for the major product, a correlation between the H-2 and C-5 was observed (Figure 16). However, for the two minor products the linkage could not be determined.

Figure 16. *HMBC correlation spectrum showing the correlation between H-2 and C-5 indicating the desired 5 membered ring had formed*

Scheme 73. *Potential products from the carbamate annulation.*

To understand more about the formation of the multiple products in the I_2 -promoted carbamate annulation, transition state models for the formation of the iodide intermediate need to be analysed. Iodocyclisation reactions are irreversible and accordingly, the relative stereochemistry of the carbamate product is determined by the relative energies of the transition states leading to the iodides.¹⁴⁰ Here, two effects can be observed. First, the stabilising electronic effect from the allylic hydroxyl (as described in Chapter 4, pg 87-89) favours the formation of a 4,5-*cis* product. Second, the presence of a hydroxymethyl group at the 2-position, represents steric bulk and may favour the 2,5-*trans* product. Indeed, it should be noted that in the work by Davies *et al.*,¹⁴¹ the presence of a $\text{CH}_2\text{CO}_2\text{tBu}$ group at the C-1 position appears to influence the stereochemical outcome of their I_2 -mediated iodoamination (with concomitant *N*-debenzylation). Though the 4,5-*cis* product is the major diastereomer (*e.g.* **I**, Scheme 74), the relative stereochemistry of the chiral-auxiliary and C-1 can reduce, or even reverse, this selectivity. This *cis*-selectivity is thought to occur via the rapid equilibrium of the two diastereomeric iodonium ions and subsequent

cyclisation as the rate-determining step. Surprisingly, halocyclisation or amidomercuration of a per-*O*-benzylated *N*-benzyloxycarbonyl protected alkenylamine derived from D-arbinose, however, resulted in preferential formation of the 4,5-*trans*-diastereomer (**II**, Scheme 74).¹⁴² This *trans*-selectivity was attributed to the differential steric requirements in the transition states, and may reflect the added steric-considerations that need to be taken into account when both a C1-substituent and an *N*-protecting group are present.^{142,143} For example, *O*-cyclisation of a similar system (where NHCbz = OH) gives the *cis*-diastereomer preferentially (9:1; *cis:trans*)(**III**, Scheme 74).¹⁴⁴ Studies within the Stocker/Timmer group, also indicate that the presence of a nitrile at the C-1 position also influences the diastereoselectivity of the carbamate annulation (see Chapter 4, pg 89), though this is thought to be due to electronic factors, as well as possible steric considerations.¹³²

Scheme 74. Examples of sterics influencing the stereoselective outcome of the halocyclisation

The transition states for all four possible carbamate diastereoisomers upon treatment of **154a/154b** to the I₂-mediated annulation are depicted in Figure 17. Here, alkenylamine **154a** can form both the 4,5-*cis* carbamate **179**, through transition state **A**, and the 4,5-*trans* carbamate **180** via transition state **B**. In transition state **A**, the allylic hydroxyl is positioned in the plane of the double bond stabilising the I₂-ethylene complex (O-in-plane), however the presence of the hydroxymethyl at the 2-position leads to negative steric interactions across the five-membered ring. In transition state **B**, the allylic hydroxyl is positioned out of the plane of the double bond (H-in-plane), and although this leads to a less stable I₂-ethylene complex, the steric interaction of the hydroxymethyl at the 2-position is minimal. Accordingly, it is likely the transition state energies of **A** and **B** will be close and that both products can be expected. For alkenylamine **154b**, two diastereoisomers can also form the 4,5-*cis* carbamate **181** from transition state **C**, and the 4,5-*trans* carbamate **182** from transition state **D**. Transition state **C** leads to the favoured 4,5-*cis* product (O-in-plane), and in addition, there is no adverse steric interaction between the 2-hydroxymethyl and the CH₂ on the 6-position of the furanose product. In comparison, transition state **D** would give the 4,5-*trans* product. This transition state would be expected to be high in energy due to the unfavourable 'H-in-plane' alignment and the steric hindrance between the hydroxymethyl and the newly formed methylene bond. Accordingly, the transition state energy of **D** relative to that of transition state **C** will be higher and as a result, only product **181** (and not **182**) is expected. Taken as a whole, one would thus anticipate carbamate **181** to be the expected major diastereomer and carbamates **179** and **180** to be the other two minor products. To determine this, the carbamates, however, need to be hydrolysed to give known azasugars as NOE (or similar) NMR studies cannot be used with certainty to predict configuration in five-membered rings.¹⁴⁵

Figure 17. Transition state models for the formation of the iodine intermediates

5.4 Hydrolysis of the carbamate product

To allow for purification of the respective diastereomers and to determine the relative stereochemistry of each product, the carbamate products were hydrolysed. This was achieved by subjecting the mixture of carbamate products to NaOH in refluxing EtOH. From the possible four carbamate products (**179**, **180**, **181** and **182**), only three potential iminosugars can be formed as carbamates **179** and **182** both lead to the formation of imine DGDP **128** upon hydrolysis (Scheme 75).

Scheme 75. *Potential product after hydrolysis of the carbamate*

To this end, the carbamates were thus subjected to NaOH in refluxing EtOH which resulted in the formation of a ninhydrin positive product on TLC, indeed suggesting that the desired imine products were formed. The crude reaction mixture was then passed over a DOWEX® H⁺ column and the column washed with water (two column lengths) The column was then washed with ethanol, and the product removed from the resin via eluting with 30% aq. ammonia. A combination of ¹H and ¹³C NMR analysis of the crude reaction mixture revealed that two products were formed in a

ratio of 3:1. Characteristic in the carbon spectrum was that for both products only three signals were observed which suggest a axes of symmetry present in both products (Figure 18). Of the three potential products only two contain a C₂ axes, these are 2,5-dideoxy-2,5-imino-L-itol **112** and DMDP (**106**) (Scheme 76). When the NMR spectra were compared to literature both products were in good agreement with the reported data. The major product could be identified as the 2,5-dideoxy-2,5-imino-L-itol **112**,¹⁴⁶ and the minor as 2,5-dideoxy-2,5-imino-D-manitol (DMDP) **106**.¹⁴⁷ Although several attempts at separating the two diastereoisomers were made by carefully columning the products on a silica column [eluent 2/1 → 0/1 brew(5/2/2/1, CH₂Cl₂/EtOH/MeOH/30% aq. Ammonia) /CH₂Cl₂] separation could not be achieved.

*Figure 18. Carbon spectrum of the crude reaction mixture, identified as 2,5-dideoxy-2,5-imino-L-itol **112**,¹⁴⁶ and the minor as 2,5-dideoxy-2,5-imino-D-manitol (DMDP) **106**.¹⁴⁷*

Scheme 76. *Imine products formed after hydrolysis of the carbamate product.*

4.5 Conclusion

In summary, the scope of the I_2 -mediated carbamate annulation has been successfully extended for the synthesis of 2,5-dihydroxymethyl-3,4-dihydropyrrolidines. During this work, it was illustrated that the presence of steric bulk, in the form of a hydroxymethyl at the 2-position, can influence the stereoselective outcome of the carbamate annulation. All this was achieved with a minimal use of protecting groups and a total of six steps.

4.6 Future directions

Although several attempts at separating the two final products were made by silica gradient flash chromatography, due to time constraints, reverse phase (HP 20, C8 and C18) remain yet to be explored. In addition, separation of the alkenylamine products could be further attempted either at the protected (**172a/172b**) or deprotected stage (**154a/154b**). Furthermore, the conditions for the reductive amination can still be optimised to eliminate the use of a protected amine and ammonia can be used as a nitrogen source instead. Finally, it would be interesting to see if the developed methodology can be applied to other ketose substrates such as Tagatose, Sorbose and Psicose.

Experimental: N-glycans

Unless otherwise stated all reactions were performed under atmospheric air. THF (Lab-Scan) was distilled from sodium wire prior to use. Triethylamine was distilled and stored over KOH pellets. Pyridine was distilled over KOH prior to use. EtOAc (Panreac), Toluene (Panreac), MeOH (Pure Science), and hexanes (Panreac). EtOH (absolute, Pure Science), AcOH (Lab Scam), Ac₂O (Acros Organics), CH₂Cl₂ (LabServ), 30% aqueous NH₃ (Fisher Science), xylose (Sigma-Aldrich), triethylsilane (Fluka), NaCNBH₃ (Aldrich), and NaOH (Pure Science) BzCl (Riedel de Haen), DMAP (Merck), DDQ (Merck) PhBCl₂ (Aldrich), MgSO₄ (Pure Science), Na₂S₂O₄ (Pure Science), sodium (Aldrich), were used as received. TMSOTf and TfOH were distilled fresh prior to use. All solvents were removed by evaporation under reduced pressure (*in vacuo*). Reactions were monitored by TLC-analysis on Macherey-Nagel silica gel coated plastic sheets (0.20 mm, with fluorescent indicator UV₂₅₄) with detection by UV-absorption (254 nm), by dipping in 10% H₂SO₄ in MeOH or 3% ninhydrin in EtOH followed by charring at ~150 °C. Column chromatography was performed on Pure Science silica gel (40-63 micron). DOWEX® H⁺ 50wx8-100 ion exchange resin was activated by overnight exposure to 1M HCl. Size exclusion column chromatography was performed on Sephadex® LH-20, bead size 25-100μ. C8 and C18 reverse phase column were performed on thermo fisher scientific HyperSep Columns. High-resolution mass spectra were recorded on a Waters Q-TOF Premier™ Tandem Mass Spectrometer using positive electrospray ionisation. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter at the sodium D-line. Infrared spectra were recorded as thin films using a Bruker Tensor 27 FTIR spectrometer, equipped with an Attenuated Total Reflectance (ATR) sampling accessory, and are reported in wave numbers (cm⁻¹). Nuclear magnetic resonance spectra were recorded at 20 °C in chloroform using either a Varian Unity-INOVA operating at 300 MHz or a Varian Unity operating at 500 MHz. Chemical shifts are given in ppm (δ) and are relative to chloroform, all given ¹³C spectra are proton decoupled. NMR peak assignments were made using COSY, HSQC, TOCSY and HMBC experiments. Melting points (Mp) were obtained on a Gallenkamp Melting Point Apparatus.

Tert-butyldimethylsilyloxy

4-O-(2-O-benzoyl-4,6-O-(R)-

benzylidene-3-O-*para*-methoxybenzyl-β-D-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamido-β-

D-glucopyranoside (54b). ManGlc dimer **55** (1.04 g, 1.0 mmol, 1 equiv.) was co-evaporated with Tol. (3x), dissolved in pyridine (2.6 mL), and cooled down to (0°C), benzoylchloride (0.24 mL, 2.0 mmol, 2.0 equiv.) was added dropwise, followed by *N,N*-dimethylaminopyridine (DMAP) (12 mg, 0.017mmol, 0.02 equiv.). The resulting mixture was allowed to warm up to rt and left to react for 14h. The reaction was then diluted with EtOAc and the organic fraction washed with sat. aq. NaHCO₃ (3x), brine, dried over MgSO₄, filtered and concentrated *in vacuo*. Purification of the residue by column chromatography (PE/EtOAc, 10/1 → EtOAc,

v/v) yielded the title compound **54b** (1.05 gr, 0.94 mmol, 92%) as a colourless oil. R_f : 0.54 (PE/EtOAc, 1/1, v/v); $[\alpha]_D^{20}$: -0.5 (c = 1.0 in CHCl_3); IR (v): 3351.2, 3033.1, 2929.9, 2858.0, 1724.0, 1585.4, 1513.5, 1453.5, 1362.1, 1314.4, 1270.0, 1249.6, 1174.3, 1151.7, 1091.5, 1068.3, 1005.0, 910.8, 732.6, 671.6 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3): δ 8.08 (dd, J = 2.0 Hz, J = 8.3, 2H, CH_{arom} Bz), 7.53 (t, J = 7.5, 1H, CH_{arom} Bz), 7.51 (dd, J = 1.9 Hz, J = 8.0, 2H, CH_{arom} Bz), 7.40-7.34 (m, 9H, CH_{arom}), 7.27-7.24 (m, 8H, CH_{arom}), 7.17-7.14 (m, 2H, CH_{arom}), 6.82 (m, $J_{a,b}$ = 8.5 Hz, 2H, CH_{arom} PMB), 5.70 (d, $J_{1',2'}$ = 2.9 Hz, 1H, H-2'), 5.60 (s, 1H, CHPh), 5.36 (d, $J_{a,b}$ = 11.5 Hz, 2H, CH-a 3-*O*-Bn, NH Troc), 4.81 (s, 1H, H-1'), 4.76 (d, $J_{1,2}$ = 7.8 Hz, 1H, H-1), 4.72 (d, $J_{a,b}$ = 11.9 Hz, CH-a 1H, 6-*O*-Bn), 4.65 (d, $J_{a,b}$ = 11.0 Hz, CH-a 1H, 3'-*O*-PMB), 4.57 (bdd, $J_{a,b}$ = 11.7, $J_{a,b}$ = 21.2, 2H, CH_2 Troc), 4.52 (d, $J_{a,b}$ = 12.0 Hz, 1H, CH-b 3'-*O*-PMB), 4.49 (d, $J_{a,b}$ = 12.2 Hz, 2H, CH-b 6-*O*-Bn), 4.43 (d, $J_{a,b}$ = 11.4 Hz, 2H, CH-b 3-*O*-Bn), 4.27 (dd, $J_{6a,6b}$ = 4.9 Hz, $J_{5,6a}$ = 10.5 Hz, 1H, H-6a), 4.07 (t, $J_{3,4}$ = $J_{4,5}$ = 9.1 Hz, 1H, H-4), 4.01 (t, $J_{3',4'}$ = $J_{4',5'}$ = 9.4 Hz, 1H, H-4'), 3.79 (dd, $J_{6a',6b'}$ = 3.2 Hz, $J_{5',6'}$ = 11.2 Hz, 1H, H-6b'), 3.77 (s, 3H OMe, PMB), 3.77 (t, $J_{6a,6b}$ = $J_{5,6}$ = 13.4, 1H, H-6a), 3.68 (dd, $J_{6a,6b}$ = 2.2 Hz, $J_{5,6}$ = 11.3, 1H, H-6b), 3.65 (bt, $J_{2,3}$ = $J_{3,4}$ = 9.5 Hz, 1H, H-3), 3.61 (dd, $J_{2',3'}$ = 3.4 Hz, $J_{3',4'}$ = 9.7 Hz, 1H, H-3'), 3.41 (bd, $J_{4,5}$ = $J_{5,6}$ = 9.6 Hz, 1H, H-5), 3.25 (dt, $J_{5',6'}$ = 4.9 Hz, $J_{5',6'}$ = $J_{4',5'}$ = 9.8 Hz, 1H, H-5'), 3.22 (1H, H-2), 0.86 (s, 9H, *t*-Bu, OTBDMS), 0.09 (s, 3H, Me, OTBDMS), 0.04 (s, 3H, Me, OTBDMS); ^{13}C NMR (125MHz, CDCl_3): δ 165.9 (C=O, Bz), 159.3 (C-*p*, PMB), 153.8 (C=O, Troc), 138.7 (C, 3-*O*-Bn), 137.9 (C, 6-*O*-Bn), 137.6 (C Bn), 133.3, 130.2, 130.1, 129.9, 129.86, 129.3, 129.1, 128.7, 128.5, 128.3, 128.3, 128.1, 128.07, 128.0, 127.6, 126.2 (16 $\times$ C_{arom}), 113.9 (C_{arom} PhCH), 101.7 (PhCH), 99.4 (C-1'), 95.6 (C-1), 78.4 (C-4'), 78.3 (C-4), 78.0 (C-3), 75.6 (C-3'), 74.5, 74.4 (CH_2 Troc, C-5, 3-*O*-Bn), 73.7 (CH_2 6-*O*-Bn), 71.4 (CH_2 , 3'-*O*-PMB), 69.7 (C-2), 68.78, 68.76 (C-6, C-6'), 67.3 (C-5'), 59.7 (C-2), 55.4 (OMe, PMB), 25.7 (Me, *t*-Bu, OTBDMS), 18.0 (C_q , *t*-Bu, OTBDMS), -4.1, -5.1 (2 $\times$ Me, TBDMS); HRMS(ESI) m/z calcd. for $[\text{C}_{57}\text{H}_{66}\text{O}_{14}\text{NCl}_3\text{Si}+\text{Na}]^+$: 1144.3216, obsd.: 1144.3216.

***Tert*-butyldimethylsilyloxy 4-*O*-(2-*O*-benzoyl-4,6-*O*-(*R*)-benzylidene- β -D-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethyloxy)cabonylamido- β -D-glucopyranoside (71).**

Benzoylated dimer **54b** (74 mg, 0.066 mmol, 1 equiv.) was dissolved in $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (0.66 mL, 10/1, v/v), followed by the addition of 2,3-chloro-5,6-cyano-1,4-benzoquinone (16.4 mg, 0.077 mmol, 1.1 equiv.) and left to stir for 2 h after which the reaction was quenched by the addition of 10% aq. $\text{Na}_2\text{S}_2\text{O}_4$. Then EtOAc was added and the organic fraction washed with 10% aq. $\text{Na}_2\text{S}_2\text{O}_4$, sat. aq. NaHCO_3 (2 $\times$), and brine (1 $\times$). The organic phase was then dried over MgSO_4 , filtered and concentrated *in vacuo*. Purification of the residue by column chromatography (PE/EtOAc, 10/1 $\rightarrow$ 3/1, v/v) yielded the title compound **71** (51.2 mg, 0.051 mmol, 77%) as a colourless oil. R_f : 0.34 (PE/EtOAc, 2/1, v/v); α_D^{20} : -0.4 (c = 1.0 in CHCl_3); IR (v): 3395.6, 3064.9, 3033.1, 2929.4, 2858.1, 1720.3, 1602.3, 1525.8, 1453.3, 1361.6, 1315.2, 1272.9, 1217.9, 1174.9, 1090.2, 1028.3, 995.0, 910.3, 838.8, 781.58, 731.1, 710.1, 698.3 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3): δ 8.08 (dd, J = 1.9 Hz, J = 8.0, 2H, CH_{arom} Bz), 7.56 (t, J = 7.6, 1H, CH_{arom} Bz), 7.54-7.49 (m, 2H, CH_{arom}), 7.44-7.36 (m, 10H,

CH_{arom}), 7.27-7.25 (m, 4H, CH_{arom}), 7.17-7.16 (m, 4H, CH_{arom}), 5.57 (s, 1H, CHPh), 5.56 (d, $J_{1',2'} = 2.2$ Hz, 1H, H-2'), 4.89 (s, 1H, H-1'), 4.88 (d, $J_{a,b} = 11.4$ Hz, 2H, CH-a 3-O-Bn, NH Troc), 4.759 (1H, H-1), 4.757 (d, $J_{a,b} = 11.9$ Hz, 1H, CH-a 6-O-Bn), 4.59 (bdd, $J_{NH,CH_2} = 12.2$ Hz, $J_{a,b} = 21.0$ Hz, 2H, CH₂, Troc), 4.53 (d, $J_{a,b} = 12.2$ Hz, 1H, CH-b 6-O-Bn), 4.45 (d, $J_{a,b} = 11.7$ Hz, CH-b 1H, 3-O-Bn), 4.27 (dd, $J_{6a',6b'} = 4.9$ Hz, $J_{5',6'} = 10.4$ Hz, 1H, H-6a'), 4.07 (t, $J_{3,4} = J_{4,5} = 9.2$ Hz, 1H, H-4), 3.88 (t, $J_{3',4'} = J_{4',5'} = 9.8$ Hz, 1H, H-4'), 3.82 (dd, $J_{6a',6b'} = 3.2$ Hz, $J_{5',6'} = 10.5$ Hz, 2H, H-6b', H-3'), 3.74 (t, $J_{6a',6b'} = J_{5',6'} = 10.2$, 1H, H-6b'), 3.64 (bt, $J_{2,3} = J_{3,4} = 9.2$ Hz, 1H, H-3), 3.39 (bd, $J_{4,5} = J_{5,6} = 9.5$ Hz, 1H, H-5), 3.28 (dt, $J_{5',6'} = 4.9$ Hz, $J_{5',6'} = J_{4',5'} = 9.7$ Hz, 1H, H-5'), 3.24 (1H, H-2), 0.86 (s, 9H, *t*-Bu, OTBDMS), 0.10 (s, 3H, Me OTBDMS), 0.04 (s, 3H, Me OTBDMS); ¹³C NMR (125MHz, CDCl₃): δ 166.0 (C=O, Bz), 153.7 (C=O, Troc), 138.6 (C, 3-O-Bn), 137.9 (C, 6-O-Bn), 137.0 (C Bn), 129.9, 129.6, 129.4, 128.6, 128.5, 128.4, 128.2, 128.04, 128.02, 127.9, 127.5, 126.3 (12 × C_{arom}) 102.2 (PhCH), 99.3 (C-1'), 95.5 (C-1, CCl₃, Troc), 79.0 (C-4), 78.6 (C-4'), 77.9 (C-3), 74.3 (CH₂, Troc, 3-O-Bn, C-5), 73.7 (CH₂, 6-O-Bn), 71.7 (C-2'), 70.0 (C-3'), 68.62, 68.57 (C-6, C-6'), 66.8 (C-5'), 59.6 (C-2), 25.6 (Me, *t*-Bu, OTBDMS), 17.9 (C_q, *t*-Bu, OTBDMS), -4.2, -5.3 (2 × Me, TBDMS); HRMS(ESI) *m/z* calcd. for [C₅₇H₆₆O₁₄NCl₃Si+Na]⁺: 1024.2641, obsd.: 1024.2641.

