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Glycomimetic Probes for the Study of Galactose-Binding Proteins

by

Frank-Michael Schweizer ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**

DEPARTMENT OF CHEMISTRY

Edmonton, Alberta

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Glycomimetic Probes for the Study of Galactose-Binding Proteins** submitted by **Frank-Michael Schweizer** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**.

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ABSTRACT

Pseudomonas aeruginosa employs pili to mediate adherence to epithelial cell surface receptors. It has previously been shown that the pilus adhesin of *P. aeruginosa* PAK recognizes the internal carbohydrate sequence β-D-GalNAc-(1→4)-β-D-Gal in the glycosphingolipid asialo-GM_1. To study the binding specificity of *P. aeruginosa*, *O*- and *N*- modified sugar analogs were synthesized, where each hydroxyl group was substituted either by *O*-methyl or *O*-propyl groups and the acetamido group was changed to a propionamido group. The sugar analogs were evaluated as inhibitors in a competitive solid phase binding assay. The results demonstrate that the pili of the *P. aeruginosa* strain PAK binds a variety of sugar analogs possessing the sequence β-D-GalNAc-(1→4)-β-D-Gal. Most sugar analogs bind with a similar order of magnitude (IC_{50} = 60 - 130 μM) except for the 2-*O*-propyl derivative **7** (IC_{50} = 8 ± 4 μM) and the 2-*O*-hexyl derivative **56** (IC_{50} < 8 ± 4 μM) compared with an IC_{50} of 79 ± 18 μM for the native compound. The significant increase in binding affinity of **7** and **56** suggests that improved inhibitors of adhesion may be prepared by introducing a hydrophobic side chain at the 2-position of galactose.

In the second part of the thesis α,α-disubstituted pyrans were synthesized as novel glycomimetic probes of galactose binding proteins. A general entry into these new glycomimetics was achieved by treating α-D-galacto-2-deoxy-oct-3-ulopyranosonate with a Lewis acid and silyl-based nucleophiles. In this way, the allyl, azido, cyano, thiophenyl and thioacetyl group could be introduced onto the pyran ring with high α-selectivity. Compound **97**α, bearing a cyano function at the α-position was reduced to yield the γ-sugar amino acid **122** which was used as a building block in solution- and solid phase-synthesis of unnatural glycopeptides. Sugar β-peptides were accessible via a one pot sequence beginning with α-D-galacto-2-deoxy-octo-3-ulopyranosonic acids. Initially, the

ketopyranose reacts under Lewis acid catalyzed conditions with a nitrile (aromatic or aliphatic) to form a glycosyliminoanhydride intermediate which can be isolated. Exposure of this intermediate to simple primary amines or amino acids produces novel sugar-β-peptides. Three different nitriles and three different amines have been used to demonstrate the generality of this reaction. Finally α-D-galacto-2-deoxy-oct-3-ulopyranosonate was used as a latent acyclic Michael donor in a model reaction with the Michael acceptor levoglucosenone.

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LIST OF ABBREVIATIONS

Ac	acetate
Ac_2O	acetic anhydride
Ala	L-alanine
Asn	L-asparagine
Asp	L-aspartic acid
Bn	benzyl
BSA	bovine serum albumin
CCC	combinatorial carbohydrate chemistry
Cys	D-cystine
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DIEA	Diisopropylethylamine
DMF	*N,N* -dimethyl formamide
Fmoc	9-fluorenylmethoxycarbonyl
Fuc	L-fucose
G	free energy of binding
Gal	D-galactose
GalNAc	*N*-acetyl-D-galactosamine
gem	geminal
GH	growth hormone
GlcA	D-glucuronic acid
Glc	D-glucose
GlcNAc	*N*-acetyl-D-glucosamine
Glu	L-glutamic acid
Gly	glycine
GPI	glycerol phosphatidyl inositol
HOBt	1-hydroxybenzotriazole
Hpy	2-hydroxypyridine
HTS	high-throughput screening
Hz	hertz
IC_{50}	concentration point when 50% inhibition is observed
Ido	L-idouronic acid
Ile	L-isoleucine
K	binding constant
KB7	*Pseudomonas aeruginosa* strain KB7
KDO	3-deoxyoctulosonic acid
Lys	L-lysine
Man	L-mannose
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
NMePhe	*N*-methylphenylalanine
NMR	nuclear magnetic resonance
OD	optical density
PI	*Pseudomonas aeruginosa* strain PI
PAI	*Pseudomonas aeruginosa* lectin I
PAII	*Pseudomonas aeruginosa* lectin II
PAK	*Pseudomonas aeruginosa* strain P
PAO	*Pseudomonas aeruginosa* strain O
PBS	phosphate buffered saline
Pfp	pentafluorophenyl