***Tert*-butyldimethylsilyloxy 4-*O*-(2-*O*-benzoyl-4-*O*-benzyl-β-*D*-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)cabonylamido-β-*D*-glucopyranoside (72). Dimer 71**

(651 mg, 0.065 mmol) was co-evaporated with toluene (3x), then dissolved in dry CH₂Cl₂ (6.5 mL). Activated molecular sieves (4Å) were added and the resulting suspension was stirred for 75 min at 0°C, after which the reaction mixture was cooled down to -78°C and Et₃SiH (0.30 mL, 1.95 mmol) was added. After stirring for 15 min, PhBCl₂ (0.29 mL, 2.2mmol) was added, and the reaction was stirred until TLC analysis showed complete disappearance of the starting material (15 min). The reaction was quenched by the addition of sat. aq. NaHCO₃ (3 mL) and EtOAc, and the organic phase washed with sat. aq. NaHCO₃ (2×) and brine (1x), dried over MgSO₄, filtered and concentrated *in vacuo*. Purification of the residue by column chromatography (silica gel, PE/EtOAc, 10/1 → 3/1, v/v) yielded the title compound **72** (530 mg, 0.53 mmol, 81%) as a colourless oil. *R*_f: 0.56 (PE/EtOAc, 1/1, v/v); [α]_D²⁰: 0.2 (*c* = 1.0 in CHCl₃); IR (ν): 3393.7, 3063.7, 3032.0, 2928.8, 2857.4, 1718.7, 1523.6, 1497.0, 1453.4, 1360.9, 1315.2, 1273.4, 1169.1, 1067.9, 1028.4, 941.4, 910.3, 838.8, 782.1, 732.8, 708.0 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 8.07-8.05 (m, 2H, CH_{arom} Bz), 7.57-7.55 (m, 1H, CH_{arom} Bz), 7.44-7.40 (m, 2H, CH_{arom} Bz), 7.40-7.25 (m, 14H, CH_{arom}), 7.13-7.12 (m, 2H, CH_{arom}), 5.44 (d, $J_{1',2'} = 2.7$ Hz, 1H, H-2'), 4.89 (d, $J_{a,b} = 11.8$ Hz, 1H, CH-a 3-O-Bn), 4.86 (d, $J_{NH,2'} = 7.4$, 1H, NH Troc), 4.81 (s, 1H, H-1), 4.76 (d, $J_{a,b} = 11.5$ Hz, 1H, CH-a 4'-O-Bn), 7.26 (d, 1H, H-1), 4.69 (d, $J_{a,b} = 11.5$ Hz, 1H, CH-a 6-O-Bn), 4.67 (d, $J_{a,b} = 11.0$ Hz, 1H, CH-a 4'-O-Bn), 4.57 (bdd, $J_{a,b} = 12.3$ Hz, $J_{a,b} = 25.2$ Hz, 2H, CH₂ Troc), 4.52 (d, $J_{a,b} = 12.2$ Hz, 1H, CH-b 6-O-Bn), 4.46 (d, $J_{a,b} = 12.0$ Hz, CH-b 1H, 3-O-Bn), 4.02 (t, $J_{3,4} = J_{4,5} = 9.3$ Hz, 1H, H-4), 3.80 (d, $J_{6a',6b'} = J_{5',6'} = 9.0$ Hz, 1H, H-6a'), 3.76 (dd, $J_{6a,6b} = 3.4$ Hz, $J_{5',6'} = 11.4$ Hz, 1H, H-6a), 3.72-3.69 (m, 2H, H-3', H-4'), 3.66 (dd, $J_{6a',6b'} = 2.5$ Hz, $J_{5',6'} = 11.5$ Hz, 1H, H-6b), 3.63 (dd, $J_{6a',6b'} = 4.6$ Hz, $J_{5,6} = 12.0$, 1H, H-6b'), 3.60

(1H, H-3), 3.40 (dt, $J_{5,6b} = 2.5$, $J_{5,6b} = 11.4$ Hz, 1H, H-5), 3.27 (bdd, $J = 8.1$ Hz, 1H, H-2), 3.25-3.22 (m, 1H, H-5'), 0.85 (s, 9H, *t*-Bu, OTBDMS), 0.09 (s, 3H, Me OTBDMS), 0.03 (s, 3H, Me OTBDMS); ^{13}C NMR (125MHz, CDCl_3): δ 166.4 (C=O, Bz), 154.0 (C=O, Troc), 137.8 (C, 3-O-Bn), 138.2 (C, 6-O-Bn), 138.0, 133.5, 130.1, 130.0, 128.74, 128.72, 128.7, 128.5, 128.18, 128.16, 128.10, 128.06, 127.7, 127.5 ($14 \times \text{C}_{\text{arom}}$), 98.8 (C-1'), 95.8 (C-1), 95.5 (CCl_3 , Troc), 78.3 (C-4, C-3), 75.79 (C-4'), 75.75 (C-5'), 75.1 (CH_2 , 4'-O-Bn), 74.6 (CH_2 , Troc, C-5), 74.2 (CH_2 , 3-O-Bn), 73.9 (CH_2 , 6-O-Bn), 73.4 (C-3'), 72.4 (C-2), 68.87 (C-6, C-6'), 25.8 (Me, *t*-Bu, OTBDMS), 18.1 (C_q , *t*-Bu, OTBDMS), -4.0, -5.1 ($2 \times \text{Me}$, TBDMS). HRMS(ESI) m/z calcd. for $[\text{C}_{57}\text{H}_{66}\text{O}_{14}\text{NCl}_3\text{Si}+\text{Na}]^+$: 1026.2797, obsd.: 1026.2797.

Tert-butyldimethylsilyloxy 4-O-(2-O-(2,3,4-tri-O-acetyl- β -D-xylopyranosyl)-4,6-O-(*R*)-benzylidene-3-O-*para*-methoxybenzyl- β -D-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)cabonylamido- β -D-glucopyranoside (54a).

ManGlc acceptor **55** (1.15 g, 1.1 mmol) and xylose imidate **69** (1.66 g, 4.0 mmol), were co-evaporated together, and dissolved in dry CH_2Cl_2 (5 mL). Activated molecular sieves (4Å) were added and the resulting suspension was stirred for 1h at 0°C, after which TMSOTf (28.3 μL , 1M in CH_2Cl_2), and the reaction was stirred for 4h and until TLC analysis showed complete consumption of the xylose donor **69**. The reaction was quenched by the addition of sat. aq. NaHCO_3 , diluted with EtOAc and the organic phase washed with sat. aq. NaHCO_3 (2x), and brine (1x), dried over MgSO_4 , filtered and concentrated *in vacuo*. Purification of the residue by column chromatography (PE/EtOAc, 6/1 $\rightarrow$ 4/1, v/v), yielded the title compound **54a** (1.03 g, 0.70 mmol, 72%) as a colourless oil. R_f : 0.51 (tol/EtOAc, 3/1, v/v); $[\alpha]_D^{20}$: +3.6 ($c = 10.0$ in CHCl_3); IR (v): 3349, 3030, 2935, 1740, 1612, 1369, 1303, 1285, 1247, 1222, 1174, 1156, 1085, 1051, 1037, 970, 911, 883, 838, 820, 782, 732, 698, 676 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3): δ 7.48-7.47 (m, 2H, CH_{arom}), 7.40-7.23 (m, 16H, CH_{arom}), 6.85 (d, $J_{a,b} = 8.8$ Hz, 2H, CH_{arom} PMB), 5.45 (s, 1H, CHPh), 5.02-5.00 (m, 5H, NH Troc, H-1'', H-2'', H-3'', CH_2 3-O-Bn), 4.81 (bd, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.79 (bs, 1H, H-4''), 4.68 (d, $J_{a,b} = 12.7$ Hz, 1H, CH-a 3'-O-PMB), 4.68 (s, 2H, CH_2 Troc), 4.61 (d, $J_{a,b} = 12.2$ Hz, 1H, CH_2 6-O-Bn), 4.60 (d, $J_{a,b} = 12.5$ Hz, CH-b 1H, 3'-O-PMB), 4.59 (H-5a) 4.57 (s, 1H, H-1'), 4.54 (d, $J_{a,b} = 11.3$ Hz, 1H, CH-b 3-O-Bn), 4.45 (d, $J_{a,b} = 12.2$ Hz, 1H, CH-b 6-O-Bn), 4.10 (dd, $J_{6a',6b'} = 4.4$ Hz, $J_{5',6'} = 10.5$ Hz, 1H, H-6a'), 4.00 (s, 1H, H-2'), 3.93 (t, $J_{3',4'} = J_{4',5'} = 9.1$ Hz, 1H, H-4'), 3.86 (t, $J_{3,4} = J_{4,5} = 9.5$, 1H, H-4), 3.83 (t, $J_{2,3} = J_{3,4} = 9.8$, 1H, H-3), 3.78 (s, 3H, OMe PMB) 3.64 (m, 2H, H-6a, H-6b), 3.44-3.38 (m, 4H, 3', 5, 5b'', 6b'), 3.80 (dd, 1H, H-2), 3.13 (dt, $J_{5',6'} = 4.6$ Hz, $J_{4',5'} = J_{5',6'} = 9.5$ Hz, 1H, H-5'), 2.11, 2.06, 1.98 (3x s, 3H, Me Ac), 0.88 (s, 9H, *t*-Bu, OTBDMS), 0.12 (s, 3H, Me OTBDMS), 0.09 (s, 3H, Me OTBDMS); ^{13}C NMR (125MHz, CDCl_3): δ 170.0, 169.4, 169.2 (C=O Ac), 159.7 (C-*p*, PMB), 154.1 (C=O, Troc), 139.2 (C_{arom}) 138.0 (C, 3-O-Bn), 137.6 (C, 6-O-Bn), 130.4, 129.3, 129.1, 128.7, 128.32, 128.26, 128.1, 127.9, 127.6, 127.5, 126.3 ($11 \times \text{C}_{\text{arom}}$), 113.9 (C_{arom}) 101.9 (C-1'), 101.7 (PhCH), 99.1 (C-1''), 96.1 (C-1), 95.7 (CCl_3 Troc), 79.8 (C-4'), 79.3 (C-3), 78.6 (C-4), 76.8 (C-5), 75.8 (C-2'), 74.8, 74.7 (CH_2 Troc, 3-O-Bn), 74.5 (C-3'),

73.9 (CH₂ 6-*O*-Bn), 72.1 (CH₂ 3'-*O*-PMB), 69.4 (C-6), 68.8 (C-6'), 68.5 (C-2''), 68.3 (C3''), 68.1 (C-4''), 67.5 (C-5'), 59.9 (C-5''), 59.7 (C-2), 55.4 (OMe, PMB), 25.8 (Me, *t*-Bu, OTBDMS), 21.10, 21.08, 20.9 (Me Ac), 18.1 (C_q, *t*-Bu, OTBDMS), -4.0, -5.0 (2 × Me, TBDMS); HRMS(ESI) *m/z* calcd. for [C₆₁H₇₆Cl₃NNaO₂₀Si]⁺: 1298.3688, obsd.: 1298.3687.

***Tert*-butyldimethylsilyloxy 4-*O*-(2-*O*-(2,3,4-tri-*O*-acetyl-β-*D*-xylopyranosyl)-4,6-*O*-(*R*)-benzylidene-β-*D*-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)cabonylamido-β-*D*-glucopyranoside (73).** Xylose trimer **54a** (59.2 mg, 0.046 mmol, 1

equiv.) was dissolved in CH₂Cl₂/MeOH (0.5 mL, 10/1, v/v), followed by the addition of 2,3-chloro-5,6-cyano-1,4-benzoquinone (16.4 mg, 0.077 mmol, 1.2 equiv.). After stirring for 2h the reaction was quenched by the addition of 10% aq. Na₂S₂O₄ and diluted with EtOAc. The organic layer was washed with 10% aq. Na₂S₂O₄, sat. aq. NaHCO₃ (2×) and brine. The organic phase was then dried over MgSO₄, filtered and concentrated *in vacuo*. Purification of the residue by column chromatography (PE/EtOAc, 10/1 → 3/1, v/v) yielded the title compound (**73**) (51.2 mg, 0.051 mmol, 77%) as a colourless oil. *R_f*: 0.31, debenzylated product *R_f*: 0.23, (PE/EtOAc, 1/1, v/v); [α]_D²⁰: -12.0 (*c* = 1.0 in CHCl₃); IR (ν): 3348.4, 3033.3, 2930.0, 2858.6, 1742.4, 1531.7, 1454.8, 1369.4, 1249.2, 1221.9, 1173.0, 1069.5, 911.8, 839.1, 783.0, 734.0, 698.8 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.47-7.45 (m, 2H, CH_{arom}), 7.40-7.31 (m, 8H, CH_{arom}), 5.45 (s, 1H, CHPh), 5.19 (t, *J*_{2'',3''} = *J*_{3'',4''} = 7.8 Hz, 1H, H-3''), 5.16 (bs, 1H, NH Troc), 4.97 (dd, *J*_{1,2} = 6.1 Hz, *J*_{2,3} = 7.9 Hz, 1H, H-2), 4.95-4.93 (m, *J*_{3'',4''} = 7.8 Hz, 1H, H-4''), 4.92 (H-1), 4.91 (d, *J*_{a,b} = 11.2 Hz, 1H, CH-a 3-*O*-Bn), 4.85 (d, *J*_{1'',2''} = 6.1 Hz, 1H, H-1''), 4.72 (d, *J*_{a,b} = 12.2 Hz, 1H, CH-a 6-*O*-Bn), 4.66 (s-1H, H-1'), 4.65 (m, *J* = 12.0 Hz, *J* = 22.0 Hz, 2H, CH₂ Troc), 4.55 (d, *J*_{a,b} = 11.2 Hz, 1H, CH-b 3-*O*-Bn), 4.51 (d, *J*_{a,b} = 12.0 Hz, 1H, CH-b 6-*O*-Bn), 4.23 (dd, *J*_{5a'',5b''} = 4.9 Hz, *J*_{4'',5''} = 12.0 Hz, 1H, H-5''), 4.02 (dd, *J*_{5',6'} = 4.9 Hz, *J*_{6a',6b'} = 10.2 Hz, 1H, H-6a'), 3.96-3.91 (m, 3H, H-2', H-3 and H-4), 3.70-3.67 (m, 2H, H-6a and H-6b), 3.60 (t, *J*_{3',4'} = *J*_{4',5'} = 9.8 Hz, 1H, H-4'), 3.56 (bs, 1H, H-3'), 3.47 (t, *J*_{5,6} = *J*_{6a',6b'} = 10.3 Hz, 1H, H-6b'), 3.44 (1H, H-5), 3.38 (dd, *J*_{4'',5''} = 8.1 Hz, *J*_{5a'',5b''} = 12.0 Hz, 1H, H-5b''), 3.28 (bs, 1H, H-2), 3.14 (dt, *J*_{5',6'} = 4.8 Hz, *J*_{4',5'} = *J*_{5',6'} = 9.5 Hz, 1H, H-5'), 2.89 (s, 1H, 3'-OH), 2.07, 2.04, 2.00 (3x s, 9H, Me Ac), 0.89 (s, 9H, *t*-Bu, OTBDMS), 0.13 (s, 3H, Me OTBDMS), 0.09 (s, 3H, Me OTBDMS); ¹³C NMR (125MHz, CDCl₃): δ 169.9, 169.8, 169.5 (3 x C=O Ac), 154.0 (C=O, Troc), 138.9 (C, 3-*O*-Bn), 137.9 (C, 6-*O*-Bn), 137.4, (11 × C_{arom}), 113.9 (C_{arom}) 102.3 (PhCH), 102.0 (C-1'), 100.3 (C-1''), 95.6 (C-1), 94.0 (CCl₃ Troc), 80.0 (C-4), 79.1 (C-4'), 78.7 (C-2'), 77.9 (C-3), 74.8 (C-5), 74.7 (CH₂ Troc), 74.0 (CH₂ 3-*O*-Bn, 6-*O*-Bn), 70.7 (C-2''), 70.6 (C-3''), 70.4 (C-3'), 69.2 (C-6), 68.6 (C-6', C-4''), 67.6 (C-5'), 61.9 (C-5''), 59.8 (C-2), 25.8 (Me, *t*-Bu, OTBDMS), 21.1, 20.9, 20.8 (3 x Me OAc), 18.1 (C_q, *t*-Bu, OTBDMS), -4.0, -5.0 (2 × Me, TBDMS); HRMS(ESI) *m/z* calcd. for [C₅₃H₆₈O₁₉NCl₃Si+Na]⁺: 1178.3118, obsd.: 1178.3118.

***Tert*-butyldimethylsilyloxy 4-O-[2-O-(2,3,4-O-acetyl-β-D-xylopyranosyl)-4-O-benzyl-β-D-mannopyranosyl]-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamido-β-D-glucopyranoside (74).**

Trimer (73) (536 mg, 0.46 mmol) was co-evaporated 3 x with toluene, and dissolved in dry CH₂Cl₂ (5 mL). Activated molecular sieves (4Å) were added and the resulting suspension was stirred for 1h at 0°C, after which the reaction mixture was cooled down to -78°C and Et₃SiH (0.37 mL 2.32 mmol) was added. After stirring for 15 min, PhBCl₂ (1.57 mL, 1M in CH₂Cl₂) was added, and the reaction was stirred until TLC analysis showed complete disappearance of the starting material. The reaction was quenched by the addition of sat. aq. NaHCO₃ (3 mL) and EtOAc, and the organic phase washed with sat. aq. NaHCO₃ (2x) and brine (1x), dried over MgSO₄, filtered and concentrated *in vacuo*. Purification of the residue by column chromatography (silica gel, PE/EtOAc, 3/1 → 1/1, v/v) yielded the title compound (74) (438 mg, 0.36 mmol, 82%) as a colourless oil. R_f: 0.54 (PE/EtOAc, 1/1, v/v); [α]_D^{18.0} = -14.4 (c 1.0, CHCl₃); IR (ν): 3363, 3065, 3031, 2954, 2928, 2858, 1742, 1537, 1498, 1498, 1455, 1370, 1222, 1168, 1066, 839, 755, 699 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.40-7.26 (m, 15H, CH_{arom}), 5.20 (t, *J*_{2'',3''} = *J*_{3'',4''} = 8.0 Hz, 1H, H-3''), 5.18 (bs, 1H, NH Troc), 4.98 (dd, *J*_{2'',3''} = 8.0 Hz, *J*_{1'',2''} = 6.2 Hz, 1H, H-2''), 4.95 (dd, *J*_{3'',4''} = 8.1 Hz, *J*_{4'',5''} = 3.4 Hz, 1H, H-4''), 4.93 (s, 1H, H-1), 4.92 (d, *J*_{a,b} = 11.1 Hz, 1H, CH-a 3-O-Bn), 4.85 (d, *J*_{a,b} = 11.2 Hz, 1H, CH-a 4'-O-Bn), 4.79 (d, *J*_{1'',2''} = 6.0 Hz, 1H, H-1''), 4.67-4.61 (m, 2H, CH₂ Troc), 4.66 (d, *J*_{a,b} = 13.3 Hz, 1H, CH-a 6-O-Bn), 4.63 (d, *J*_{a,b} = 12.1 Hz, 1H, CH-b 3-O-Bn), 4.60 (1H, H-1'), 4.59 (d, *J*_{a,b} = 10.6 Hz, 1H, CH-b 4'-O-Bn), 4.53 (d, *J*_{a,b} = 12.1 Hz, 1H, CH-b 6-O-Bn), 4.21 (dd, *J*_{4'',5a''} = 4.8 Hz, *J*_{5a'',5b''} = 11.9 Hz, 1H, H-5a''), 3.93 (bd, *J* = 7.9 Hz, 1H, H-3), 3.87 (t, *J*_{3,4} = *J*_{3,4} = 9.1 Hz, 1H, H-4), 3.83 (d, *J*_{1,2} = 2.4 Hz, 1H, H-2'), 3.72 (d, *J*_{6a,6b} = 10.0 Hz, 1H, H-6a), 3.66 (dd, *J*_{5,6b} = 3.6 Hz, *J*_{6a,6b} = 11.0 Hz, 1H, H-6b), 3.61 (d, *J*_{6a',6b'} = 9.9 Hz, 1H, H-6a'), 3.53-3.48 (m, 2H, H-3' and H-5), 3.43 (t, *J*_{3',4'} = *J*_{4',5'} = 9.1 Hz, 1H, H-4'), 3.41 (dd, *J*_{5',6b'} = 5.0 Hz, 1H, H-6b'), 3.36 (dd, *J*_{4'',5b''} = 8.2 Hz, *J*_{5a'',5b''} = 11.9 Hz, 1H, H-5b''), 3.28 (bd, *J*_{2,3} = 9.4 Hz, 1H, H-2), 3.13-3.10 (m, 1H, H-5'), 2.09, 2.06, 2.02 (3 x s, 9H, Me Ac), 0.89 (s, 9H, Me OTBDMS), 0.13, 0.10 (2 x s, 6H, Me OTBDMS); ¹³C NMR (125 MHz, CDCl₃): δ 170.0, 169.9, 169.6 (3x C=O Ac), 154.0 (C=O Troc), 138.7 (C, 3-O-Bn), 138.3 (C, 4'-O-Bn), 137.7 (C, 6-O-Bn), 128.7, 128.57, 128.56, 128.2, 128.1, 128.0, 127.8, 127.2 (7x C_{arom}), 100.5 (C-1', C1''), 95.5 (CCl₃ Troc), 95.3 (C-1), 79.1 (C-4), 78.2 (C-2), 78.1 (C-3), 76.0 (C-4'), 75.9 (C-5'), 75.0 (CH₂ 4'-O-Bn), 74.7 (C-5), 74.5 (CH₂ Troc), 73.9 (CH₂ 3-O-Bn, 6-O-Bn), 73.7 (C-3'), 70.52, 70.46 (C-2'' and C-3''), 69.0 (C-6), 68.4 (C-4''), 62.1 (C-6'), 61.7 (C-5''), 59.7 (C-2), 25.7 (Me *t*-Bu, OTBDMS), 21.1, 20.9, 20.8 (3 x Me Ac), 18.1 (C_q *t*-Bu, OTBDMS), -4.1, -5.1 (2 x Me, OTBDMS); HRMS (ESI) *m/z* calcd. for [C₅₃H₇₀Cl₃NNaO₁₉Si]⁺: 1180.3269, *odsd.*: 1180.3270.

***Tert*-butyldimethylsilyloxy 2-acetamido-2-deoxy-4-O-[2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-4,6-O-(R)-benzylidene-3-O-**

***para*-methoxybenzyl- β -D-mannopyranosyl]-3,6-O-benzyl- β -D-glucopyranoside (90).** Troc protected trimer (**54a**) (128.7 mg, 0.104 mmol) was dissolved in THF (0.4 mL), followed by the addition of activated zinc (350.0 mg, 5.35 mmol) and aq. Na₂HPO₄ (0.1 mL, 1M). The suspension was stirred vigorously until TLC analysis showed complete disappearance of the starting material. The reaction mixture was then diluted with EtOAc, filtered over celite to remove unreacted zinc, and co-concentrated with pyridine. The residue was dissolved in pyridine/Ac₂O (1 mL, 2/1) and left to stir until TLC analysis, [*R*_f: 0.08, (Pe/EtOAc, 2/1, v/v, ninhydrin stain)], showed complete disappearance of the amine. The reaction mixture was diluted with EtOAc, washed with sat. aq. NaHCO₃ (4x) until no further gas was formed. The organic fraction was then washed with brine (1x), dried over MgSO₄, filtered and concentrated. Purification of the residue by column chromatography (silica gel, PE/EtOAc, 2/1, v/v) yielded the title compound (**90**) (100 mg, 0.087 mmol, 84%) as a colourless oil. *R*_f: 0.42 (Pe/EtOAc, 2/1, v/v) ; [α]_D^{17.7} = -33 (c 1.0 CHCl₃); IR (ν): 3294, 3065, 3034, 2955, 2929, 2858, 1749, 1658, 1613, 1558, 1514, 1455, 1371, 1303, 1249, 1223, 1175, 1086, 1053, 839 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.51-7.49 (m, 2H, CH_{arom}), 7.41-7.36 (m, 3H, CH_{arom}), 7.35-7.25 (m, 13H, CH_{arom}), 6.86 (d, *J* = 8.8 Hz, *J* = 7.5 Hz, 2H, CH_{arom} OPMB), 5.47 (s, 1H, CHPh), 5.45 (bd, *J*_{2,NH} = 8.0 Hz, 1H, NHAc), 5.05 (d, *J*_{1'',2''} = 6.6 Hz, 1H, H-1''), 5.04-5.00 (m, 2H, H-2'' and H-4''), 4.99 (d, *J*_{1,2} = 7.6 Hz, 1H, H-1), 4.94 (d, *J*_{a,b} = 11.5 Hz, 1H, CH-a 3-O-Bn), 4.80 (dd, *J* = 3.4 Hz, *J* = 7.3 Hz, 1H, H-3''), 4.53 (dd, *J*_{a,b} = 12.0 Hz, *J*_{a,b} = 21.9 Hz, 2H, CH₂ 4'-O-PMB), 4.62 (d, *J*_{a,b} = 12.0 Hz, 1H, CH-a 6-O-Bn), 4.568 (s, 1H, 1'), 4.567 (dd, *J*_{4'',5a''} = 3.4 Hz, *J*_{5a'',5b''} = 12.9 Hz, 1H, H-5a''), 4.51 (d, *J*_{a,b} = 11.7 Hz, 1H, CH-b 3-O-Bn), 4.46 (d, *J*_{a,b} = 12.0 Hz, 1H, CH-b 6-O-Bn), 4.13 (dd, *J*_{5',6a'} = 4.9 Hz, *J*_{6a',6b'} = 10.5 Hz, 1H, H-6a'), 4.04 (d, *J*_{1',2'} = 3.1 Hz, 1H, H-2'), 4.00 (dd, *J*_{3,4} = 8.5 Hz, *J*_{2,3} = 9.8 Hz, 1H, H-3), 3.883 (t, *J* = 8.3 Hz, 1H, H-4), 3.879 (t, *J* = 9.8 Hz, 1H, H-4'), 3.79 (s, 3H, OMe OPMB), 3.47 (m, 2H, H-5 and H-6b'), 3.43 (dd, *J*_{4'',5b''} = 3.1 Hz, *J*_{5a'',5b''} = 12.9 Hz, 2H, H-5b'' and H-3), 3.40 (m, *J*_{2,3} = 9.5 Hz, 1H, H-2), 3.14 (dt, *J*_{5',6a'} = 4.9 Hz, *J*_{5',6b'} = *J*_{4',5'} = 9.8 Hz, 1H, H-5'), 2.12, 2.07, 2.00 (3 x s, 9H, Me O-Ac), 1.82 (s, 3H, Me NHAc), 0.89 (s, 9H, Me *t*-Bu OTBDMS), 0.12, 0.09 (2 x s, 6H, Me OTBDMS); ¹³C NMR (125 MHz, CDCl₃): δ 169.95, 169.93, 169.3, 169.2 (4 x C=O O-Ac and N-Ac), 159.2 (C, 3'-O-PMB), 139.3 (C, 3-O-Bn), 137.9 (C, 6-O-Bn), 137.4 130.2, 129.2, 128.9, 128.5, 128.21, 128.19, 128.1, 127.9, 127.7, 127.6, 127.3, 126.1 (13x CH_{arom}), 113.7 (CH_{arom} PMB), 101.9 (C-1'), 101.5 (C-CHPh), 99.0 (C-1''), 95.2 (C-1), 79.9 (C-4), 78.36, 78.32 (C-3 and C-4'), 76.4 (C-3'), 75.3 (C-2'), 74.4 (C-5), 74.2, 73.6 (CH₂ 6-O-Bn and CH₂ 4-O-Bn), 71.8 (CH₂ PMB), 69.3 (C-6), 68.6 (C-6'), 68.4, 68.2 (C-2'' and C-4''), 68.0 (C-3''), 67.3 (C-5'), 59.8 (C-5''), 58.4 (C-2), 55.3 (OMe PMB), 25.6 (Me *t*-Bu, OTBDMS), 23.5 (Me N-Ac), 21.0, 21.0, 20.8 (3 x Me O-Ac), 17.9 (C_q *t*-Bu, OTBDMS), -4.2, -5.2 (2 x Me, OTBDMS); HRMS (ESI) *m/z* calcd. for [C₆₀H₇₇NNaO₁₉Si]⁺: 1166.4751, odstd.: 1166.4763.

2-*N*-acetamido-2-deoxy-4-*O*-(2-*O*- β -D-xylopyranosyl- β -D-mannopyranosyl)- β -glucopyranose (99). Acetamido trimer (**90**) (81.9 mg, 0.072 mmol) was dissolved in dry THF (0.5 mL), NH₃ (l) (15 mL) was added followed by the careful addition of sodium (s) (200 mg) until the blue colour remained. The

solution was stirred for 1.5 h under refluxing NH_3 , then MeOH was added (4 mL) and resulting solution allowed to warm up to rt and the NH_3 was evaporated. The solution was neutralized by the addition of activated DOWEX® H^+ , after 15 min the organic phase was filtered and concentrated under reduced pressure. Purification by reverse phase column chromatography (HyperSep C8 Column, 0→50% MeOH/ H_2O) yielded title compound (**99**) as a white foam (21.9 mg, 0.042 mmol, 59%) and TBDMS protected trimer (**100**) as a colorless oil (18.9 mg, 0.30 mmol, 41%); R_f : 0.05 (EtOAc/ isopropanol/ H_2O , 6/4/1 v/v) ; $[\alpha]_D^{19.8} = -20$ (C 0.1 CHCl_3); ratio $\alpha/\beta = 2/1$ IR (v): 3295, 2923, 2853, 1636, 1557, 1427, 1375, 1318, 1245, 1155, 1032, 897, 807 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3): δ 5.18 (d, $J_{1,2} = 3.2$ Hz, 1H, H-1 α), 4.82 (s, 1H, H-1' α), 4.81 (s, 1H, H-1' β), 4.69 (d, $J_{1,2} = 7.6$ Hz, 1H, H-1 β), 4.48 (d, $J_{1'',2''} = 7.8$ Hz, 1H, H-1'' α), 4.45 (d, $J_{1'',2''} = 7.6$ Hz, 1H, H-1'' β), 4.23 (d, $J_{2',3'} = 3.4$ Hz, 1H, H-2' α), 4.21 (d, $J_{2',3'} = 3.5$ Hz, 1H, H-2' β), 3.96-3.82 (m, 8H), 3.81-3.79 (m, 1.4H), 3.78-3.68 (m), 3.66-3.53 (m, 7H, H-2, H-3, H-3', H-3'', H-4, H-4', H-4'', H-5, H-5', H-6 and H-6'), 3.35 (d, $J_{1'',2''} = 7.8$ Hz, H-2'' α), 3.34 (d, $J_{1'',2''} = 7.6$ Hz, H-2'' β), 3.23 (t, $J = 11.5$ Hz, H-5b'' α), 3.22 (t, $J = 11.2$ Hz, H-5b'' β), 2.02 (s, 6H, Me NHAc); ^{13}C NMR (125 MHz, CDCl_3): δ 174.6, 174.3 (2x C=O, NHAc), 104.0, 103.9 (2x C-1''), 100.1 (C-1'), 94.8 (C-1 β), 90.4 (C-1 α), 79.1, 78.8, 77.77, 77.69, 76.27, 74.6, 73.11, 73.06, 72.2, 71.7, 70.0, 69.2, 69.1, 66.7 (C-2', C-2'', C-3, C-3', C-3'', C-4, C-4', C-4'', C-5, C-5'), 64.9 (C-5''), 60.3, 60.2, 60.1 (C-6, C-6'), 56.1 (C-2 β), 53.6 (C-2 α), 22.0, 21.7 (2 x Me NHAc); HRMS (ESI) m/z calcd. for $[\text{C}_{19}\text{H}_{33}\text{NNaO}_{15}]^+$: 538.1742, obsd.: 538.1738.

Tert-Butyldimethylsilyloxy 2-N-acetamido-2-deoxy-4-O-(2-O- β -D-xylopyranosyl- β -D-mannopyranosyl)- β -D-glucopyranoside (100**).** ^1H NMR (500 MHz, CDCl_3): δ 4.82 (s, 1H, H-1'), 4.72 (d, $J_{1,2} = 7.6$ Hz, 1H, H-1), 4.45 (d, $J_{1'',2''} = 7.6$ Hz, 1H, H-1''), 4.22 (s, 1H, H-2'), 3.93 (dd, $J_{4'',5''} =$

5.7 Hz, $J_{5a'',5b''} = 12.0$ Hz, 1H, H-5a''), 3.88 (t, $J = 12.7$ Hz, 2H, H-6a and H-6a'), 3.76-3.53 (m, 9H, H-2, H-3, H-3', H-4, H-4', H-4'', H-5, H-6b, H-6b'), 3.42 (t, $J = 7.8$ Hz, 2H, H-3'', H-5'), 3.35 (t, $J_{1'',2''} = J_{2'',3''} = 7.8$ Hz, 1H, H-2''), 3.23 (t, $J_{4'',5''} = J_{5a'',5b''} = 11.2$ Hz, 1H, H-5b''), 2.01 (s, 3H, Me NHAc), 0.86 (s, 9H, *t*-Bu, OTBDMS), 0.12, 0.11 (2x 6H Me, OTBDMS); ^{13}C NMR (125 MHz, CDCl_3): δ 174.2 (C=O, NHAc), 104.1 (C-1''), 100.2 (C-1'), 95.9 (C-1), 79.2 (C-4), 77.9 (C-2), 76.3 (C-5'), 75.3, (C-3''), 74.5 (C-5), 73.1 (C-2''), 72.1 (C-3), 71.7 (C-3'), 69.2 (C-4''), 66.7 (C-4'), 64.9 (C-5''), 60.3 (C-6 and C-6'), 56.9 (C-2), 24.6 (Me, *t*-Bu, OTBDMS), 22.2 (Cq, *t*-Bu, OTBDMS), -5.4, -6.4 (2x Me, OTBDMS); HRMS (ESI) m/z calcd. for $[\text{C}_{25}\text{H}_{47}\text{NNaO}_{15}\text{Si}]^+$: 652.2607, obsd.: 652.2607.

2-N-acetamido-2-deoxy-4-O-(2-O- β -D-xylopyranosyl- β -D-mannopyranosyl)- β -D-glucopyranose (99**).** Silane protected trimer (**100**) (14.6 mg, 0.023 mmol) was dissolved in MeOH (0.3mL), activated DOWEX® H^+ added (20 granules) and left for 2 days. The DOWEX® H^+ was then filtered off and the organic

fraction concentrated. Purification by reverse phase column chromatography (HyperSep C8 column, H_2O)

yielded title compound (**99**) as a white foam (11.3 mg, 0.023 mmol, 96%); Data matched those reported above.

***Tert*-butyldimethylsilyloxy 2-*N*-acetamido-2-deoxy-4-*O*-(2-*O*-acetyl-4,6-*O*-[*R*]-benzylidene-3-*O*-*para*-methoxybenzyl- β -D-glucopyranosyl)-3,6-*O*-benzylidene- β -D-glucopyranoside (**89**).** Troc

protected dimer (**87**) (41.8 mg, 0.039 mmol) was dissolved in THF (0.15 mL), followed by the addition of activated zinc (60 mg, 0.92 mmol) and aq. Na₂HPO₄ (0.03 mL, 1M). The suspension was stirred vigorously until TLC analysis showed complete disappearance of the starting material (1.5 h). The reaction mixture was then diluted with EtOAc, filtered over celite to remove unreacted zinc. The residue was dissolved in pyridine/Ac₂O (1 mL, 2/1) and left to stir until TLC analysis, [*R_f*: 0.34 (1/1, Pe/EtOAc, v/v, ninhydrine stain)], showed complete disappearance of the amine. The reaction mixture was diluted with EtOAc, washed with sat. aq. NaHCO₃ (3x) until no further gas was formed. The organic fraction was then washed with brine (1x), dried over MgSO₄, filtered and concentrated. Purification by flash chromatography (silica gel, 33% EtOAc in PE) gave the title compound (**89**) as a colorless oil (23.1mg, 0.025 mmol, 64%); TLC *R_f*: 0.23 (Pe/EtOAc, 2/1, v/v); [α]_D^{23.3} = -15 (c 0.1, CHCl₃); IR (v): 3281, 3066, 3033, 2956, 2930, 2857, 1751, 1654, 1559, 13.72, 1246, 1231, 1095, 1063, 839, 752, 698 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 7.52-7.48 (m, 2H, CH_{arom}), 7.44-7.38 (m, 3H, CH_{arom}), 7.36-7.28 (m, 10H, CH_{arom}), 7.21-7.19 (m, 2H, CH_{arom} 3'-O-PMB), 6.88-6.85 (m, 2H, CH_{arom} 3'-O-PMB), 5.79 (d, *J*_{NH,2} = 8.6 Hz, 1H, NH NHAc), 5.53 (s, 1H, CHPh), 4.95 (d, *J*_{2',3'} = 6.1 Hz, 1H, H-2'), 4.94 (d, *J*_{1,2} = 8.9 Hz, 1H, H-1), 4.78 (d, *J*_{a,b} = 11.6 Hz, 1H, CH-a 3'-O-Bn), 4.75 (d, *J*_{a,b} = 11.8 Hz, 1H, CH-a 3-O-Bn), 4.64 (d, *J*_{a,b} = 12.1 Hz, 1H, CH-a 6-O-Bn), 4.63 (d, *J*_{a,b} = 11.9 Hz, 1H, CH-b 3-O-Bn), 4.60 (d, *J*_{a,b} = 11.8 Hz, 1H, CH-b 3'-O-Bn), 4.55 (d, *J*_{1',2'} = 7.9 Hz, 1H, H-1'), 4.48 (d, *J*_{a,b} = 12.0 Hz, 1H, CH-b 6-O-Bn), 4.26 (dd, *J*_{5',6'} = 5.1 Hz, *J*_{6a',6b'} = 10.5 Hz, 1H, H-6a'), 3.97 (t, *J*_{3,4} = *J*_{4,5} = 6.7 Hz, 1H, H-4), 3.88 (t, *J*_{2,3} = *J*_{3,4} = 7.0 Hz, 1H, H-3), 3.83-3.80 (m, 1H, H-6a), 3.81 (s, 3H, OMe OPMB), 3.69 (t, *J* = 9.7 Hz, 2H, H-4' and H-6b), 3.65-3.54 (m, 4H, H-2, H-3', H-5, H-6b'), 3.29 (dt, *J* = 5.1 Hz, *J* = 9.7 Hz, 1H, H-5'), 2.00 (s, 3H, Me, OAc), 1.90 (s, 3H, Me, NHAc), 0.89 (s, 9H, *t*-Bu, OTBDMS), 0.09, 0.08 (2x Me, OTBDMS); ¹³C NMR (125 MHz, CDCl₃): δ 170.1 (C=O NHAc), 169.7 (C=O OAc), 159.3 (C-OPMB), 138.7, 138.1, 137.2 (3x C-OBn), 130.3, 129.5, 129.1, 128.5, 128.29, 128.26, 127.8, 127.7, 127.5, 126.0, 113.7 (11x CH_{arom}), 101.2 (CHPh), 100.3 (C-1'), 94.7 (C-1), 81.6 (C-4'), 77.8 (C-3'), 77.2 (C-3), 75.6 (C-4), 74.3 (C-5), 73.8 (CH₂ 3'-O-Bn), 73.6 (CH₂ 3-O-Bn, CH₂ 6-O-Bn), 73.3 (C-2'), 68.9 (C-6), 68.6 (C-6'), 66.1 (C-5'), 55.5 (C-2), 55.3 (Me OPMB), 25.6 (Me, *t*-Bu, OTBDMS), 23.4 (Me NHAc), 20.9 (Me OAc), 17.9 (Cq, *t*-Bu, OTBDMS), -4.4, -5.3 (2 x Me, OTBDMS); HRMS (ESI) *m/z* calcd. for [C₅₁H₆₅NNaO₁₃Si]⁺: 950.4117, obsd.: 950.4124.

2-*N*-acetamido-2-deoxy-4-*O*- β -D-mannopyranosyl- β -D-glucopyranose (98**).**

Acetamido dimer (**89**) (27.8 mg, 0.030 mmol) was dissolved in dry THF (0.3

mL), NH₃ (l)(15 mL) was added followed by the careful addition of sodium (s) until the blue colour remained. The solution was stirred for 45 min under refluxing NH₃, then MeOH was added (4 mL) and resulting solution allowed to warm up to rt and the NH₃ was evaporated. The solution was neutralized by the addition of activated DOWEX® H⁺, after 15 min the organic phase was filtered and concentrated under reduced pressure. Purification by column chromatography (HyperSep C18 column, H₂O) yielded title compound (**98**) as a white foam (10.8 mg, 0.028 mmol, 94%). ¹H NMR (500 MHz, CDCl₃): δ 5.18 (s, 1H, H-1α), 4.69 (d, *J*_{1,2} = 7.8 Hz, 1H, H-1β), 4.51 (d, *J*_{1,2} = 8.1 Hz, 1H, H-1''), 3.96-3.78 (m, 8H), 3.73-3.64 (m, 7H), 3.51-3.44 (m, 4H), 3.41-3.37 (m, 2H), 3.34-3.27 (m, 2H), 2.02 (s, 5H, Me NHAc); ¹³C NMR (125 MHz, CDCl₃): δ 174.6, 174.4 (2x Cq NHAc), 102.4, 94.7, 90.4 (C-1α, C-1β, C-1'), 79.0, 78.6, 75.91, 75.88, 75.4, 74.7, 73.1, 72.3, 10.2, 69.3, 69.1, 62.4, 60.5, 59.9, 59.8, 56.2, 53.7, 48.8 (C-2, C-2', C-3, C-3', C-4, C-4', C-5, C-5', C-6, C-6'), 22.1, 21.8 (2x Me NHAc); HRMS (ESI) *m/z* calcd. for [C₁₄H₂₅NNaO₁₁]⁺: 406.1320, odsd.: 406.1319.

4-O-(2-O-benzoyl-3,6-di-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-4-O-benzyl-β-D-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamido-α/β-D-glucopyranose (77**).** Compound **58b** (233.1 mg, 0.112 mmol) was transferred to a Falcon tube (25 mL) then dissolved in THF (0.33mL) and treated with HF·pyridene (70%, 133μL) for 6h. The

reaction was then diluted with EtOAc and the organic layer washed with saturated aq. NaHCO₃ (3x) and Brine (1x). The organic layer was then dried over MgSO₄, Filtered, and concentrated *in vacuo*. Purification by flash chromatography (silica gel, 33% EtOAc in PE) yielded the title compound **77** as an amorphous white solid (103mg, 103mmol, 92%, α/β = 10/1); TLC R_f: 0.05 (Pe/EtOAc, 2/1, v/v); α_D^{19.8} = 1.3 (c 0.1, CHCl₃); IR (ν): 3419, 3088, 3064, 3031, 2926, 2865, 1724, 1453, 1268, 1097, 1028, 736, 711, 698 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 8.14-8.13 (m, 2H, CH_{arom} OBz), 8.05-8.03 (m, 4H, CH_{arom} OBz), 7.58-7.53 (m, 3H, CH_{arom}), 7.50-7.47 (m, 2H, CH_{arom}), 7.39-7.21 (m, 36H, CH_{arom}), 7.19-7.06 (m, 12H, CH_{arom}), 5.72 (s, 1H, H-2'''), 5.67 (s, 1H, H-2'), 5.59 (s, 1H, H-2''), 5.25 (s, 1H, H-1''), 4.98 (s, 1H, H-1'''), 4.87-4.65 (m, 10H, CH₂ O-Bn), 4.85 (s, H-1), 4.81 (s, H-1'), 4.59-4.41 (m, 9H, CH₂ O-Bn), 4.35-4.32 (m, 2H, CH₂ O-Bn), 4.19-4.00 (m, 5H, H-3, H-3'', H-4'', H-4''', H-5''), 3.96 (dd, *J* = 2.9 Hz, *J* = 9.5 Hz, 1H, H-3'''), 3.88-3.70 (m, 7H, H-2, H-3', H-4', H-5, H-5''', H-6'), 3.64-3.63 (m, 1H, CH-a H-6), 3.57 (d, *J* = 11.2 Hz, 1H, CH-b H-6), 3.35 (dd, *J* = 5.4 Hz, *J* = 8.5 Hz, 1H, H-5'); ¹³C NMR (125 MHz, CDCl₃): δ 165.6, 165.5, 165.4 (3x C=O Bz), 154.2 (C=O Troc), 138.7, 138.6, 138.5, 138.2, 138.1, 138.0, 137.9, 137.8, 137.4 (9x CH_{arom}), 133.4, 133.2, 133.1 (3x CH_{arom} OBz), 129.99, 129.97, 129.94, 129.8 (4x CH_{arom} OBz), 128.7, 128.5, 128.4, 128.35, 128.28, 128.18, 128.15, 128.1, 128.0, 127.9, 127.82, 127.77, 127.73, 127.65, 127.54, 127.48, 127.44, 127.36 (18x CH_{arom}), 99.7 (C-1''), 98.2 (C-1'''), 97.2 (C-1'), 95.4 (CCl₃ Troc),

91.8 (C-1), 79.0 (C-3'), 77.8 (C-3''), 77.5 (C-3'''), 76.0 (C-3), 75.5, 75.3 (2x CH₂ Bn), 74.7 (C-4'), 74.6 (3x CH₂ Bn), 74.5 (C-4), 74.3 (C-4'''), 73.9 (C-4''), 73.4, 73.3 (2x CH₂ Bn), 72.7 (C-5''), 71.8 (C-5'''), 71.7 (C-2'), 71.5, 71.2 (2x CH₂ Bn), 70.1 (C-5), 69.4 (C-2''), 68.9, 68.8 (C-6, C-6'', C-6'''), 68.5 (C-2'''), 66.9 (C-6'), 54.3 (C-2); HRMS (ESI) *m/z* calcd. for [C₁₁₁H₁₁₀Cl₃NNaO₂₅]⁺: 1984.6325, *obsd.*: 1984.6325

4-O-(2-O-benzoyl-3,6-di-O-[2-O-benzoyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl]-4-O-benzyl-β-D-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamido-1-O-(N-[phenyl]trifluoroacetimidoyl)-α/β-D-glucopyranoside (82). Lactol (77)