LIST OF ABBREVIATIONS--CONT.

Phe	L-phenylalanine
Ph	phenyl
Phth	phthalimido
Pro	L-proline
Pyr	pyridine
ROE	rotating frame overhauser effect
S	entropy
SAA	sugar amino acid
Ser	L-serine
SRIF	peptide hormone somastatin
T	temperature
$TBAHSO_4$	tetrabutylammonium hydrogen sulfate
TentaGel	polyethylene glycol-crafted polystyrene
TFA	trifluoroacetic acid
TfOH	trifluoromethanesulfonic acid
Thr	L-threonine
THF	tetrahydrofuran
tlc	thin layer chromatography
TMS	trimethylsilyl
TMSAll	allyltrimethylsilane
TMSCN	trimethylsilyl cyanide
$TMSN_3$	trimethylsilyl azide
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TMSSMe	methylthiotrimethylsilane
TROESY	transfer overhauser effect spectroscopy
Trp	L-tryptophan
TsOH	para toluensulfonic acid
Tyr	L-tyrosine
Val	L-valine
vic	vicinal
Xyl	D-xylose
Neu5Ac	sialic acid

CHAPTER 1

Introduction

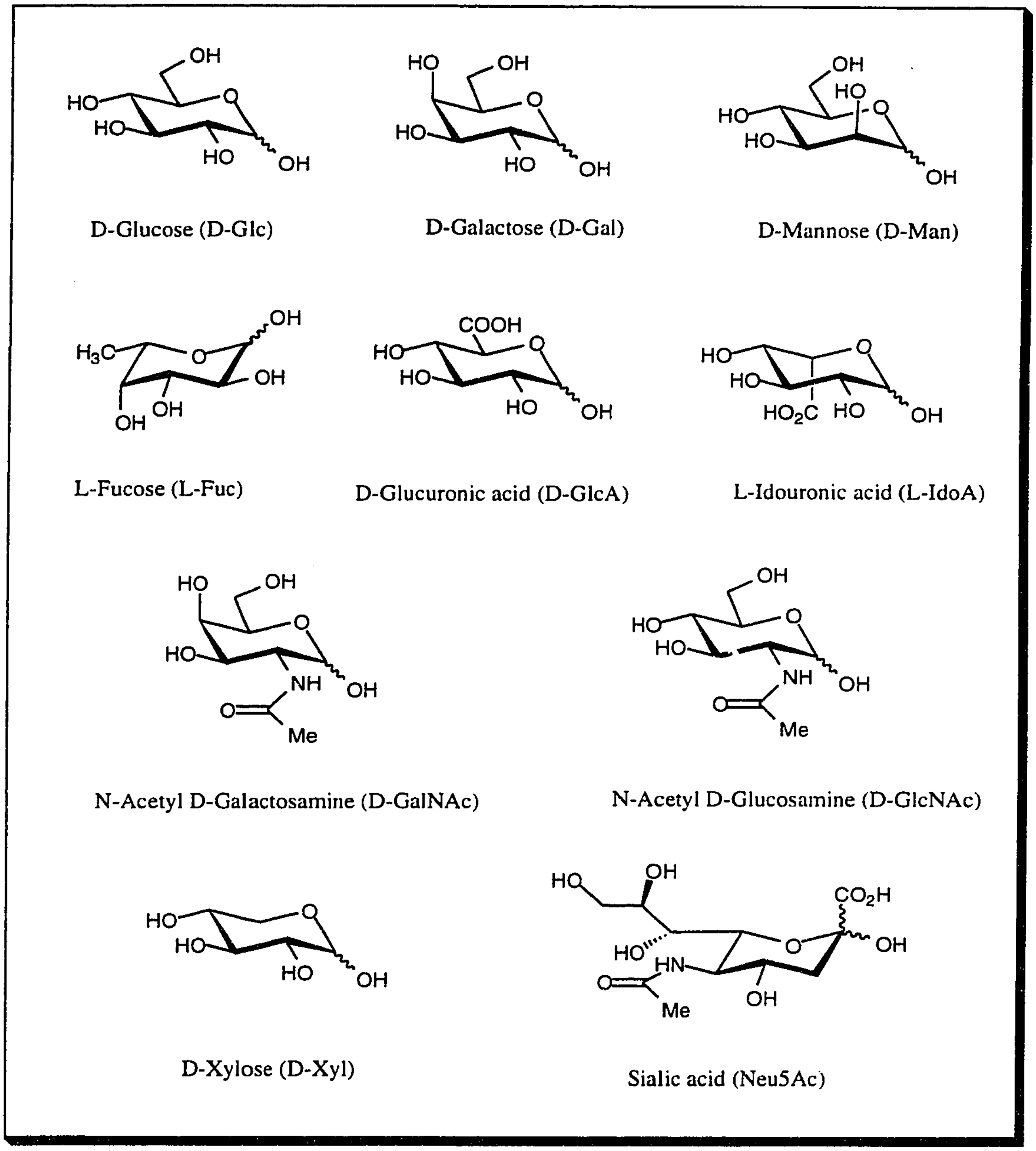
A. Glycobiology:

The past decade has seen a renaissance in carbohydrate biology and chemistry. The common perception of carbohydrates as being limited to energy and structural functions have been challenged by the recent and rapid emergence of the field of glycobiology[1,2]. This discipline promises to contribute to our knowledge of the intricate workings of the immune system, the means by which bacteria, viruses and cancer cells interact within their hosts, and the mechanisms of tissue growth and repair[1]. As a result, multidisciplinary research teams are now exploring what has been called "one of the last frontiers of biochemistry"[3].