(125.7 mg, 0.064 mmol) was dissolved in dry acetone (0.7 mL), N-(phenyl)trifluoroacetimidoyl chloride (26 μL, 0.19 mmol) was at 0°C followed by the addition of Cs₂CO₃ (31 mg, 0.096 mmol). The resulting suspension was allowed to warm up to rt and stirred for 2 h and until side product formation was observed [*R_f* side: 0.63 (toluene/EtOAc, 6/1, v/v)]. The reaction was diluted with EtOAc, then filtered over celite to remove the unreacted Cs₂CO₃ and concentrated *in vacuo*. Purification using flash chromatography (silica gel, 3% → 6% EtOAc in toluene) yielded title compound **82** as a colourless oil. (108.7 mg, 0.051 mmol, 80%); α/β = 2/1; TLC α *R_f*: 0.57, β *R_f*: 0.50 (toluene/EtOAc, 6/1, v/v); IR (ν): 3050, 3030, 2930, 2873, 1560, 1728, 1355, 1268, 1108, 1042, 752 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 8.19-8.18 (m, 2H, CH_{arom} Bz), 8.06-8.04 (m, 2H, CH_{arom} Bz), 7.96-7.94 (m, 2H, CH_{arom} Bz), 7.58-7.49 (m, 5H, 3x CH_{arom} Bz, 2x CH_{arom} Bn), 7.40-7.05 (m, 50H, CH_{arom}), 6.87 (m, 2H, CH_{arom} Bn), 6.70-6.69 (m, 2H, CH_{arom} Bn), 5.76 (s, 1H, H-2'''), 5.67 (d, *J*_{1',2'} = 3.0 Hz, 1H, H-2'), 5.62 (t, *J*_{1'',2''} = 2.2 Hz, 1H, H-2''), 5.26 (d, *J*_{1''',2'''} = 1.5 Hz, 1H, H-1'''), 5.10 (d, *J*_{1''',2'''} = 1.4 Hz, 1H, H-1'''), 4.87-4.81 (m, 4H, 3x CH₂ Bn, NH Troc), 4.76-4.59 (m, 6H, 6x CH₂ Bn, H-1'), 4.69 (bs, 1H, H-1'), 4.59-4.46 (m, 8H, 6x CH₂ Bn, 2 x CH₂ Troc), 4.41 (d, *J*_{a,b} = 12.2 Hz, 2H, CH₂ Bn), 4.33 (d, *J*_{a,b} = 11.4 Hz, 2H, CH₂ Bn), 4.19-4.07 (m, 6H, H-3, H-3''', H-4, H-4'' H-4'''), 4.00-3.98 (m, 1H, H-3''), 3.96 (t, *J* = 10 Hz, 1H, H-4'), 3.92 (dd, *J*_{5',6a'} = 11.7 Hz, 1H, H-6a'), 3.87-3.77 (m, 6H, H-2, H-3', H-5'', 2 x H-6, H-6b'), 3.72-3.57 (m, 5H, H-5'', 4x H-6), 3.46-3.41 (m, 1H, H5), 3.24-3.22 (m, 1H, H5); ¹³C NMR (125 MHz, CDCl₃): δ 165.7, 165.5, 165.3 (3x C=O Bz), 153.9 (C=O Troc), 146.6, 143.2, 140.2, 138.7, 138.6, 138.5, 137.9, 137.8, 137.7, 137.6 (10 x CH_{arom}), 133.4, 133.2, 133.0 (3x CH_{arom} Bz), 129.97, 129.95, 129.9, 129.83, 129.79, 129.7, 128.9, 128.73, 128.69, 128.50, 128.47, 128.39, 128.36, 128.3, 128.23, 128.18, 128.17, 128.1, 128.0, 128.0, 127.8, 127.74, 127.69, 127.64, 127.56, 127.52, 127.47, 127.446, 127.36 (29x CH_{arom}), 119.3 (CH_{arom}), 99.7 (C-1''), 99.0 (C-1'), 98.3 (C-1'''), 95.3 (CCl₃ Troc), 79.0 (C-3'), 78.0 (C-3'''), 77.9 (C-3''), 77.3 (C-3), 75.3, 75.2 (2x CH₂ Bn), 74.7, 74.61, 74.56, 74.5, 74.4 (C-4 or C-4''', C-4', C-5, C-5', CH₂ Bn), 74.2, 74.1 (2x CH₂ Bn), 73.9 (C-4''), 73.5, 73.41, 74.37, 72.9 (4x (CH₂ Bn), 72.6 (C-4 or C-4''', C-5''), 71.7 (CH₂ Bn), 71.6 (C-2'), 71.3 (CH₂ Bn), 69.3 (C-2''), 69.1, 68.9 (2x C-6), 68.5 (C-2'''), 67.7 (C-6), 65.7 (C-6'), 53.9 (C-2); HRMS (ESI) *m/z* calcd. for [C₁₁₉H₁₁₄Cl₃F₃N₂NaO₂₅]⁺: 2155.6621, *obsd.*: 2155.6626

Tert-butyldimethylsilyloxy 4-O-(2-O-benzoyl-3,6-di-O-[2-O-benzoyl-3,4,6-tri-O-benzyl- α -D-mannopyranosyl]-4-O-benzyl- β -D-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)cabonylamido- β -D-glucopyranoside (58b).

Diol acceptor (**72**) (628 mg, 0.63 mmol) and mannose donor (**53**) (1.68 g, 2.4 mmol), were co-evaporated with toluene (3x), dissolved in dry CH_2Cl_2 (12 mL) and activated molecular sieves (4Å) added. The resulting suspension was left to stir at 0°C for 45 min after which TMSOTf (328 μL , 1M in CH_2Cl_2) was added. The reaction was then allowed to warm up to rt. After 4h a second portion of mannose donor (**53**) (407 mg, 0.58 mmol) was added in CH_2Cl_2 (1 mL) and left an additional 1.5h. the reaction was diluted with CH_2Cl_2 and the organic phase washed with sat. aq NaHCO_3 (2x) and Brine (1x), dried over MgSO_4 , filtered and concentrated *in vacuo*. Purification of the residue by column chromatography (silica gel, 0% $\rightarrow$ 5% EtOAc in CH_2Cl_2) and size exclusion chromatography (Sephadex® LH-20, bead size 25-100 μ , $\text{CH}_2\text{Cl}_2/\text{MeOH}$, 1/1, v/v) yielded the title compound (**58b**) (1178.2 mg, 0.567 mmol, 91 %) as a colourless oil. R_f : 0.60 ($\text{CH}_2\text{Cl}_2/\text{EtOAc}$, 20/1, v/v); $\alpha_D^{16.0}$: -0.6 ($c = 1.0$ in CHCl_3); IR (v): 3064, 3031, 2928, 2859, 1724, 1602, 1515, 1497, 1453, 1362, 1316, 1267, 1097, 1070, 1028, 839, 737, 711, 698 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3): δ 8.15-8.13 (m, 2H, CH_{arom} Bz), 8.09-8.06 (m, 2H, CH_{arom} Bz), 8.05-8.03 (m, 2H, CH_{arom} Bz), 8.0-7.98 (m, 2H, CH_{arom} Bz), 7.58-7.40 (m, 3H, CH_{arom}), 7.38-7.23 (m, 33H, CH_{arom}), 7.19-7.14 (m, 8H, CH_{arom}), 7.10 (s, 6H, CH_{arom}), 6.91-6.90 (m, 2H, CH_{arom}), 5.68 (s, 2H, H-2' and H-2''), 5.59 (t, $J_{1''',2'''} = J_{2''',3'''} = 2.2$ Hz, 1H, H-2'''), 5.24 (d, $J_{1''',2'''} = 1.5$ Hz, 1H, H-1'''), 5.06 (s, 1H, H-1''), 4.82 (s, 1H, H-1'), 4.81 (d, $J_{a,b} = 10.9$ Hz, 1H, CH_2 Bn), 4.77 (d, $J_{a,b} = 12.2$ Hz, 1H, CH_2 Bn), 4.75 (d, $J_{a,b} = 11.0$ Hz, 1H, CH_2 Bn), 4.73 (d, $J_{a,b} = 12.2$ Hz, 1H, CH_2 Bn), 4.68 (bs, 1H, H-1), 4.65 (d, $J_{a,b} = 11.7$ Hz, 1H, CH_2 Bn), 4.63 (d, $J_{a,b} = 10.9$ Hz, 1H, CH_2 Bn), 5.54 (d, $J_{a,b} = 11.3$ Hz, 1H, CH_2 Bn), 4.53 (d, $J_{a,b} = 11.5$ Hz, 1H, CH_2 Bn), 4.50 (d, $J_{a,b} = 11.9$ Hz, 2H, CH_2 Troc), 4.46 (d, $J_{a,b} = 12.2$ Hz, 1H, CH_2 Bn), 4.42 (d, $J_{a,b} = 11.5$ Hz, 1H, CH_2 Bn), 4.39 (d, $J_{a,b} = 11.0$ Hz, 1H, CH_2 Bn), 4.36 (d, $J_{a,b} = 11.2$ Hz, 1H, CH_2 Bn), 4.31 (d, $J_{a,b} = 11.2$ Hz, 1H, CH_2 Bn), 4.12 (t, $J_{3''',4'''} = J_{4''',5'''} = 9.5$ Hz, 1H, H-4'''), 4.07-4.05 (m, 3H, H-5''', H-3'', H-4' or H-4''), 4.01 (t, $J_{3,4} = J_{4,5} = 9.3$ Hz, 1H, H-4), 3.95 (dd, $J_{2''',3'''} = 3.0$ Hz, $J_{3''',4'''} = 9.2$ Hz, 1H, H-3'''), 3.86 (dd, $J_{6a',6b'} = 5.1$ Hz, $J_{5',6'} = 11.2$ Hz, 1H, H-6a'), 3.85 (s, 3H, H-3', H-5'', H-4' or H-4''), 3.82 (dd, $J_{6a'',6b''} = 2.9$ Hz, $J_{5'',6''} = 11.2$ Hz, 1H, H-6a'''), 3.79 (t, $J_{6a',6b'} J_{6a'',6b''} = 11.1$ Hz, 2H, H-6b' and H-6b'''), 3.66 (dd, $J_{6a'',6b''} = 3.4$ Hz, $J_{5'',6''} = 11.0$ Hz, 1H, H-6a''), 3.69 (dd, $J_{6a,6b} = 2.9$ Hz, $J_{5,6} = 11.7$ Hz, 1H, H-6a), 3.58 (dd, $J_{6a,6b} = 0.8$ Hz, $J_{5,6} = 11.0$, 1H, H-6b), 3.57 (bt $J_{2,3} = J_{3,4} = 9.2$ Hz, 1H, H-3), 3.52 (t, $J_{5,6} = J_{6a'',6b''} = 12.7$ Hz, 1H, H-6b''), 3.34-3.30 (m, 2H, H-5 and H-5'), 3.02 (bdd, $J_{1,2} = 7.2$ Hz, 1H, H-2), 0.82 (s, 9H, Me *t*-Bu OTBDMS), 0.04, -0.01 (2x s, 3H, Me OTBDMS); ^{13}C NMR (125 MHz, CDCl_3): δ 165.8, 165.7, 165.6 (3 x C=O, OBz), 153.6 (C=O, Troc), 138.94, 138.86, 138.3, 138.2, 138.1, 137.8, 133.4, 133.2, 133.1, 130.1, 128.9, 128.7, 128.62, 128.59, 128.57, 128.52, 128.50, 128.47, 128.44, 128.43, 128.37, 128.29, 128.28, 128.2, 128.13, 128.09, 128.03, 127.98, 127.96, 127.91, 127.83, 127.78, 127.7, 127.64, 127.61, 127.59, 127.57, 127.54, 127.52, 127.45, 127.3 (41 $\times$ CH_{arom}),

100.0 (C-1'''), 98.8 (C-1'), 98.2 (C-1''), 95.6 (C-1), 95.5 (CCl₃, Troc), 79.6 (C-3'), 78.4 (C-3''), 78.2 (C-4), 78.0 (C-3'''), 77.4 (C-3), 75.4, 75.2 (CH₂, Bn), 74.8, 74.6 (C-5, C-5', C-4', C-4''), 74.1 (C-4'''), 74.0, 73.61, 73.59, 73.5 (CH₂, Bn), 72.8 (C-5'''), 72.3 (CH₂ Bn), 71.9 (C-2'), 71.6 (CH₂ Bn), 69.6 (C-2'''), 69.3, 69.13, 69.07, 68.9 (C-2'', C-6, C-6'', C-6'''), 66.5 (C-6'), 59.6 (C-2), 25.8 (Me, *t*-Bu, OTBDMS), 18.1 (C_q, *t*-Bu, OTBDMS), -4.1, -5.1 (2 x Me, TBDMS). HRMS(ESI) *m/z* calcd. for [C₅₇H₆₆O₁₄NCl₃Si+Na]⁺: 2098.7195, obsd.: 2098.7184.

***Tert*-butyldimethylsilyloxy 4-*O*-(2-*O*-[2,3,4-tri-*O*-acetyl- β -D-xylopyranosyl]-3,6-di-*O*-[2-*O*-benzoyl-3,4,6-tri-*O*-benzyl- α -D-mannopyranosyl]-4-*O*-benzyl- β -D-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamido- β -D-glucopyranoside (58a).**

Trimer acceptor (**74**) (438 mg, 0.38 mmol) and mannose imidate donor (**53**) (1.01 g, 1.5 mmol) were co-evaporated together with toluene (3x), dissolved in CH₂Cl₂ (7.5 mL) and activated molecular sieves (4 Å) were added. The resulting suspension was stirred at 0°C for 45 min after which TMSOTf (205 μ l, 1M in CH₂Cl₂) was added. the resulting mixture was kept at 0°C until TLC analysis indicated complete disappearance of donor (1h), after which an additional portion of mannose donor (**53**) was added (132mg, 0.19 mmol) and left for another 1.5h. the reaction was then diluted with EtOAc, the organic fraction washed with sat aq. NaHCO₃ (2x), Brine (1x), dried over MgSO₄, filtered and concentrated *in vacuo*. Purification of the residue by column chromatography (silica gel, PE/EtOAc, 5/1 $\rightarrow$ 4/1 $\rightarrow$ EtOAc, v/v) yielded the title compound (**58a**) (559 mg, 0.25 mmol, 66%) as a colourless oil and 6-mannosylated tetramer (**76**) (153 mg, 0.090 mmol, 24%) as a colourless oil.

***Tert*-butyldimethylsilyloxy 4-*O*-(2-*O*-[2,3,4-tri-*O*-acetyl- β -D-xylopyranosyl]-6-*O*-[2-*O*-benzoyl-3,4,6-tri-*O*-benzyl- α -D-mannopyranosyl]-4-*O*-benzyl- β -D-mannopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamido- β -D-glucopyranoside (76). R_f:**

0.26 (toluene/EtOAc, 6/1, v/v) ¹H NMR (500 MHz, CDCl₃): δ 8.09-8.07 (m, 2H, CH_{arom} OBz), 7.57-7.54 (m, 1H, CH_{arom} Bz), 7.38-7.14 (m, 37H, CH_{arom}), 5.25 (t, $J_{2'',3''} = J_{3'',4''} = 8.8$ Hz, 1H, H-3''), 5.00-4.93 (m, 3H, H-2'', H-4'', CH₂ Bn), 4.94 (s, 1H, H-1'''), 4.90-4.82 (m, 4H, 3x CH₂ Bn, NH Troc), 4.88 (1H, H-1), 4.83 (1H, H-1'), 4.73-4.47 (m, 11H, 2x CH₂ Troc, 9x CH₂ Bn), 4.64 (1H, H-1'), 4.38 (d, $J_{a,b} = 12.0$ Hz, 1H, CH₂ Bn), 4.15 (dd, $J_{4'',5a''} = 5.1$ Hz, $J_{5a'',5b''} = 12.0$ Hz 1H, H-5a''), 4.07-4.05 (m, 2H, H-2', H-3), 3.96 (t, $J_{3,4} = J_{4,5} = 8.3$ Hz, 1H, H-4), 3.90-3.85 (m, 2H, H-2' and H-3), 3.77-3.69 (m, 3H, H-4''', H-6a''', H-6b'''), 3.67-3.58 (m, 3H, H-6a, H-6a', H-6b'), 3.51-3.44 (m, 3H, H-3', H-4', H-6b), 3.38-3.29 (m, 3H, H-5, H-5', H-5b''), 3.20-3.15 (m, 1H, H-2), 3.00 (bd, $J_{3'',OH} = 9.8$ Hz, 1H,

Tert-butyltrimethylsilyloxy 4-O-[2-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-3,6-di-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-4-O-benzyl-β-D-mannopyranosyl]-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamido-β-D-glucopyranoside (58a). R_f: 0.50 (toluene/EtOAc, 6/1, v/v) α_D^{16.0}: -1.2 (c = 1.0 in CHCl₃); IR (ν): 3064, 3031, 2931, 2859, 1756, 1725, 1454, 1267, 1250, 1220, 1097, 1071, 839, 752, 698 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 8.10-8.05 (m, 4H, CH_{arom} OBz), 7.58-7.54 (m, 2H, CH_{arom} OBz), 7.42-7.14 (m, 49H, CH_{arom}), 5.73 (s, 1H, H-2'''), 5.66 (s, 1H, H-2'''), 5.22 (s, 1H, H-1'''), 5.17 (t, J_{2'',3''} = J_{3'',4''} = 9.0 Hz, 1H, H-3''), 5.07-5.04 (m, J_{1'',2''} = 7.1 Hz, J_{2'',3''} = 9.0 Hz, 2H, H-2'' and NH Troc), 4.93-4.82 (m, 5H, 4 x CH₂ Bn, H-4''), 4.92 (1H, H-1'''), 4.89 (1H, H-1''), 4.84 (1H, H-1), 4.79-4.40 (m, 16H, CH₂ Bn), 4.65 (1H, H-1'), 4.26-4.21 (m, 2H, H-3''' and H-5'''), 4.13 (bs, 1H, H-2'), 4.09-4.02 (m, 4H, H-3''', H-4''', H-4''', H-5a''), 3.94 (t, J_{3,4} = J_{4,5} = 9.0 Hz, 1H, H-4), 3.85-3.82 (m, 1H, H-3), 3.79-3.64 (m, 3H, H-3', H-4', H-5'''), 3.58-3.47 (m, 3H, H-5, H-6a', H-6b'), 3.85-3.82, 3.79-3.64, 3.58-3.47 (m, 6H, H-6a, H-6b, H-6a''', H-6b''', H-6a''', H-6b'''), 3.36-3.30 (m, 1H, H-2), 3.28-3.22 (m, 1H, H-5'), 3.19-3.14 (m, 1H, H-5b''), 2.03, 2.00, 1.95 (3 x s, 9H, Me Ac), 0.87 (s, 9H, *t*-Bu OTBDMS), 0.10, 0.06 (2 x s, 6H, Me OTBDMS); ¹³C NMR (125 MHz, CDCl₃): δ 170.0, 169.5, 169.4 (3 x C=O Ac), 165.9, 165.4 (C=O Bz), 153.8 (C=O Troc), 138.7, 138.61, 138.57, 138.5, 138.4, 138.2, 138.1, 137.0, 137.6 (9x CH_{arom}), 133.14, 133.06 (2x CH_{arom} Bz), 130.00, 129.96, 129.9, 128.44, 128.41, 128.36, 128.34, 128.31, 128.24, 128.16, 128.03, 127.97, 127.92, 127.87, 127.6, 127.53, 127.50, 127.47, 127.42, 127.39, 127.35 (24x CH_{arom}), 100.3 (C-1''), 100.0 (C-1'), 99.7 (C-1'''), 96.8 (C-1'''), 95.5 (CCl₃ Troc), 95.3 (C-1), 79.0 (C-3'), 77.7, 77.6, 77.43, 77.40 (C-3, C-3''', C-3''', C-4), 75.6 (C-2'), 75.1 (CH₂ Bn), 74.43, 74.38, 74.1, 73.8, 73.5 (C-4', C-4''', C-4''', C-5, C-5'), 73.4, 73.34, 73.31 (CH₂ Bn), 72.4 (C-5'''), 72.3, 72.0 (CH₂ Bn), 71.8 (C-5'''), 71.6 (C-3''), 71.1 (CH₂ Bn), 71.0 (C-2''), 69.5, 69.3, 69.18, 69.15, 68.8 (C-2''', C-2''', C-4'', C-6, C-6''', C-6'''), 66.3 (C-6'), 61.8 (C-5''), 58.8 (C-2), 25.6 (Me *t*-Bu OTBDMS), 20.80, 20.78, 20.76 (3 x Me OAc), 17.9 (C_q *t*-Bu OTBDMS), -4.2, -5.2 (2 x Me OTBDMS); HRMS (ESI) *m/z* calcd. for [C₁₂₁H₁₃₄Cl₃NNaO₃₁Si]⁺: 2252.7667, found.: 2252.7673.

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mannopyranosyl]-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamido- α/β -D-glucopyranose (84). Pentasaccharide (**58a**) (208 mg, 0.093 mmol) was transferred to a falcon tube, dissolved in THF (0.9 mL) and treated with HF-pyridene (70%, 110 μ L) for 4.5h after which EtOAc was added and the organic layer was washed with saturated aq. NaHCO₃ until no gas erupted (3x) and Brine (1x). The organic layer was dried over MgSO₄, Filtered, and concentrated *in vacuo*. Purification by flash chromatography (silica gel, 33% EtoAc in PE) yielded the title compound (**84**) as colourless oil (185 mg, 0.087 mmol, 94%, α/β = 3/1); TLC R_f: 0.26 (PE/EtOAc, 1/1, v/v); IR(v): 3064, 3031, 2924, 1754, 1724, 1602, 1514, 1497, 1453, 1365, 1266, 1220, 1097, 1070, 1044, 909, 818, 733, 711, 698 cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ 8.10-8.07 (m, 4H, CH_{arom} OBz), 7.59-7.55 (m, 2H, CH_{arom} OBz), 7.43-7.15 (m, 50H, 4x CH_{arom} Bz, 46x CH_{arom} Bn), 5.66 (s, 2H, H-2''', H-2'''), 5.21 (s, 1H, H-1'''), 5.11 (t, $J_{2'',3''} = J_{3'',4''} = 8.3$ Hz, 2H, H-3'', NH Troc), 5.02 (t, $J_{1'',2''} = J_{2'',3''} = 8.8$ Hz, 1H, H-2''), 4.95-4.88 (m, 3H, H-1'' and H-4'', CH₂ Bn), 4.84-4.76 (m, 5H, 4x CH₂ Bn, CH₂ Troc), 4.76 (s, 1H, H-1'''), 4.71-4.55 (m, 11H, 10x CH₂ Bn, CH₂ Troc), 4.69 (s, 1H, H-1'), 4.49-4.46 (m, 2H, 2x CH₂ Bn), 4.47 (bs, 1H, H-1), 4.40 (d, $J_{a,b} = 12$ Hz, 1H, CH-b Bn), 4.33 (d, $J_{a,b} = 12$ Hz, 1H, CH-b Bn), 4.24-3.95 (m, 11H, H-2, H-2' H-3, H-3'', H-3''', H-4, H-4', H-4'', H-4''', H-4''', H-5a''), 3.85-3.73, 3.61-3.58, 3.50-3.41 (m, 11H, H-3', H-5 or H-5', H-5'', CH₂ H-6, CH₂ H-6', CH₂ H-6'', CH₂ H-6'''), 3.33 (t, $J = 8.8$ Hz, 1H, H-5 or H-5'), 3.11 (dd, $J = 9.3$ Hz, $J = 11.5$ Hz, 1H, H-5b''), 2.08, 2.02, 2.00 (3x s, 9H, Me OAc); ¹³C NMR (125 MHz, CDCl₃): δ 171.2, 169.9, 169.5 (3x C=O OAc), 165.7, 165.5 (3x C=O Bz), 154.1 (C=O Troc), 138.8, 138.7, 138.6, 138.2, 137.93, 137.90, 137.8, 137.31, 137.28 (9x CH_{arom}) 133.3, 133.1 (CH_{arom} Bz), 130.2, 130.0 (2x CH_{arom} Bz), 129.9, 129.7, 128.60, 128.56, 128.49, 128.45, 128.404, 128.397, 128.36, 128.34, 128.32, 128.27, 128.2, 128.1, 127.9, 127.8, 127.8, 127.6, 127.50, 127.46, 127.40, 127.37, 127.2 (23 x CH_{arom}), 99.9 (C-1'''), 99.6 (C-1''), 97.8 (C-1'''), 96.7 (C-1'), 95.4 (CCl₃ Troc), 91.5 (C-1), 79.7 (C-3'), 77.3 (C-3'''), 76.5 (C-3'''), 75.8 (CH₂ Bn), 75.3 (CH₂ Bn), 75.0 (CH₂ Bn), 74.7 (C-2', C-5 or C-5'), 74.6 (CH₂ Bn), 74.34, 74.29, 74.2, 73.8 (C-4, C-4', C-4''', C-5 or C-5'), 73.4, 73.3, 73.2 (CH₂ Bn), 72.9 (C-5'''), 72.4 (C-4'''), 71.8 (CH₂ Bn), 71.42, 71.36 (C-3'', C-2''), 71.1 (C-2''), 71.0 (CH₂ Bn), 70.2 (C-3), 69.4, 69.3, 68.2, 69.21, 68.9, 68.6, 68.44, 68.40 (C-2''', C-4'', C-2''', C-6, C-6', C-6'', C-6'''), 61.7 (C-5''), 54.3 (C-2), 20.9, 20.84, 20.77 (3 x Me Ac); HRMS (ESI) *m/z* calcd. for [C₁₁₅H₁₂₀Cl₃NNaO₃₁]⁺: 2138.6802, odsd.: 2138.6807

4-O-[2-O-(2,3,4-tri-O-acetyl- β -D-xylopyranosyl)-3,6-di-O-(2-O-benzoyl-3,4,6-tri-O-benzyl- α -D-mannopyranosyl)-4-O-benzyl- β -D-mannopyranosyl]-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamido-1-O-(N-(phenyl)trifluoroacetimidoyl)- α/β -D-glucopyranoside (85**).**

Lactol Pentasaccharide (**84**) (185 mg, 0.087 mmol) was dissolved in acetone (0.7 mL), N-(phenyl)trifluoroacetimidoyl chloride (36 μ L, 0.26 mmol) was added. The resulting solution was cooled to 0°C and Cs₂CO₃ (43 mg, 0.131 mmol) added. The resulting suspension was stirred for 2 h at rt after which

EtOAc was added and the unreacted Cs₂CO₃ filtered off over celite. The organic phase was then concentrated *in vacuo*. Purification using flash chromatography (silica gel, 4% → 7% EtOAc in toluene) yielded title compound (**85**) as a colourless oil. (161 mg, 0.070 mmol, 80%), α/β = 5/1; TLC R_f: 0.54 (toluene/EtOAc, 6/1, v/v); IR(v): 3064, 3030, 2927, 2866, 1755, 1724, 1600, 1497, 1454, 1267, 1219, 1099, 1047, 752, 698 cm⁻¹; ¹H NMR (600 MHz, CDCl₃): δ 8.08-8.03 (m, 4H, CH_{arom} OBz), 7.58-7.52 (m, 2H, CH_{arom} OBz), 7.42-7.10 (m, 52H, CH_{arom}), 6.76-6.75 (m, 2H, CH_{arom}), 5.76 (s, 1H, H-2'''), 5.73 (s, 1H, H-2'''), 5.25 (s, 1H, H-1'''), 5.13 (t, $J_{2'',3''}$ = 7.6 Hz, 1H, H-3''), 5.06 (t, $J_{2'',3''}$ = 7.4 Hz, 1H, H-2''), 4.95-4.40 (m, 22H, NH Troc, CH₂ Troc, H-4'', 9x CH₂ Bn), 4.91 (s, 1H, H-1'''), 4.88 (1H, H-1''), 4.56 (1H, H-1') 4.23-4.21 (m, 2H), 4.14 (dd, $J_{4'',5a''}$ = 4.7 Hz, $J_{5a'',5b''}$ = 11.7 Hz, 1H, H-5a''), 4.08-3.95 (m, 6H), 3.84-3.57 (m, 10H), 3.54-3.52 (m, 2H), 3.22 (dd, $J_{4'',5b''}$ = 7.4 Hz, $J_{5a'',5b''}$ = 11.8 Hz, H-5b''), 3.19-3.16 (m, 1H, H-5'), 2.01, 1.99, 1.98 (3 x s, 3H, Me Ac); ¹³C NMR (150 MHz, CDCl₃): δ 169.9, 169.6, 169.2 (3 x C=O Ac), 165.6, 165.5 (3x C=O Bz), 154.2 (C=O Troc), 143.3 (C=NPh), 138.73, 138.71, 138.63, 138.61, 138.5, 138.3, 138.23, 138.17, 138.04, 138.00, 137.8, 137.7, 133.4, 133.2, 133.1, 130.14, 130.10, 130.0, 129.5, 128.9, 128.8, 128.72, 128.68, 128.63, 128.56, 128.54, 128.51, 128.49, 128.46, 128.44, 128.41, 128.39, 128.37, 128.31, 128.29, 128.2, 128.1, 128.04, 128.01, 128.00, 127.95, 127.91, 127.89, 127.85, 127.8, 127.74, 127.69, 127.62, 127.59, 127.58, 127.53, 127.51, 127.48, 127.3, 126.5, 124.6, 120.5, 119.4 (58x CH_{arom}), 101.1 (C-1'), 100.4 (C-1''), 99.9 (C-1'''), 97.7 (C-1'''), 95.4 (CCl₃), 79.0, 77.74, 77.72, 77.4, 77.2, 76.9, 76.8, 75.3, 75.23, 75.20, 75.18, 75.16, 74.7, 74.6, 74.5, 74.4, 74.1, 73.6, 73.52, 73.50, 73.47, 73.44, 73.39, 72.5, 72.0, 71.1, 70.7, 70.2, 69.7, 69.4, 69.34, 69.26, 69.1, 69.0, 68.9, 68.8, 68.3, 66.4, 53.6, 20.9, 20.8 (3x Me Ac); HRMS (ESI) m/z calcd. for [C₁₂₃H₁₂₄Cl₃F₃N₂NaO₃₁]⁺: 2309.7098, odstd.: 2309.7104

Tert-butyldimethylsilyloxy 2-azido-4-O-(2-O-(2,3,4-tri-O-acetyl- β -D-xylopyranosyl)-3,6-di-O-(2-O-benzoyl-3,4,6-tri-O-benzyl- α -D-mannopyranosyl)-4-O-benzyl- β -D-mannopyranosyl)-3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxy)carbonylamido- α/β -D-glucopyranosyl]-3,6-di-O-benzyl-deoxy- α/β -D-glucopyranoside (49b**).**

Traces of water were removed from Penta imidate (**85**) (38.1 mg, 0.0166 mmol) and acceptor (**52**) (24.6 mg, 0.0499 mmol), by co-evaporation with toluene (3x), then dissolved in dry toluene and activated molecular sieves (4 Å) were added. The resulting suspension was left to stir at rt. for 1h, then cooled to -40°C followed by the careful addition of TfOH (8.3 μ L, 0.1M in toluene). The reaction was then allowed to warm up to rt (3h), then diluted with EtOAc, filtered over cotton wool, and the organic fraction was washed with sat. aq. NaHCO₃ (2x), Brine (1x). the organic phase was dried over MgSO₄, filtered and concentrated *in vacuo*. Purification using flash chromatography (silica gel, 4% → 7% EtOAc in toluene) yielded title compound (**49b**) as a colourless oil. (28.7 mg, 0.0116 mmol, 70%), α/β = 1/1; TLC R_f: 0.34 (toluene/EtOAc, 6/1, v/v); α_D^{16} : -1.2 (c = 1.0 in CHCl₃); IR (v): 3060, 3032, 2926, 2867, 1754, 1723, 1600,

1499, 1454, 1266, 1220, 1097, 1047, 752 cm^{-1} ; ^1H NMR (500 MHz, CDCl_3): δ 8.11-8.05 (m, 8H, α/β CH_{arom} OBz), 7.59-7.54 (m, 4H α/β CH_{arom} Bz), 7.44-7.05 (m, 118H, α/β CH_{arom}), 6.10 (d, $J_{\alpha 2', \alpha \text{NH}} = 9.8$ Hz, 1H, α -NH Troc), 5.89, 5.80 (s, 4H, α/β H-2'''' and H-2'''''), 5.75 (s, 4H, α/β H-2''''', H-2'''''), 5.28 (d, $J_{\alpha 1', \alpha 2'} = 3.4$ Hz, 1H, α -H-1'), 5.26, 5.22 (s, 2H, 2x α/β H-1'''' and/or H-1'''''), 5.20-5.16 (m, 2H, α/β H-2'''' or H-3'''), 5.12-5.08 (m, 2H, α/β H-2'''' or H-3'''), 5.03 (d, $J_{a,b} = 11.7$ Hz, 1H, CH-a Bn), 4.99 (d, $J_{a,b} = 11.4$ Hz, 1H, CH-a Bn), 4.96-4.38 (m, 26H, α/β CH_2 Bn, CH_2 Troc), 4.88, 4.81, 4.57, 4.45 (9H, α/β H-1, β H-1', α/β H-1'', α/β H-1''', α/β H-1'''' and/or H-1'''''), 4.32 (dd, $J = 2.9$ Hz, $J = 9.3$ Hz, 1H), 4.26 (dd, $J = 2.9$ Hz, $J = 9.3$ Hz, 1H), 4.23-4.20 (m, 2H), 4.17-4.02 (m, 8H), 3.96 (t, $J = 9.3$ Hz, 1H), 3.92-3.81 (m, 5H), 3.76-3.39 (m, 20H), 3.38-3.17 (m, 5H, α or β H-5b'''), 3.11-3.02 (m, 5H, α or β H-5b'''), 2.10, 2.036, 2.025, 2.02 (4x s, 12H, Me Ac), 1.99 (2x s, 6H, Me Ac), 0.97, 0.96 (2x s, 18H, Me t-Bu OTBDMS), 0.20, 0.184, 0.175, 0.16 (4x s, 12H, Me OTBDMS); ^{13}C NMR (125 MHz, CDCl_3): δ 171.2, 170.3, 170.0, 169.5, 169.4, 169.30, 169.27 (6x C=O Ac), 165.7, 165.39, 165.35 (4x C=O Bz), 154.3, 153.9 (2x C=O Troc), 138.8, 138.73, 138.70, 138.67, 138.6, 138.52, 138.49, 138.3, 138.24, 138.19, 138.1, 138.02, 137.97, 137.95, 137.90, 137.85, 137.8, 137.7, 137.3 (18x CH_{arom}) 133.09, 133.05 (CH_{arom} Bz), 130.1, 130.00, 129.97, 129.9, 129.1, 128.9, 128.7, 128.63, 128.61, 128.54, 128.50, 128.47, 128.46, 128.39, 128.37, 128.34, 128.33, 128.31, 128.30, 128.26, 128.23, 128.21, 128.18, 128.16, 128.14, 128.11, 128.05, 128.03, 127.98, 127.9, 127.81, 127.79, 127.77, 127.73, 127.67, 127.6, 127.5, 127.446, 127.43, 127.41, 127.36, 127.33, 127.30, 127.2, 126.5, 125.3 (48x CH_{arom}), 101.3, 100.9, 100.5, 99.7, 99.5, 98.1, 97.4, 97.3, 97.1 (α/β C-1, β C-1', α/β C-1'', α/β C-1''', α/β C-1''''', α/β C-1'''''), 99.0 (α C-1'), 95.5 2x (α/β CCl_3 NHTroc), 82.3, 81.2, 81.1, 78.8, 78.3, 78.0, 77.8, 77.5, 76.2, 75.9, 75.2, 75.14, 75.06, 75.0, 74.9, 74.7, 74.6, 74.3, 74.3, 74.2, 74.02, 73.98, 73.9, 73.7, 73.5, 73.4, 73.30, 73.28, 73.2, 72.5, 72.4, 72.1, 72.02, 71.98, 71.9, 71.8, 71.5, 71.2, 71.0, 70.7, 70.4, 69.3, 69.2, 69.1, 68.84, 68.76, 68.7, 68.5, 68.2, 68.1, 66.3, 61.8, 61.6, 60.4, 57.2, 54.9, 25.62, 25.60 (6x Me t-Bu OTBDMS),), 21.5, 21.1, 20.93, 20.86, 20.84, 20.80, 20.77 (6x Me O-Ac), 18.00 17.98 (2x C_q t-Bu, OTBDMS), -4.2, -4.3, -5.16, -5.19 (4x Me, OTBDMS); HRMS (ESI) m/z calcd. for $[\text{C}_{141}\text{H}_{155}\text{Cl}_3\text{N}_4\text{NaO}_{35}\text{Si}]^+$: 2619.9199, odstd.: 2619.9204.

Tert-butyltrimethylsilyloxy 2-azido-4-O-[(2,2,2-trichloroethoxy)carbonylamido- α -D-glucopyranosyl]-3,6-di-O-benzyl-2-deoxy- β -D-glucopyranoside (49d).

(2,2,2-trichloroethoxy)carbonylamido- α -D-glucopyranosyl]-3,6-di-O-benzyl-2-deoxy- β -D-glucopyranoside (49d). Tetra imidate (**82**) (109 mg, 0.051 mmol) and acceptor (**52**) (78 mg, 0.15 mmol), were co-evaporated together with toluene (3x), dissolved in dry toluene, activated molecular sieves (4 Å) added and the resulting suspension stirred at rt for 1h. The solution was then cooled to 0°C followed by the careful addition of TfOH (10 μL , 1M in toluene) after which the reaction was allowed to warm up to rt. Two

additional portions of TFOH (50 μ L, 1M in toluene) were added over 5h at 0°C and the resulting mixture was left to stir for a total 14h. The reaction was diluted with EtOAc, filtered over cotton wool, and the organic fraction was washed with sat. aq. NaHCO₃ (2x), Brine (1x), dried over MgSO₄, Filtered and concentrated *in vacuo*. Purification using flash chromatography (silica gel, 4% → 7% EtOAc in toluene) yielded title compound (**49d**) as a colourless oil. (77.4 mg, 0.032 mmol, 63%), α/β = 1/1; TLC R_f α : 0.47, R_f β : 0.53 (toluene/EtOAc, 6/1, v/v); IR (v): 3062, 3034, 2928, 2864, 1750, 1602, 1498, 1266, 1096, 1047, 752 cm⁻¹; α_D^{16} : -1.2 (c = 1.0 in CHCl₃); Spectroscopic data α : ¹H NMR (500 MHz, CDCl₃): δ 8.18-8.16 (m, 2H, CH_{arom} OBz), 8.06-8.03 (m, 2H, CH_{arom} OBz), 8.00-7.88 (m, 2H, CH_{arom} OBz), 7.57-7.52 (m, 5H, CH_{arom}), 7.37-7.09 (m, 54H, CH_{arom}), 7.01-6.98 (m, 2H, CH_{arom}), 6.94-6.91 (m, 3H, CH_{arom}), 5.72 (s, 1H, H-2'''), 5.62-5.61 (m, 2H, H-2'', H-2'''), 5.41 (d, J_{NH,2} = 9.8 Hz, 1H, NH' Troc), 5.25 (d, J_{1',2'} = 3.4 Hz, 1H, H-1'), 5.18 (s, 1H, H-1'''), 4.88 (s, 1H, H-1'''), 4.87-4.81 (m, 3H, CH₂ Bn), 4.76-4.65 (m, 6H), 4.66 (H-1''), 4.60-4.41 (m, 17H), 4.46 (H-1), 4.33-4.26 (m, 3H), 4.16 (t, J = 9.5 Hz, 1H), 4.07-4.04 (m, 3H), 4.00-3.52 (m, 17H), 3.41 (d, J = 10.5 Hz), 3.32-3.15 (m, 5H), 0.91 (s, 9H, Me *t*-Bu OTBDMS), 0.14, 0.12 (2x s, 6H, Me OTBDMS); ¹³C NMR (125 MHz, CDCl₃): δ 165.8, 165.6, 165.4 (3x C=O Bz), 154.2 (C=O NHTroc), 138.8, 138.73, 138.68, 138.6, 138.3, 138.0, 137.9, 137.4, 133.6, 133.3, 133.2, 130.3, 130.1, 130.04, 129.98, 129.9, 129.0, 128.8, 128.6, 128.54, 128.49, 128.46, 128.44, 128.43, 128.33, 128.3, 128.27, 128.25, 128.09, 128.05, 128.0, 127.91, 127.88, 127.8, 127.7, 127.59, 127.56, 127.53, 127.50, 127.48, 127.3, 127.1 (41x CH_{arom}), 99.8 (C-1'''), 99.5 (C-1''), 98.5 (C-1'), 98.4 (C-1'''), 97.4 (C-1), 95.5 (CCl₃ NHTroc), 82.0, 79.9, 78.1, 78.01, 77.97, 77.7, 75.3, 75.2, 74.9, 74.8, 74.5, 74.2, 74.1, 74.0, 73.50, 73.47, 73.2, 72.8, 72.2, 71.9, 71.7, 71.32, 71.28, 69.3, 69.0, 68.8, 68.4, 68.2, 65.9, 55.1 25.7 (Me, *t*-Bu OTBDMS), 18.1 (C_q *t*-Bu OTBDMS), -4.2, -5.1 (2x Me OTBDMS); Coupling constants; 99.8 (C-1''', J = 173.4 Hz), 99.5 (C-1'', J = 159.8 Hz), 98.5 (C-1', J = 176.5 Hz), 98.4 (C-1''', J = 175.6 Hz), 97.4 (C-1, J = 159.6 Hz); Spectroscopic data β : ¹H NMR (500 MHz, CDCl₃): δ 8.23-8.21 (m, 2H, CH_{arom} OBz), 8.07-8.03 (m, 2H, CH_{arom} OBz), 8.00-7.88 (m, 2H, CH_{arom} OBz), 7.58-7.55, 7.40-6.88 (m, 64H, CH_{arom}), 5.72 (s, 1H, H-2'''), 5.62 (d, J = 3.6 Hz, 1H, H-2''), 5.61 (s, 1H, H-2'''), 5.15 (s, 1H, H-1'''), 4.99 (d, J_{a,b} = 11.7 Hz, 1H, CH-a Bn), 4.92 (bs, 1H, H-1'''), 4.90-4.82, 4.77-4.64, 4.62-4.38, 4.34-4.27 (m, 27H, NHTroc, CH₂ Troc, CH₂ Bn), 4.69 (H-1''), 4.40 (H-1), 4.22 (d, J_{a,b} = 12.0 Hz, 1H, CH-b Bn), 4.18-4.12, 4.07-3.52 (m, 19H), 4.12 (H-1'), 3.45 (bd, J = 10.2 Hz, 1H), 3.34 (bd, J_{2',3'} = 8.5 Hz, J_{NH,2'} = 16.4 Hz, 1H, H-2'), 3.26-3.15 (M, 4H), 3.09 (bd, J = 9.3 Hz, 1H), 2.94 (bt, J = 8.5 Hz, 1H, H-3'), 0.92 (s, 9H, Me *t*-Bu OTBDMS), 0.14, 0.12 (2x s 6H, Me OTBDMS); ¹³C NMR (125 MHz, CDCl₃): δ 165.8, 165.6, 165.4 (3x C=O Bz), 153.8 (C=O NHTroc), 139.0, 1387, 138.6, 138.0, 137.9, 137.8, 133.6, 133.3, 133.2, 130.14, 130.10, 130.02, 129.98, 129.94, 129.89, 129.21, 129.17, 129.1, 129.0, 128.9, 128.81, 128.76, 128.6, 128.54, 128.49, 128.47, 128.43, 128.35, 128.31, 128.27, 128.23, 128.21, 128.16, 128.1, 128.0, 127.94, 127.90, 127.89, 127.86, 127.84, 127.81, 127.76, 127.7, 127.64, 127.6, 127.53, 127.48, 127.4, 125.4 (50x CH_{arom}), 100.8 (C-1'), 99.9 (C-1'''), 98.7 (C-1''), 98.3 (C-1'''), 97.2 (C-1), 95.7 (CCl₃ NHTroc), 81.1, 80.2, 79.3, 78.1, 78.0, 77.8, 75.4, 75.2, 74.9, 74.6, 74.4, 74.2, 74.0, 73.6, 73.5, 73.5, 73.3, 72.8, 72.1, 71.9, 71.8, 71.7, 71.4, 69.3, 68.9, 68.7, 68.5, 68.4, 67.8, 53.6, 25.7 (Me, *t*-Bu OTBDMS), 18.1 (C_q *t*-Bu OTBDMS), -4.2, -5.1 (2x

Me OTBDMS); Coupling constants; 100.8 (C-1', $J = 160.0$ Hz), 99.9 (C-1''', $J = 173.6$ Hz), 98.7 (C-1'', $J = 159.7$ Hz), 98.3 (C-1''', $J = 176.3$ Hz), 97.2 (C-1, $J = 164.5$ Hz); HRMS (ESI) m/z calcd. for $[C_{137}H_{145}Cl_3N_4NaO_{29}Si]^+$: 2465.8722, odstd.: 2465.8728.

***Tert*-butyldimethylsilyloxy 2-acetamido-2-deoxy-4-O-(2-acetamido-2-deoxy-4-O-(2-O-benzoyl-3,6-di-O-(2-O-benzoyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-4-O-benzyl-β-D-mannopyranosyl)-3,6-di-O-benzyl-β-D-**

glucopyranosyl)-3,6-di-O-benzyl-β-D-glucopyranoside (95). Pentamer (**49d**) (21.4 mg, 0.0087 mmol) was dissolved in THF (0.2 mL), activated zinc was added (110 mg, 1.68 mmol) followed by aq. KH_2PO_4 (0.04 mL, 1M) after which the suspension was stirred vigorously for 2h. The suspension was then diluted with EtOAc, filtered over celite to remove unreacted zinc, and co-concentrated in presence of pyridine. The resulting yellow oil was dissolved in pyridine/ Ac_2O (0.3mL, 2/1) and left to stir until ninhydrin stain showed complete disappearance of amine product (1 h). The reaction was diluted with EtOA, and organic fraction washed with sat. aq. $NaHCO_3$ (3x) and until no further gas was formed and brine (1x). The organic phase was dried over $MgSO_4$, filtered and concentrated. Purification of the residue by column chromatography (silica gel, PE/EtOAc, 2/1→1/1, v/v) yielded the title compound (**95**) (6 mg, 0.0258 mmol, 30%) as a yellow oil. R_f : 0.42 (Pe/EtOAc, 1/2, v/v); α_D^{17} : -0.03 ($c = 1.0$ in $CHCl_3$); 1H NMR (500 MHz, $(CD_3)_2CO$): δ 8.27-8.23 (m, 2H, CH_{arom} Bz), 8.08-8.03 (m, 4H, CH_{arom} Bz), 7.68-7.60 (m, 5H, CH_{arom}), 7.47-7.02 (m, 60H, 59x CH_{arom} , NHAc), 6.66 (bd, $J_{NH',2'} = 7.8$ Hz, 1H, NHAc'), 5.84 (s, 1H, H-2''), 5.81 (s, 1H, H-2'''), 5.66 (s, 1H, H-2'''), 5.29 (s, 1H, H-1'''), 5.03-5.00 (m, 3H, H-1'', H-1''', CH_2 Bn), 4.95 (d, $J_{a,b} = 11.7$ Hz, 1H, CH_2 Bn), 4.90-4.80 (m, 3H, H-1, 2x CH_2 Bn), 4.78-4.44 (m, 17H, 17x CH_2 Bn), 4.70 (1H, H-1'), 4.34 (d, $J_{a,b} = 11.4$ Hz, 1H, $CH-b$ Bn), 4.21-3.95 (m, 10H, H-3'', H-3''', H-3''', H-4, H-4', H-4'', H-4''', H-4''', H-5''' or H-5''', H-6a''), 3.90-3.61 (m, 14H, H-2, H-2', H-3, H-3', H-5''' or H-5''', 2x H-6, 2x H-6', H-6b'', 2x H-6''', 2x H-6'''), 3.53-3.50 (m, 1H, H-5), 3.46-3.41 (m, 1H, H-5''), 3.28-3.24 (m, 1H, H-5'), 1.82 (s, 3H, Me NHAc), 1.69 (s, 3H, Me NHAc), 0.87 (s, 9H, Me *t*-Bu OTBDMS), 0.10, 0.09 (2x s, 6H, Me OTBDMS); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.3 (C=O NHAc'), 168.8 (C=O NHAc), 165.4, 165.1, 165.0 (3 x C=O Bz), 139.8, 139.6, 139.1, 139.0, 138.94, 138.87, 138.8, 138.4, 138.2, 138.1, 133.33, 133.27, 133.2, 130.5, 130.1, 130.0, 129.8, 129.7, 129.6, 128.9, 128.61, 128.57, 128.4, 128.3, 128.24, 128.20, 128.19, 128.11, 128.09, 128.07, 128.05, 127.88, 127.87, 127.8, 127.63, 127.58, 127.56, 127.5, 127.41, 127.37, 127.34, 127.31, 127.28, 127.24, 127.19, 126.8, 126.7 (47x CH_{arom}), 100.1 (C-1''), 99.4 (C-1'''), 98.7 (C-1''), 98.0 (C-1'''), 96.1 (C-1), 80.3 (C-3), 80.0 (C-3'), 79.4 (C-3''), 78.2 (C-4'), 78.0 (C-3'''), 77.6 (C-3'''), 75.3 (C-4), 74.9, 74.84, 74.82, 74.44, 74.39, 74.3, 74.1, 73.7 (C-4'', C-4''', C-4''', C-5, C-5', C-5'', CH_2 Bn), 73.1, 73.0, 72.9, 72.8, 72.7, 72.5 (C-5''', C-5''', CH_2 Bn), 72.3, 72.2 (C-2'', CH_2 Bn), 71.3, 70.7 (2x CH_2 Bn), 69.4 (C-2'''), 69.1, 69.0, 68.7 (C-6, C-6', C-6''', C-6'''), 68.6 (C-2'''), 66.0 (C-6''), 56.5 (C-2), 55.9 (C-2'), 25.1 (Me *t*-Bu

OTBDMS), 22.62, 22.57 (2x Me NHAc), 17.6 (C_q *t*-Bu OTBDMS), -4.8, -5.9 (2x Me OTBDMS); HRMS (ESI) *m/z* calcd. for [C₁₃₈H₁₅₀N₂NaO₂₉Si]⁺: 2349.9986, odsd.: 2349.9990

***Tert*-butyldimethylsilyloxy 2-acetamido-4-*O*-(2-acetamido-4-*O*-[2-*O*-{2,3,4-tri-*O*-acetyl- β -D-xylopyranosyl}-3,6-di-*O*-{2-*O*-benzoyl-3,4,6-tri-*O*-benzyl- α -D-mannopyranosyl}-4-*O*-benzyl- β -D-mannopyranosyl]-3,6-di-*O*-benzyl-2-deoxy- β -D-**

glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy- β -D-glucopyranoside (96). A 1/1 α/β mixture of xylose hexasaccharide (**49b**) (18.1mg, 0.0070 mmol) was dissolved in THF (0.2 mL), then activated zinc (110 mg, 1.68 mmol) was added, followed by aq. KH₂PO₄ (0.04 mL, 1M). The resulting suspension was stirred vigorously for 3h and then diluted with EtOAc, filtered over celite to remove the unreacted zinc and concentrated in presence of pyridine under reduced pressure. The resulting yellow oil was taken up in pyridine/Ac₂O (0.3 mL, 2/1) and left to stir until ninhydrin stain showed complete disappearance of amine product (2.5 h). the reaction was diluted with EtOAc, and organic fraction washed with sat. aq. NaHCO₃ (3x) and until no further gas was formed, and brine (1x).The organic phase was then dried over MgSO₄, filtered and concentrated *in vacuo*. Purification of the residue by column chromatography (silica gel, PE/EtOAc, 1/1, v/v) yielded the title compound (**X**) (8.4 mg, α/β , 0.0338 mmol, 48%) of which the beta product could be separated by a long silica column (silica gel, PE/EtOAc, 1/1, v/v): R_f α : 0.10 R_f β : 0.15 (PE/EtOAc, 1/1, v/v): α_D^{20} : -0.6 (*c* = 1.0 in CHCl₃); IR (v): 2925, 2855, 1726, 1662, 1454, 1366, 1251, 1218, 1074, 843, 753, 698 cm⁻¹; ¹H NMR (500 MHz, (CDCl₃): δ 8.05-8.00 (m, 4H, CH_{arom} OBz), 7.57-7.51 (m, 2H, CH_{arom} OBz), 7.38-7.08 (m, 59H, CH_{arom}), 6.08 (bs, 1H, NH' NHAc), 5.72 (s, 1H, H-2'''), 5.70 (s, 1H, H-2'''), 5.20 (s, 1H, H-1'''), 5.41 (t, $J_{2''',3'''} = J_{2'',3''} = 8.2$ Hz, 1H, H-3'''), 5.05 (dd, $J_{1''',2'''} = 6.3$ Hz $J_{2''',3'''} = 8.2$ Hz, 1H, H-2'''), 5.00 (bd, $J_{NH,2} = 2.6$ Hz, 1H, NH NHAc), 4.91-4.81 (m, 4H, CH₂ Bn), 4.90 (1H, H-1'''), 4.88 (2H, H-4''' and H-1'''), 4.81 (H, H-1'), 4.74-4.38 (m, 18H, CH₂ Bn), 4.57 (1H, H-1''), 4.46 (1H, H-1), 4.22-4.18 (m, 2H, H-3''' and H-5''' or H-5'''), 4.12-4.09 (m, 1H, H-5a'''), 4.07-4.00 (m, 4H, H-2'', H3''', H-4''', H-4'''), 3.95-3.93 (m, 1H, H-4), 3.89-3.88 (m, 1H, 4'), 3.83-3.76, 3.71-3.63, 3.62-3.57, 3.54-3.51 (m, 18H, H-2, H-2', H-3, H-3', H-3'', H-4'', H-5, H-5''' or H-5'''), H-6, H-6', H-6'', H-6''', H-6'''), 3.26 (bd, $J = 10.9$ Hz, 1H, H-5'), 3.12 (dd, $J = 7.8$ Hz, $J = 10.9$ Hz, 1H, H-5b'''), 3.16-3.13 (m, 1H, H-5''), 2.00, 1.92 (3x s, 9H, Me OAc), 1.61, 1.25 (2x s, 6H, Me NHAc), 0.83 (s, 9H, Me *t*-Bu OTBDMS), 0.05 (s, 6H, Me OTBDMS); ¹³C NMR (125 MHz, CDCl₃): δ 170.7, 170.0, 169.6, 169.4 (5x C=O, NHAc, OAc), 165.7, 165.5 (2x C=O OBz), 138.9, 138.8, 138.75, 138.71, 138.6, 138.4, 138.3, 138.0, 137.3, 137.7, 133.21, 133.18, 130.07, 130.05, 129.97, 128.7, 128.6, 128.54, 128.51, 128.48, 128.42, 128.39, 128.32, 128.28, 128.06, 127.34, 127.90, 127.87, 127.34, 127.78, 127.76, 127.69, 127.65, 127.6, 127.54, 127.50, 127.4 (37x CH_{arom}), 101.4 (C-1'', $J_{CH} = 157.3$ Hz), 100.2 (C-1''', $J_{CH} = 168.8$ Hz), 100.0 (C-1', $J_{CH} = 162.2$ Hz), 99.9 (C-1''', $J_{CH} = 174.5$ Hz), 97.7 (C-1''', $J_{CH} = 173.7$ Hz), 95.0 (C-1, $J_{CH} = 163.9$ Hz), 79.2 (C-3''), 79.0 (C-4'), 78.7 (C-

3'), 77.8 (C-3, C-3''', C-3'''), 76.3 (C-2''), 75.2, 75.1, 74.9 (C-5', CH₂ Bn), 74.62, 74.57, 74.4, 74.3, 74.2 (C-4'', C-4''', C-4''', C-5, C-5''), 73.51, 73.48, 73.47, 73.4, 73.0, 72.7 (CH₂ Bn), 72.5, 72.0 (C-5''', C-5''', CH₂ Bn), 71.2, 71.0, (CH₂ Bn), 70.5 (C-3'''), 69.8 (C-2''), 69.5, 69.3, 69.0, 68.9 (C-2''', C-2''', C-4'', C-6, C-6', C-6''', C-6'''), 66.6 (C-6''), 61.5 (C-5'''), 55.3 (C-2'), 54.5 (C-2), 25.7 (Me *t*-Bu OTBDMS), 23.5, 23.1 (2x Me NHAc), 20.9 (3x Me OAc), 18.0 (C_q *t*-Bu OTBDMS), -4.3, -5.2 (2x Me OTBDMS); HRMS (ESI) *m/z* calcd. for [C₁₄₂H₁₆₀N₂NaO₃₅Si]⁺: 2504.0463, *obsd.*: 2504.0469.

***Tert*-butyldimethylsilyloxy 2-azido-4-*O*-(2-*O*-Benzoyl-3,6-di-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl- α -D-mannopyranosyl)-4-*O*-benzyl- β -D-mannopyranosyl]-3,6-di-*O*-benzyl-2-deoxy-2-[2,2,2-trichloroethoxycarbonylamido]- β -D-glucopyranosyl)-6-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-**

benzyl- α -L-fucopyranosyl)-2-deoxy- β -D-glucopyranoside (49c). Traces of water were removed from tetra imidate (**82**) (63.1 mg, 0.030 mmol) and acceptor (**52**) (24.6 mg, 0.044 mmol), by co-evaporated with toluene (3x), then dissolved in dry toluene and activated molecular sieves (4 Å) were added. The resulting suspension was left to stir at rt for 1h and then cooled to 0°C followed by the careful addition of TfOH (75 μ L, 0.1M in toluene). The resulting mixture was then kept at 0°C for 3h, after which 5 drops of triethylamine were added, then diluted with EtOAc, filtered over cotton wool, and the organic fraction was washed with sat. aq. NaHCO₃ (2x), Brine (1x), dried over MgSO₄, filtered and concentrated *in vacuo*. Purification using flash chromatography (silica gel, 20% EtOAc in CH₂Cl₂) and size exclusion to yield title compound (**49c**) as a colourless oil. (26.8 mg, 0.0092 mmol, 31%); *R*_f: 0.73 (toluene/EtOAc, 6/1, v/v); α_D^{19} : -0.5 (*c* = 1.0 in CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ 8.25-8.22 (m, 2H, CH_{arom} OBz), 8.06-8.04 (m, 2H, CH_{arom} OBz), 7.99-7.97 (m, 2H, CH_{arom} OBz), 7.58-7.51 (m, 5H, CH_{arom}), 7.36-7.13, 7.11-7.03, 7.0-6.98 (m, 69 H, CH_{arom}), 5.75 (s, 1H, H-2'''), 5.52 (t, *J*_{1''',2''''} = *J*_{2''',3''''} = 2.3 Hz, 1H, H-2'''), 5.50 (d, *J* = 2.6 Hz, 1H, H-2'''), 5.45 (d, *J*_{1',2'} = 3.3 Hz, 1H, H-1'), 4.97 (s, 1H, H-1'''), 4.85-4.76 (m, 5H, CH₂ Bn), 4.79 (H-1'''), 4.72-4.61 (m, 5H), 4.58 (dd, *J* = 6.8 Hz, *J* = 13.2 Hz, 1H, H-5'), 4.54 (s, 1H, H-1'''), 4.53-4.39 (m, 17H), 4.49 (H-1, H-1''), 4.23 (d, *J*_{a,b} = 11.4 Hz, CH₂ Bn), 4.14-4.11, 4.07-4.03, 4.01-3.88, 3.86-3.80, 3.75-3.49 (m, 25H), 3.46 (dd, *J* = 2.7 Hz, *J* = 9.6 Hz, 1H H-3'''), 3.34 (dd, *J* = 7.7 Hz, *J* = 10.1 Hz, 1H, H-2), 3.34 (s, 1H), 3.26 (d, *J* = 9.9 Hz, 1H), 3.12 (bs, 1H), 3.04 (bd, *J* = 9.6 Hz, 1H), 1.15 (bd, *J*_{5',6'} = 4.9 Hz, 3H, H-6'), 0.90 (s, 9H, Me *t*-Bu OTBDMS), 0.11, 0.10 (2x s 6H, Me OTBDMS); ¹³C NMR (125 MHz, CDCl₃): δ 166.5, 165.5 (3x C=O Bz), 154.0 (C=O NHTroc), 139.2, 139.1, 138.63, 138.56, 138.48, 138.45, 138.4, 138.2, 138.1, 137.8, 137.8, 137.7, 133.6, 133.2, 133.1, 130.2, 130.0, 129.77, 129.76, 128.8, 128.7, 128.54, 128.48, 128.4, 128.34, 128.27, 128.24, 128.23, 128.17, 128.11, 128.07, 128.01, 127.97, 127.8, 127.7, 127.64, 127.62, 127.57, 127.51, 127.48, 127.46, 127.44, 127.40, 127.31, 127.27, 127.2, 127.1, 127.0 (48 CH_{arom}), 100.2 (C-1 or C-1''), 99.7 (C-1'''), 98.4 (C-1'''), 97.4 (C-1 or C-1'' and C-1'''), 96.9 (C-1'), 95.8

(CCl₃ NHTroc), 80.8, 80.4, 79.5, 78.9, 78.0, 77.8, 75.8, 75.3, 75.1, 74.9, 74.8, 74.6, 74.3, 74.1, 73.8, 73.7, 73.6, 73.4, 73.3, 73.2, 73.0, 72.8, 72.6, 72.0, 71.9, 71.4, 71.3, 69.5, 69.0, 68.8, 68.7, 68.5, 68.5, 68.4, 66.3, 65.8, 56.2, 25.6 (Me *t*-Bu OTBDMS), 18.0 (C_q *t*-Bu OTBDMS), 16.6 (C-6'), -4.3, -5.0 (2x Me OTBDMS); Coupling constant: (C-1 or C-1'', C-1''' *J* = 158.0 Hz, *J* = 160.0 Hz, *J* = 163.0 Hz), (C-1''', *J* = 173.8 Hz), (C-1''''', *J* = 174.8 Hz), (C-1', *J* = 177.4 Hz) HRMS (ESI) *m/z* calcd. for [C₁₅₇H₁₆₇Cl₃N₄NaO₃₃Si]⁺: 2792.0240, odstd.: 2792.0244.

***Tert*-butyldimethylsilyloxy 2-acetamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-4-*O*-(2-*O*-benzoyl-3,6-di-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl- α -D-mannopyranosyl)-4-*O*-benzyl- β -D-mannopyranosyl)-3,6-di-*O*-benzyl- β -D-glucopyranosyl)-6-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-**

benzyl- α -L-fucopyranosyl)- β -D-glucopyranoside (94). Fucose hexamer (**49c**) (17.2 mg, 0.0062 mmol) was dissolved in THF (0.2 mL), activated zinc was added (93.3 mg, 1.43 mmol) followed by aq. KH₂PO₄ (0.04 mL, 1M), the resulting suspension was stirred vigorously for 2 h and until complete disappearance of starting material by TLC analysis. The suspension was diluted with EtOAc, filtered over celite, and co-concentrated with pyridine. The resulting oil was taken up in pyridine/Ac₂O (0.3mL, 2/1) and left to stir until ninhydrin stain showed complete disappearance of amine product (2 h). Then EtOAc was added, and organic fraction washed with sat. aq. NaHCO₃ (3x) until no further gas was formed and brine (1x). The organic phase was dried over MgSO₄, filtered and concentrated. Purification of the residue by column chromatography (silica gel, PE/EtOAc, 2/1→1/1, v/v) yielded the title compound (**94**) (10.1 mg, 0.0038 mmol, 62%) as a colorless oil. *R*_f: 0.11 (PE/EtOAc, 1/1, v/v); α_D^{19} : -0.3 (*c* = 1.0 in CHCl₃); IR (ν): 3060, 3030, 2926, 2857, 1724, 1670, 1454, 1362, 1268, 1099, 773, 698 cm⁻¹; ¹H NMR (600 MHz, (CDCl₃): δ 8.15-8.12 (m, 2H, CH_{arom} Bz), 7.94-7.91 (m, 4H, CH_{arom} OBz), 7.52-7.49 (m, 5H CH_{arom}), 7.23-6.88 (m, 68H, CH_{arom}), 6.94-6.90 (m, 2H, CH_{arom}, NHAc), 6.67 (bd, *J*_{NH,2} = 9.1 Hz, NH NHAc), 5.70 (s, 2H, H-2''' and H-2'''''), 5.52 (s, 1H, H-2'''), 5.14 (s, 1H, H-1'), 5.11 (s, 1H, H-1'''), 4.91 (s, 1H, H-1'''''), 4.88 (s, 1H, H-1'''), 4.86 (d, *J*_{a,b} = 12.3 Hz, 1H, CH-a Bn), 4.76-4.35 (m, 21H, CH₂ Bn), 4.66 (H-1), 4.45 (H-1''), 4.22 (d, *J*_{a,b} = 11.5 Hz, 1H, CH-b Bn), 4.20 (d, *J*_{a,b} = 11.5 Hz, 1H, CH-b Bn), 4.11 (bd, *J* = 5.9 Hz, 1H, H-5'), 4.06 (t, *J*_{3''',4''''} = *J*_{4''',5''''} = 9.6 Hz, 1H, H-4'''''), 4.03-3.91 (m, 7H), *J*_{2''',3''''} = 3.0 Hz, *J*_{3''',4''''} = 9.4 Hz, 1H, H-3'''''), 3.85-3.79 (m, 5H), 3.74-3.58 (m, 10H), 3.56-3.52 (m, 2H), 3.49-3.45 (m, 2H), 3.25-3.22 (m, 2H), 1.75 (s, 3H, Me NHAc), 1.63 (s, 3H, Me NHAc), 0.98 (d, *J*_{5',6'} = 6.4 Hz, 3H, H-6'), 0.75 (s, 9H, Me *t*-Bu OTBDMS), -0.04, -0.05 (2x s, 6H, Me OTBDMS); ¹³C NMR (150 MHz, ((CD₃)₂CO): δ 169.7, 169.0 (2x C=O NHAc), 165.6, 165.1 (3x C=O Bz), 139.60, 139.56, 139.4, 139.3, 139.1, 139.02, 139.00, 139, 138.7, 138.6, 138.4, 138.2, 138.0, 133.34, 133.26, 130.5, 130.0, 129.9, 129.8, 129.7, 129.6, 129.0, 128.61, 128.58, 128.4, 128.31, 128.30, 128.25, 128.2, 128.14, 128.10, 128.09, 128.05, 127.9, 127.92, 127.86, 127.8, 127.7, 127.62, 127.56, 127.54, 127.50, 127.48, 127.41, 127.38, 127.34, 127.33, 127.29, 127.28, 127.24, 127.23, 127.19,

127.1, 127.0, 126.9 (55x CH_{arom}), 99.4 (C-1'''), 99.3 (C-1''), 98.7 (C-1'''), 98.0 (C-1'''), 96.4 (C-1'), 95.8 (C-1), 80.0, 79.6, 78.8, 78.6, 78.3, 78.1, 77.94, 77.92, 77.5, 76.2, 75.7, 75.4, 74.9, 74.8, 74.6, 74.53, 74.46, 74.4, 74.33, 74.28, 74.0, 73.4, 73.1, 72.9, 72.80, 72.77, 72.6, 72.4, 72.29, 72.25, 71.4, 71.2, 70.7, 69.6, 69.3, 69.11, 69.06, 68.9, 68.5, 66.4, 65.8, 56.7, 55.1, 25.2 (Me *t*-Bu OTBDMS), 22.7, 22.5 (2x Me NHAc), 17.6 (C_q *t*-Bu OTBDMS), 16.2 (C-6'), -4.8, -5.8 (2x Me OTBDMS); HRMS (ESI) *m/z* calcd. for [C₁₅₈H₁₇₂N₂NaO₃₃Si]⁺: 2676.1504, odstd.: 2676.1509.

***Tert*-butyldimethylsilyloxy 2-deoxy-2-azido-4-*O*-(4-*O*-[2-*O*-(2,3,4-tri-*O*-acetyl- β -D-xylopyranosyl)-3,6-di-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl- α -D-mannopyranosyl)-4-*O*-benzyl- β -D-mannopyranosyl]-3,6-di-*O*-benzyl-2-deoxy-2-[2,2,2-trichloroethoxycarbonylamido]- β -D-**

glucopyranosyl)-6-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl- α -L-fucopyranosyl)- β -D-glucopyranoside (49a). Penta imideate (**85**) (89.3 mg, 0.039 mmol) and acceptor (**51**) (99 mg, 0.12 mmol), were co-evaporated together with toluene (3x), then dissolved in dry toluene and activated molecular sieves (4 Å) where added. The resulting suspension was stirred at rt for 1h, cooled to 0°C and TfOH (20 μ L, 1M in toluene) was added. the resulting solution was allowed to warm up to rt and two additional portions of TfOH (20 μ L and 10 μ L, 1M in toluene) were added after 3,5h and 7h cooling the reaction to 0°C before each subsequent addition. The resulting mixture was then left to stir for an additional 15h after which EtOAc was added, then the organic fraction was filtered over cotton wool, washed with sat. aq. NaHCO₃ (2x), Brine (1x), dried over MgSO₄ filtered and concentrated *in vacuo*. Purification using flash chromatography (silica gel, 2% → 3% EtOAc in toluene) to remove acceptor followed b (silica gel, PE/EtOAc, 3/1, v/v) to remove oxazoline TLC R_f: 0.32 (Pe/EtOAc, 2/1, v/v); to yield the title compound (**49a**) as a colourless oil. (43 mg); TLC R_f: 0.43 (Pe/EtOAc, 2/1, v/v); HRMS (ESI) *m/z* calcd. for [C₁₆₁H₁₇₇Cl₃N₄NaO₃₉Si]⁺: 2946.0717, odstd.: 2946.0723

***Tert*-butyldimethylsilyloxy 2-acetamido-2-deoxy-4-*O*-(2-acetamido-2-deoxy-4-*O*-(2-*O*-(2,3,4-tri-*O*-acetyl- β -D-xylopyranosyl)-3,6-di-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl- α -D-mannopyranosyl)-4-*O*-benzyl- β -D-mannopyranosyl)-3,6-di-*O*-benzyl- β -D-**

glucopyranosyl)-6-*O*-benzyl-3-*O*-(2,3,4-tri-*O*-benzyl- α -L-fucopyranosyl)- β -D-glucopyranoside (97). heptamer (**49a**) (7.0 mg, 0.00239 mmol) was dissolved in THF (0.2 mL), activated zinc was added (104 mg,

1.50 mmol) followed by aq. KH_2PO_4 (0.04 mL, 1M), the resulting suspension was stirred vigorously for 5h and until completion by TLC analysis. The reaction mixture was diluted with EtOAc, filtered over celite to remove unreacted zinc, and co-concentrated with pyridine. The resulting oil was dissolved in pyridine/ Ac_2O (0.3mL, 2/1) and left to stir until ninhydrin stain showed complete disappearance of amine (1.5 h). The reaction was diluted with EtOAc, and the organic fraction washed with sat. aq. NaHCO_3 (3x) until no further gas was formed and brine (1x), dried over MgSO_4 , filtered and concentrated. Purification of the residue by column chromatography (silica gel, PE/EtOAc, 2/1→1/1, v/v) yielded the title compound (**97**) (4.6 mg, 0.0164 mmol, 68%) as a colourless oil. R_f : 0.49 (Pe/EtOAc, 1/2, v/v) ; IR (v): 3063, 3032, 2928, 1756, 1725, 1670, 1541, 1497, 1454, 1366, 1266, 1221, 1099, 1073, 839, 738, 698, 633 cm^{-1} ; ^1H NMR (600 MHz, $(\text{CD}_3)_2\text{CO}$): δ 8.1-8.04 (m, 4H, CH_{arom} OBz), 7.66-7.62 (m, 2H, CH_{arom} OBz), 7.51-7.06 (m, 71H, 2x NH NHAc, CH_{arom}), 5.88 (t, $J = 2.3$ Hz, 1H, H-2'''''' or H-2'''''''), 5.81 (t, $J = 2.3$ Hz, 1H, H-2'''''' or H-2'''''''), 5.30 (d, $J_{1',2'} = 1.9$ Hz, 1H, H-1'), 5.20 (t, $J_{2''',3'''} = J_{3''',4'''} = 9.0$ Hz, 1H, H-3'''''), 5.17 (s, 1H, H-1'''''), 5.10 (dd, $J_{1''',2'''} = 7.1$ Hz, $J_{2''',3'''} = 9.0$ Hz, 1H, H-2'''''), 5.01 (d, $J_{\text{ab}} = 11.5$ Hz, CH_2 Bn), 4.97 (d, $J_{1''',2'''} = 7.1$ Hz, 1H, H-1'''''), 4.95-4.53, 4.46-4.38 (m, 29H, CH_2 Bn), 4.83 (H-1'''), 4.82 (H-1'), 4.74 (H-1), 4.71 (H-1'''''), 4.27-4.24 (m, 1H, H-5'), 4.22 (d, $J_{1''',2'''} = 3.0$ Hz, 1H, H-2'''''), 4.21-4.17, 4.16-4.09, 4.04-3.95, 3.92-3.70, 3.69, 3.66-3.49, 3.39 (d, $J = 8.8$ Hz, 1H), 3.22, (dd, $J = 9.3$ Hz, $J = 11.6$ Hz, 1H), 3.2-3.18 (m, 1H), (H-2, H-2'', H-2''''', H-2''''''', H-3, H-3', H-3'', H-3''', H-3''''', H-3''''''', H-4, H-4', H-4'', H-4''', H-4''''', H-4''''''', H-5, H-5'', H-5''', H-5''''', H-5''''''', H-5''''''', H-6, H-6'', H-6''', H-6''''', H-6'''''''), 2.12, 1.99, 1.98, 1.89, 1.85 (5x s, 15H, Me NHAc, OAc), 1.12 (d, $J_{5',6'} = 6.3$ Hz, 3H, H-6'), 0.88 (s, 9H, Me *t*-Bu OTBDMS), 0.10, 0.09 (2x s, 6H, Me OTBDMS); ^{13}C NMR (150 MHz, CDCl_3): δ 170.0, 169.6, 169.5, 169.4, 169.2, 169.1 (5x Me 2 x NHAc, 3 x OAc), 165.1, 165.0 (2x C=O Bz), 139.6, 139.3, 139.04, 139.02, 139.00, 138.98, 138.84, 138.80, 138.77, 138.6, 138.4, 138.38, 138.37, 133.3, 133.2, 130.3, 130.1, 129.64, 129.61, 129.1, 129.0, 128.6, 128.5, 128.4, 128.32, 128.29, 128.28, 128.24, 128.21, 128.19, 128.17, 128.16, 128.14, 128.11, 128.06, 128.04, 128.02, 128.01, 127.99, 127.97, 127.95, 127.94, 127.87, 127.7, 127.6, 127.52, 127.51, 127.48, 127.44, 127.39, 127.34, 127.30, 127.24, 127.23, 127.22, 127.19, 127.14, 127.10, 127.08, 127.0, 126.9, 126.4 (62x CH_{arom}), 101.23, 101.17 (C-1''', C-1'''''), 99.31, 99.28 (C-1, C-1'''''), 97.7 (C-1'''''''), 96.5 (C-1'), 95.8 (C-1''), 79.8, 79.2, 78.9, 78.6, 78.23, 78.17, 78.1, 77.0, 76.7, 76.6, 76.3, 75.8, 75.5, 75.0, 74.9, 74.85, 74.74, 74.68, 74.60, 74.58, 74.5, 74.3, 74.2, 74.0, 73.94, 73.91, 73.12, 73.07, 73.04, 73.00, 72.96, 72.81, 72.80, 72.72, 72.68, 72.6, 72.5, 72.4, 72.1, 72.0, 71.9, 71.8, 71.5, 71.4, 71.3, 71.1, 71.0, 70.1, 70.0, 69.9, 69.69, 69.65, 69.61, 69.33, 69.26, 69.2, 69.0, 68.5, 66.5, 66.2, 66.1, 61.8, 56.7, 54.9, 54.1, 51.8, 25.2 (Me *t*-Bu OTBDMS), 22.7, 22.5 (2x Me NHAc), 20.4, 19.92, 19.90 (3x Me OAc), 17.6 (C_q *t*-Bu OTBDMS), 16.3 (C-6'), -4.8, -5.8 (2x Me OTBDMS); HRMS (ESI) m/z calcd. for $[\text{C}_{162}\text{H}_{182}\text{N}_2\text{NaO}_{39}\text{Si}]^+$: 2830.1981, obsd.: 2830.1987 (coupling constant) 101.23, 101.17 (C-1''', C-1'''''), 99.31, 99.28 (C-1, C-1'''''), 97.7 (C-1'''''''), 96.5 (C-1'), 95.8 (C-1'')

2-acetamido-2-deoxy-3-O- α -L-fucopyranosyl-4-O-(2-deoxy-2-acetamido-4-O-[3,6-di-O- α -D-mannopyranosyl]-2-O- β -D-xylopyranosyl- β -D-mannopyranosyl)- β -D-glucopyranose (104**).** Heptamer (**97**) (5.0 mg, 0.0018 mmol) was dissolved in dry THF (2 mL), NH_3 (l)(15

mL) was added followed by the careful addition of sodium (s) until the blue colour remained. The solution was stirred for 1.5 h under refluxing NH_3 , then MeOH was added (4 mL) and resulting solution allowed to warm up to rt and the NH_3 was evaporated. The solution was neutralized by the addition of activated Dowex H^+ , after 15 min the organic phase was filtered and concentrated under reduced pressure. Purification by size exclusion (biorad, BioGel P-2 extra fine, H_2O) and reverse phase column chromatography (HyperSep C8 Column, H_2O) yielded title compound (**104**). ^1H NMR (500 MHz, CDCl_3): δ 5.10 (s, 1H), 4.89 (s), 4.53 (t, $J = 8.0$ Hz, 1H), 4.45-4.41 (m), 4.44 (s), 4.02-3.32 (m), 3.23 (t, $J = 10.8$ Hz, 1H), HRMS (ESI) m/z calcd. for $[\text{C}_{45}\text{H}_{76}\text{N}_2\text{NaO}_{34}]^+$: 1211.4172, found.: 1211.4165

2-acetamido-2-deoxy-4-O-(2-acetamido-2-deoxy-4-O-[3,6-di-O- α -D-mannopyranosyl]-2-O- β -D-xylopyranosyl- β -D-mannopyranosyl)- β -D-glucopyranose (103**).** Xylose hexasaccharide (**96**) (5.5 mg, 0.0022 mmol) was

dissolved in dry THF (3 mL), NH_3 (l)(15 mL) was added followed by the careful addition of sodium (s) until the blue colour remained. The solution was stirred for 1.5 h under refluxing NH_3 , then MeOH was added (4 mL) and resulting solution allowed to warm up to rt and the NH_3 was evaporated. The solution was neutralized by the addition of activated Dowex H^+ , after 30 min the organic phase was filtered and concentrated under reduced pressure. Purification by size exclusion (biorad, BioGel P-2 extra fine, H_2O) and reverse phase column chromatography (HyperSep C8 Column, H_2O) yielded title compound (**103**) (1.3 mg, 65%). ^1H NMR (500 MHz, CDCl_3): δ 5.16 (s, 1H), 5.10 (s, 1H), 4.89 (s), 4.57 (m, 1H), 4.42 (d, $J = 7.3$ Hz, 1H), 4.24 (d, $J = 2.3$ Hz, 1H), 4.02-3.59, 3.56-3.47 (m), 3.41 (t, $J = 9.0$ Hz, 1H), 3.35 (t, $J = 8.0$, 1H), 3.22 (t, $J = 11.0$ Hz 1H), 2.05, 2.01 (2x s, 3H Me NHAc);

2-acetamido-2-deoxy-4-O-(2-acetamido-2-deoxy-4-O-[3,6-di-O- α -D-mannopyranosyl]-2-O- β -D-xylopyranosyl- β -D-mannopyranosyl)- β -D-glucopyranose (103**).**

glucopyranose (102). Pentasaccharide (**95**) (5.5 mg, 0.0024 mmol) was dissolved in dry THF (0.5 mL), NH₃ (l)(15 mL) was added followed by the careful addition of sodium (s) until the blue colour remained. The solution was stirred for 1.0 h under refluxing NH₃, then MeOH was added (1 mL) and resulting solution allowed to warm up to rt and the NH₃ was evaporated. The solution was neutralized by the addition of activated Dowax H⁺, after 30 min the organic phase was filtered and concentrated under reduced pressure. Purification by size exclusion (biorad, BioGel P-2 extra fine, H₂O) and reverse phase column chromatography (HyperSep C8 Column, H₂O) yielded title compound (**102**) (1.4mg, 70%). ¹H NMR (500 MHz, (CDCl₃): δ 5.17 (s, 1H), 5.09 (s, 1H), 4.90 (s), 4.60 (m, 1H), 4.25 (s, 1H), 4.09 (dd, *J* = 7.3 Hz, *J* = 14.4 Hz), 4.06 (s, 1H), 3.89 (s, 1H), 3.92-3.52 (m), 2.07, 2.03 (2x s 3H, Me NHAc); HRMS (ESI) *m/z* calcd. for [C₃₄H₅₈N₂NaO₂₆]⁺: 933.3170, odsd.: 933.3169.

2-acetamido-2-deoxy-3-*O*-α-L-fucopyranosyl-4-*O*-(2-acetamido-2-deoxy-4-*O*-[3,6-di-*O*-α-D-mannopyranosyl-β-D-mannopyranosyl]-β-D-glucopyranosyl)-α/β-D-glucopyranose (101). Fucose hexasaccharide (**94**) (10 mg, 0.0038 mmol) was

dissolved in dry THF (0.5 mL), NH₃ (l)(15 mL) was added followed by the careful addition of sodium (s) until the blue colour remained. The solution was stirred for 1.0 h under refluxing NH₃, then MeOH was added (4 mL) and resulting solution allowed to warm up to rt and the NH₃ was evaporated. The solution was neutralized by the addition of activated Dowax H⁺, after 20 min the organic phase was filtered and concentrated under reduced pressure. Purification by size exclusion (biorad, BioGel P-2 extra fine, H₂O) and reverse phase column chromatography (HyperSep C8 Column, H₂O) yielded title compound (**101**) (1.5 mg, 37%) together with pentasaccharide (**102**) (1.2mg, 35%); ¹H NMR (500 MHz, (CDCl₃): δ 5.21 (s), 5.17 (s), 4.53 (d, *J* = 8.0 Hz, 1H), 4.24, (s, 1H) 4.17 (m, 1H), 4.09 (m, 1H), 4.05 (s, 1H), 3.95-3.40 (m), 2.04, 2.01 (2x s, 3H, Me NHAc), 1.22 (d, *J* = 6.3 Hz, 3H, H-6'), 1.18 (d, *J* = 6.3 Hz, 3H, H-6'); HRMS (ESI) *m/z* calcd. for [C₄₀H₆₈N₂NaO₃₀]⁺: 1079.3749, odsd.: 1079.3744.

2,3,4-Tri-*O*-acetyl-β-D-xylopyranosyl acetate (60). D-xylose **59** (25 g, 166 mmol) was suspended in acetic anhydride (60 mL, 0.66 mol, 4 equiv.) followed by addition of sodium acetate (3.5 g, 0.042 mol) the resulting suspension was left to stir at 35°C until all solids dissolved. Then sat. aq. NaHCO₃ (60 mL) was added, followed by NaHCO₃ (s) until solids remained and no additional gas is formed. EtOAc was added and the organic phase washed with sat. aq NaHCO₃ (3×), and brine (1×), then dried over MgSO₄, filtered, and concentrated *in vacuo*. Purification by crystallisation (PE/EtOAc, 3x) yielded

title compound **60** as transparent needles. (15.2 g, 47.9 mmol, 29%) and also alpha product was isolated (15.7g, 50.0 mmol, 30%). ^1H NMR (500 MHz, CDCl_3): δ 5.71 (d, $J_{1,2} = 6.9$ Hz, 1H, H-1), 5.20 (t, $J_{3,4} = J_{4,5} = 8.3$ Hz, 1H, H-3) 5.02 (dd, $J_{1,2} = 6.9$ Hz, $J_{2,3} = 8.3$ Hz, 1H, H-2), 4.97 (dt, $J_{4,5a} = 4.9$ Hz, $J_{3,4,5b} = 8.3$, 1H, H-4), 4.14 (dd, $J_{4,5a} = 4.9$ Hz, $J_{5a,5b} = 12.0$ Hz, 1H, H-5a), 3.52 (dd, $J_{4,5b} = 8.3$ Hz, $J_{5a,5b} = 12.0$ Hz, 1H, H-5b) 2.10, 2.052, 2.045, 2.04 (4 x, s, 12H, CH_3 Ac); ^{13}C NMR (125MHz, CDCl_3): δ 169.85, 169.83, 169.3, 169.1 (4x, C=O, Ac), 92.0 (C-1), 71.0 (C-3), 69.5 (C-2), 68.3 (C-4), 62.6 (C-5), 20.8, 20.72, 20.66, 20.6 (4x CH_3 Ac). HRMS(ESI) m/z calcd. for $[\text{C}_{11}\text{H}_{16}\text{O}_8+\text{Na}]^+$: 299.0743, obsd.: 299.0743.

2,3,4-Tri-O-acetyl- α/β -D-xylopyranose (68**).** To a cooled solution (0°C) of $\text{H}_2\text{NNH}_2\cdot\text{H}_2\text{O}$ (0.40 mL, 8.2 mmol, 1.3 equiv.) in DMF (15 mL), AcOH (0.47mL, 8.2mmol, 1.3 equiv.) was added drop wise, followed by addition of peracetylated xylose **60** (2.0 g, 6.3 mmol,) in DMF (15 mL). The resulting solution was then allowed to warm up to rt. and left to stir until TLC analysis (PE/EtOAc, 1/3, v/v) showed complete conversion of starting material (45 min). Then EtOAc was added and the organic phase washed with sat. aq. NaHCO_3 (2x), and brine (1x). The combined aqueous layers were extracted with EtOAc (2x), and the organic fractions combined, then dried over MgSO_4 , filtered, and concentrated *in vacuo*. Purification by column chromatography (PE/EtOAc, 6/1 $\rightarrow$ 1/1, v/v) and crystallisation (PE/EtOAc) yielded the title compound **68** (1.3 mg, 4.9 mmol, α/β , 2/1, 60%) as white needles. R_f : 0.37 (PE/EtOAc, 1/3, v/v); $[\alpha]_D^{20}$: +2.6 ($c = 10.0$ in CHCl_3); ^1H NMR (500 MHz, CDCl_3) α -product: δ 5.51 (t, $J = 9.5$ Hz, 1H, H-3), 5.38 (d, $J_{1,2} = 3.5$ Hz, 1H, H-1), 4.96 (dt, $J_{4,5} = 6.1$ Hz, $J_{3,4} = 9.5$ Hz, 1H, H-4) 4.84 (dd, $J_{1,2} = 3.4$ Hz, $J_{2,3} = 9.5$ Hz, 1H, H-2), 3.88 (t, $J = 10.5$ Hz, 1H, H-5a), 3.81 (dd, $J_{5a,5b} = 5.8$ Hz, $J_{4,5} = 11.0$ Hz, 1H, H-5b), 2.05, 2.042, 2.039 (3x, s, 3H, CH_3 Ac); ^{13}C NMR (125MHz, CDCl_3) α -product: δ 170.9 170.1, 170.0 (3x, C=O, Ac), 90.3 (C-1), 71.2 (C-2), 69.24 (C-3), 69.18 (C-2), 58.5 (C-5), 20.8, 20.70, 20.69 (3x CH_3 Ac); ^1H NMR (500 MHz, CDCl_3) β -product: δ 5.23 (t, $J = 9.3$ Hz, 1H, H-3), 4.98 (dt, $J_{4,5} = 4.6$ Hz, $J = 10.0$ Hz, 1H, H-4), 4.84 (d, $J = 9.2$ Hz, 1H, H-2), 4.68 (d, $J_{1,2} = 7.6$ Hz, 1H, H-1), 4.13 (dd, $J_{4,5} = 5.4$ Hz, $J_{5a,5b} = 11.9$ Hz, 1H, H-5a), 3.37 (dd, $J_{4,5} = 10.0$ Hz, $J_{5a,5b} = 11.7$ Hz, 1H, H-5b), 2.09, 2.05, 2.042, (3 x, s, 9H, CH_3 Ac); ^{13}C NMR (125MHz, CDCl_3) β -product: δ 170.3, 170.15, 170.10 (3x, C=O, Ac), 85.9 (C-1), 76.8 (C-2), 73.1 (C-3), 69.0 (C-4), 62.7 (C-5), 20.8, 20.7, 20.69 (3x CH_3 Ac); HRMS(ESI) m/z calcd. for $[\text{C}_{11}\text{H}_{16}\text{O}_8+\text{Na}]^+$: 299.0743, obsd.: 299.0743.

O-(2,3,4-tri-O-acetyl- α/β -D-xylopyranosyl) N-(phenyl)trifluoroacetimidoyl (70**).** Hemiacetal **68** (52.6 mg, 0.19 mmol) was co-evaporated with toluene (3x), dissolved in acetone (1 mL), and N-(phenyl)trifluoroacetimidoyl chloride (75 μL , 0.56 mmol, 3 equiv.) was added at 0°C , followed by Cs_2CO_3 (120 mg, 0.37 mmol, 2 eq). The resulting suspension was left to stir until TLC analysis (PE/EtOAc, 2/1, v/v) showed complete conversion of starting material. The reaction was then filtered over celite to remove unreacted Cs_2CO_3 and the organic fraction concentrated *in vacuo*. Purification of the residue by column chromatography (PE/EtOAc, 20/1 $\rightarrow$ 10/1, v/v) yielded the title compound **70** (73.7

mg, 0.16 mmol, 89%, α/β 2/1) as an colourless oil. R_f : 0.35 (PE/EtOAc, 2/1, v/v); ^1H NMR (500 MHz, CDCl_3) α -product: δ 7.32-7.26 (m, 2H, CH_{arom} *m*-N-Ph), 7.11 (t, $J = 7.5$ Hz, 1H, CH_{arom} *p*-N-Ph), 6.79 (d, $J = 7.5$ Hz, 1H, CH_{arom} *o*-N-Ph), 5.54 (t, $J_{2,3} = J_{2,3} = 9.8$ Hz, 1H, H-3), 5.12-5.02 (m, 2H, H-2 and H-4), 4.01-3.96 (m, 1H, H-5a), 4.80-3.72 (m, 1H, H-5b), 2.08 (s, 3H, Me OAc), 2.06 (2x s, 6H, Me OAc); ^{13}C NMR (125MHz, CDCl_3) α -product: δ 170.02, 169.97, 169.9 (3x, C=O OAc), 143.1 128.9, 124.7, 119.4 (4x CH_{arom} NPh), 92.4 (C-1), 69.7 (C-2), 69.4 (C-3), 68.6 (C-4), 60.8 (C-5), 20.8, 20.7, 20.6 (3x Me OAc); ^1H NMR (500 MHz, CDCl_3) β -product: δ 7.32-7.26 (m, 2H, CH_{arom} *m*-N-Ph), 7.11 (t, $J = 7.5$ Hz, 1H, CH_{arom} *p*-N-Ph), 6.83 (d, $J = 7.5$ Hz, 1H, CH_{arom} *o*-N-Ph), 5.12-5.10 (m, 1H, H-3), 5.12-5.02 (m, 1H, H-4), 4.95-4.90 (m, 1H, H-4), 4.23 (bd, $J = 10.5$ Hz, 1H, H-5a), 3.62 (bs, 1H, H-5b), 2.10, 2.09, 2.08 (3x s, 9H, Me OAc); ^{13}C NMR (125MHz, CDCl_3) β -product: δ 169.9, 169.6, 169.2 (3x, C=O OAc), 143.1 128.9, 124.7, 119.4 (4x CH_{arom} NPh), 94.1 (C-1), 68.5 (C-3), 67.7 (C-4), 67.4 (C-2), 61.7 (C-5), 20.9, 20.7, 20.6 (3x Me OAc); HRMS(ESI) m/z calcd. for $[\text{C}_{11}\text{H}_{16}\text{O}_8+\text{Na}]^+$: 470.1033, obsd.: 470.1030.

O-(2,3,4-tri-O-acetyl- α/β -D-xylopyranosyl) trichloroacet-imidate (69**).** Hemiacetal

68 (203 mg, 0.74 mmol) was co-evaporated with toluene (3 $\times$), dissolved in dry CH_2Cl_2 (2.5 mL), and trichloroacetonitrile (0.40 mL, 3.7 mmol, 5 equiv.) was added at 0°C, followed by 1,8-diazabicycloundec-7-ene (DBU) (11 μL , 0.074 mmol, 0.1eq). The resulting solution was left to stir until TLC analysis (PE/EtOAc, 2/1, v/v) showed complete conversion of starting material. The reaction was then concentration *in vacuo* and purification of the residue by column chromatography (PE/EtOAc, 10/1 $\rightarrow$ 6/1, v/v) yielded the title compound **69** (250 mg, 0.059 mmol, 80%, α/β) as an amorphous yellow solid. R_f α : 0.23, R_f β : 0.34 (PE/EtOAc, 2/1, v/v); ^1H NMR (500 MHz, CDCl_3) α -Imidate: δ 8.65 (s, 1H, NH, CNHCCl_3), 6.44 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1), 5.53 (t, $J = 10.0$ Hz, 1H, H-3), 5.04 (dt, $J_{4,5} = 4.8$ Hz, $J_{3,4} = 9.5$ Hz 1H, H-4), 5.03 (d, $J_{1,2} = 3.7$ Hz, $J_{2,3} = 10.2$ Hz 1H, H-2), 3.95 (dd, $J_{5a,5b} = 3.9$ Hz, $J_{4,5} = 11.2$ Hz, 1H, H-5a), 3.78 (t, $J_{4,5} = 11.0$ Hz, 1H, H-5b), 2.02, 2.01, 1.99 (3 $\times$, s, 3H, CH_3 Ac); ^{13}C NMR (125MHz, CDCl_3) α -Imidate: δ 170.09, 170.08, 170.0 (C=O, Ac), 161.1 (CN, CNHCCl_3), 93.3 (C-1), 69.5 (C-4), 68.7 (C-3), 68.5 (C-2), 60.9 (C-5), 20.9, 20.8, 20.6 (3 $\times$ CH_3 Ac); ^1H NMR (500 MHz, CDCl_3) β -Imidate: δ 8.70 (s, 1H, NH, CNHCCl_3), 5.99 (d, $J_{1,2} = 4.4$ Hz, 1H, H-1), 5.13 (t, $J = 5.9$ Hz, 1H, H-3), 5.07 (t, $J_{2,3} = 4.2$ Hz, 1H, H-2), 4.90 (dt, $J_{4,5} = 3.9$ Hz, $J_{3,4} = 5.6$ Hz 1H, H-4), 4.26 (dd, $J_{5a,5b} = 3.9$ Hz, $J_{4,5} = 12.5$ Hz, 1H, H-5a), 3.69 (dd, $J_{5a,5b} = 5.4$ Hz, $J_{4,5} = 12.7$ Hz, 1H, H-5b), 2.07, 2.063, 2.060 (3 $\times$, s, 3H, CH_3 Ac); ^{13}C NMR (125MHz, CDCl_3) β -Imidate: δ 169.9, 169.7, 169.2 (C=O, Ac), 160.6 (CN, CNHCCl_3), 95.0 (C-1), 68.5 (C-3), 67.8 (C-2), 67.5 (C-4), 61.8 (C-5), 21.0, 20.9, 20.8 (3 $\times$ CH_3 Ac); HRMS(ESI) m/z calcd. for $[\text{C}_{13}\text{H}_{16}\text{O}_8\text{NCl}_3+\text{Na}]^+$: 441.9839, obsd.: 441.9839.

Experimental: Azasugars

Unless otherwise stated all reactions were performed under atmospheric air. THF (Lab-Scan) was distilled from sodium wire prior to use. Triethylamine was distilled and stored over KOH pellets. Pyridine was distilled over KOH prior to use. EtOAc (Panreac), MeOH (Pure Science), EtOH (absolute, Pure Science), AcOH (Lab Scam), CH₂Cl₂ (LabServ), 30% aqueous NH₃ (Fisher Science), triethylsilane (Fluka), D-fructose, NaCNBH₃ (Aldrich), and NaOH (Pure Science), sodium (Aldrich), imidazole (Aldrich), I₂ (BDH), aminodiphenylmethane (Aldrich), were used as received. Zn dust was activated by the careful addition of conc. H₂SO₄. All solvents were removed by evaporation under reduced pressure (*in vacuo*). Reactions were monitored by TLC-analysis on Macherey-Nagel silica gel coated plastic sheets (0.20 mm, with fluorescent indicator UV₂₅₄) with detection by UV-absorption (254 nm), by dipping in 10% H₂SO₄ in MeOH or 3% ninhydrin in EtOH followed by charring at ~150 °C. Column chromatography was performed on Pure Science silica gel (40-63 micron). DOWEX® H⁺ 50wx8-100 ion exchange resin was activated by overnight exposure to 1M HCl. C8 and C18 reverse phase column were performed on thermo fisher scientific HypoerSep Columns. High-resolution mass spectra were recorded on a Waters Q-TOF Premier™ Tandem Mass Spectrometer using positive electro-spray ionisation. Optical rotations were recorded using a Perkin-Elmer 241 polarimeter at the sodium D-line. Infrared spectra were recorded as thin films using a Bruker Tensor 27 FTIR spectrometer, equipped with an Attenuated Total Reflectance (ATR) sampling accessory, and are reported in wave numbers (cm⁻¹). Nuclear magnetic resonance spectra were recorded at 20 °C in chloroform or deuterium oxide using either a Varian Unity-INOVA operating at 300 MHz or a Varian Unity operating at 500 MHz. Chemical shifts are given in ppm (δ) and are relative to chloroform or water, all given ¹³C spectra are proton decoupled. NMR peak assignments were made using COSY, HSQC, TOCSY and HMBC experiments.

Methyl D-fructofuranoside (170).¹²⁸ To a solution of D-fructose (**156**) (3.6 g, 20 mmol) in MeOH (200 mL), H₂SO₄ (1mL, 18 mmol) was added. The resulting solution was stirred at rt for 15 min, after which triethylamine (6 mL, 40 mmol) was added, and the solvent was removed *in vacuo*. The resulting oil was used without further purification. R_f for the alpha product: 0.57 R_f, and for the beta product: 0.70 (EtOAc/isopropanol/H₂O, 6/4/1, v/v/v).

Methyl 6-deoxy-6-iodo-D-fructofuranoside (155).¹²⁸ To a solution of **170** (368 mg, 1.9 mmol) in dry THF (15 mL) were added PPh₃ (748 mg, 2.85 mmol) and imidazole (259 mg, 3.8 mmol). The mixture was heated to reflux and a solution of I₂ (723 mg, 2.85 mmol) in dry THF (7 mL) was added over 30 min. After an additional 15 min the reaction mixture was cooled to room temperature, filtered and concentrated. The residue was purified by column chromatography (PE/EtOAc, 1/1→1/2) followed by reverse phase flash chromatography to remove

remaining $\text{Ph}_3\text{P}=\text{O}$ ($\text{H}_2\text{O} \rightarrow \text{MeOH}$, 9/1 v/v) to yield the title compound **155** as a colourless oil (399 mg, 1.3 mmol 69% over 2 steps). R_f for the alpha product : 0.34 R_f for the beta product: 0.29 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 5/1, v/v); IR (v): cm^{-1} ; NMR data of Methyl 6-deoxy-6-iodo- α -D-fructoside: Spectroscopic data matched with previously reported.¹²⁸ ^1H NMR (500 MHz, CDCl_3): δ 4.13 (d, $J_{3,4} = 3.1$ Hz, 1H, H-3), 3.91-3.88 (m, 2H, H-4 and H-5), 3.77 (d, $J_{a,b} = 12.2$ Hz, 1H, H-1a), 3.66 (d, $J_{a,b} = 12.5$ Hz, 1H, H-1b), 3.47 (dd, $J_{5,6} = 4.6$ Hz, $J_{a,b} = 11.0$ Hz, 1H, H-6a), 3.38-3.34 (m, 1H, H-6b), 3.30 (s, 3H, Me OMe); ^{13}C NMR (125 MHz, CDCl_3): δ 108.1 (C-2), 81.7, 80.8 (C4 and C-5), 80.4 (C-3), 57.6 (C-1), 48.2 (OMe), 5.2 (C-6); NMR data of Methyl 6-deoxy-6-iodo- β -D-fructoside: ^1H NMR (500 MHz, CDCl_3): δ 4.18 (d, $J_{3,4} = 8.2$ Hz, 1H, H-3), 4.04 (t, $J_{3,4} = J_{4,5} = 7.9$ Hz, 1H, H-4), 3.84-3.82 (m, 1H, H-5), 3.71 (d, $J_{a,b} = 12.4$ Hz, 1H, H-1a), 3.64 (d, $J_{a,b} = 12.4$ Hz, 1H, H-1b), 3.47 (dd, $J_{5,6} = 4.6$ Hz, $J_{a,b} = 11.0$ Hz, 1H, H-6a), 3.38-3.34 (m, 1H, H-6b), 3.34 (s, 3H, Me OMe); ^{13}C NMR (125 MHz, CDCl_3): δ 103.7 (C-2), 80.1 (C-5), 78.6 (C-4), 77.0 (C-3), 59.4 (C-1), 49.27 (OMe), 6.8 (C-6); HRMS(ESI) m/z calcd. for $[\text{C}_7\text{H}_{13}\text{INO}_5]^+$: 326.9700, obsd.: 326.9705.

(3R,4R)-2-(R/S)-(diphenylmethyl)amino-hex-5-ene-1,3,4-triol (172a-b). To a solution of methyl iodoglycoside (**155**) (179 mg, 0.59 mmol) in EtOH (11 mL) was added water (0.25 mL), activated Zn (192 mg, 2.94 mmol), aminodiphenylmethane (120 μL , 0.71 mmol), NaCNBH_3 (111 mg, 1.76 mmol), and AcOH (0.6 mL). The mixture was stirred at reflux for 3 h, then cooled, and the solvent removed *in vacuo*. Purification by silica gel column chromatography [$(\text{CH}_2\text{Cl}_2/\text{EtOH}/\text{MeOH}/30\%$ aqueous NH_3 , 5/2/2/1, v/v/v/v)/ CH_2Cl_2 5/1 v/v] gave **172a** and **172b** as a mixture of diphenylmethylamine and product, which was used without further purification. R_f : 0.88, 0.80 [$(\text{CH}_2\text{Cl}_2/\text{EtOH}/\text{MeOH}/30\%$ aqueous NH_3 , 5/2/2/1, v/v/v/v)/ CH_2Cl_2 1/1 v/v]; IR (v): cm^{-1} ; ^1H NMR (500 MHz, CDCl_3): δ 5.86 (ddd, $J_{4,5} = 4.9$ Hz, $J_{5,6}\text{-cis} = 10.5$ Hz, $J_{5,6}\text{-trans} = 17.3$ Hz, 1H, H-5), 5.45 (d, $J_{5,6}\text{-trans} = 17.4$ Hz, 1H, H-6-trans), 5.25 (d, $J_{5,6}\text{-cis} = 10.5$ Hz, 1H, H-6-cis), 5.10 (s, 1H, CH NHCHPh_2), 4.07-4.06 (m, 1H, H-4), 3.92 (dd, $J_{1,2} = 4.9$ Hz, $J_{a,b} = 11.7$ Hz, 1H, H-1a), 3.77-3.66 (m, 2H, H-3, H-1b), 2.77 (t, $J_{1,2} = J_{2,3} = 2.0$ Hz, 1H, H-2); ^1H NMR (500 MHz, CDCl_3): δ 5.81 (ddd, $J_{4,5} = 6.1$ Hz, $J_{5,6}\text{-cis} = 10.5$ Hz, $J_{5,6}\text{-trans} = 17.8$ Hz, 1H, H-5), 5.32 (d, $J_{5,6}\text{-trans} = 17.4$ Hz, 1H, H-6-trans), 5.16 (d, $J_{5,6}\text{-cis} = 10.5$ Hz, 1H, H-6-cis), 5.08 (s, 1H, CH NHCHPh_2), 4.24 (t, $J_{3,4} = J_{3,4} = 4.9$ Hz, 1H, H-4), 3.77-3.66 (m, 3H, H-3, H-1a H-1b), 2.72 (dd, $J = 4.4$ Hz, $J = 9.3$ Hz, 1H, H-2); HRMS(ESI) m/z calcd. for $[\text{C}_{19}\text{H}_{24}\text{NO}_3]^+$: 314.1751, obsd.: 314.1752.

(3R,4R)-2(R/S)-Amino-hex-5-ene-1,3,4-triol hydrochloride(154a-b). To a solution of (3R,4R)-2-(R/S)-(diphenylmethyl)amino-hex-5-ene-1,3,4-triol (**172a-b**) and (diphenylmethyl)amine in TFA (4 mL) triethylsilane (200 μL , 1.25 mmol) was added and the mixture stirred at reflux for 3 h. The solution was cooled and the solvent removed under reduced pressure. The product was dissolved in water (50 mL) and washed with EtOAc (three times 50 mL). The water layer was then concentrated and purified by reverse phase (HP20, H_2O) and

gradient flash chromatography (CH₂Cl/EtOH/MeOH/30% aqueous NH₃, 5/2/2/1, v/v/v/v) to give **154a-b** (70% from **170**, 61 mg, 0.41 mmol) which was converted to the HCl salt by co-evaparting with 1M HCl. R_f = 0.30 ((CH₂Cl₂/EtOH/MeOH/30% aqueous NH₃, 5/2/2/1, v/v/v/v)/ CH₂Cl₂, 2/1); $[\alpha]_D^{20}$: 0.4 (c = 1.0 in H₂O); IR (v): 3219, 3069, 2959, 2925, 1668, 1437, 1200, 1136, 841.8, 801, 772, 724 cm⁻¹; ¹H NMR (500 MHz, D₂O): δ 5.94 (ddd, $J_{4,5}$ = 5.8 Hz, $J_{5,6-cis}$ = 10.7 Hz, $J_{5,6-trans}$ = 17.3 Hz, 1H, H-5), 5.34 (d, $J_{5,6-trans}$ = 17.6 Hz, 1H, H-6-*trans*), 5.31 (d, $J_{5,6-cis}$ = 10.7 Hz, 1H, H-6-*cis*), 4.31-4.29 (m, 1H, H-4), 3.87 (dd, $J_{1,2}$ = 4.4 Hz, $J_{a,b}$ = 12.2 Hz, 1H, H-1a), 3.82-3.78 (m, 1H, H-3), 3.77 (dd, $J_{1,2}$ = 7.1 Hz, $J_{a,b}$ = 12.2 Hz, 1H, H-1b), 3.53-3.47 (m, 1H, H-2); ¹³C NMR (125MHz, CDCl₃): δ 136.3 (C-5), 117.2 (C-6), 72.7 (C-4), 69.1 (C-3), 59.0 (C-1), 54.9 (C-2); ¹H NMR (500 MHz, D₂O): δ 5.90 (ddd, $J_{4,5}$ = 6.6 Hz, $J_{5,6-cis}$ = 10.5 Hz, $J_{5,6-trans}$ = 17.1 Hz, 1H, H-5), 5.34 (t, $J_{5,6-trans} = J_{5,6-cis} = 17.6$ Hz, 2H, H-6a and H-6b), 4.21(dd, $J_{3,4}$ = 5.6 Hz, $J_{4,5}$ = 6.8 Hz, 1H, H-4), 3.93 (dd, $J_{1,2}$ = 4.1 Hz, $J_{a,b}$ = 12.2 Hz, 1H, H-1a), 3.87-3.85 (m, 1H, H-3), 3.80-3.77 (m, 1H, H-1b), 3.53-3.47 (m, 1H, H-2); ¹³C NMR (125MHz, CDCl₃): δ 135.5 (C-5), 118.5 (C-6), 72.7 (C-4), 70.7 (C-3), 57.5 (C-1), 54.5 (C-2); HRMS(ESI) m/z calcd. for [C₆H₁₄NO₃]⁺: 148.0968, obsd.: 148.0974.

(5R/S,6R,7R,7aR/S)-6,7-Dihydroxy-5-(hydroxymethyl)-tetrahydro-pyrrolo[1,2-c]oxazol-3-

one. To a solution of alkenylamine hydrochloride **172a-b** (10.6 mg, 0.72 mmol) in sat. aq. NaHCO₃ (1.0 mL) was added I₂ (30 mg, 0.11 mmol). The solution was stirred for 18 h at room temperature then filtered and concentrated *in vacuo*. The product was purified by

silica gel chromatography (EtOAc/MeOH, 10/1, v/v) followed by removal of the excess salt by the addition of DOWEX® H⁺ to give the carbamates as an amorphous white solid. NMR data of the major product ¹H NMR (500 MHz, D₂O): δ 4.64 (t, $J_{5,6} = J_{a,b} = 9.1$ Hz, 1H, H-6a), 4.51 (dd, $J_{5,6} = 3.4$ Hz, $J_{a,b} = 9.1$ Hz, 1H, H-6b), 4.48 (d, $J = 4.9$ Hz, 1H, H-3), 4.34 (dt, $J = 2.7$ Hz, $J = 8.6$ Hz, 1H, H-5), 4.10-4.08 (m, 1H, H-4), 3.92 (dd, $J_{2,3} = 5.4$ Hz, $J_{1,2} = 12.7$ Hz, 1H, H-2); ¹³C NMR (125 MHz, CDCl₃): δ 164.8 (C7), 77.2 (C-3), 75.5 (C-4), 64.0 (C-6), 62.0 (C-2), 61.7 (C-5), 59.7 (C-1); NMR data of the minor product ¹H NMR (500 MHz, D₂O): δ 4.70-4.67 (m, 1H, H-6a), 4.50-4.46 (m, 1H, H-6b), 4.13 (dd, $J_{2,3} = 5.9$ Hz, $J_{3,4} = 12.4$ Hz, 1H, H-3), 4.02-3.98 (m, 2H, H-4 and H-5), 3.86-3.82 (m, 1H, H-1a), 3.71 (dd, $J_{1,2} = 5.7$ Hz, $J_{a,b} = 12.0$ Hz, 1H, H-1b), 3.66 (dd, $J_{1,2} = 4.0$ Hz, $J_{1,2} = 5.9$ Hz, 1H, H-2); ¹³C NMR (125 MHz, CDCl₃): δ 163.2 (C7), 78.7 (C-4), 78.1 (C-3), 67.7 (C-6), 64.8 (C-2), 91.2 (C-5), 60.8 (C-1); HRMS(ESI) m/z calcd. for [C₇H₁₁NNaO₅]⁺: 212.0529, obsd.: 212.0533.

2,5-dideoxy-2,5-imino-L-iditol hydrochloride 112 and 2,5-dideoxy-2,5-imino-D-mannitol hydrochloride 106. To a solution of the carbamates (4.1 mg, 0.022 mmol) in EtOH (1 mL) was added NaOH (7 mg, 0.18 mmol). The solution was stirred at reflux

for 2 h then cooled and purified directly using DOWEX® H⁺. Products **112** and **106** were eluted 30% aqueous NH₃, concentrated *in vacuo*. Purification was attempted on silica column chromatography (CH₂Cl/EtOH/MeOH/30% aqueous NH₃, 5/2/2/1, v/v/v/v)/CH₂Cl₂, 2/1→1/0) to give compounds **106** and **112** as a clean mixture of diastereoisomers. (2.1 mg, 0.013 mmol, 60%) white solid. R_f : 0.71 (CH₂Cl/EtOH/MeOH/30% aqueous NH₃, 5/2/2/1, v/v/v/v); NMR data of 2,5-dideoxy-2,5-imino-L-iditol¹⁴⁶; ¹H NMR (500 MHz, CDCl₃): δ 4.47 (d, *J* = 3.3 Hz, 2H), 4.02-3.96 (m, 4H), 3.92-3.88 (m, 2H); ¹³C NMR (125 MHz, CDCl₃): δ 74.5, 62.8, 57.4; NMR data of 2,5-dideoxy-2,5-imino-D-mannitol hydrochloride¹⁴⁷; ¹H NMR (500 MHz, CDCl₃): δ 4.13-4.08 (m, 2H), 3.95-3.85 (m, 4H), 3.60-3.57 (m, 2H); ¹³C NMR (125 MHz, CDCl₃): δ 74.1, 62.3, 57.7 ; HRMS(ESI) *m/z* calcd. for [C₆H₁₄NO₄]⁺: 164.0917, obsd.: 164.0926.

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Appendix

Iminosugars