A key property displayed by carbohydrates is their ability to generate tremendous diversity using only a small set of monosaccharide residues (Figure 1). Their polyhydroxylated structures allow for numerous connectivities. For example four carbohydrate residues can be linked in over 35,000 different combinations, as opposed to four amino acid residues being linked to twenty-four combinations[4,5]. This makes oligosaccharide structures ideal candidates for "talking" to their environment[6].

The following examples demonstrate the diversity and importance of carbohydrate functions.

Figure 1: Monosaccharides Commonly Found in Nature:



D-Glucose (D-Glc)

D-Galactose (D-Gal)

D-Mannose (D-Man)

L-Fucose (L-Fuc)

D-Glucuronic acid (D-GlcA)

L-Idouronic acid (L-IdoA)

N-Acetyl D-Galactosamine (D-GalNAc)

N-Acetyl D-Glucosamine (D-GlcNAc)

D-Xylose (D-Xyl)

Sialic acid (Neu5Ac)

1. Numerous pathogenic organisms exploit oligosaccharides on cell surfaces to initiate recognition and binding (adhesion). For instance, the influenza virus employs a sialidase (an enzyme that cleaves terminal sialic acid), which is located on its surface in order to adhere to sialic acid terminating oligosaccharides (Figure 1) expressed on mammalian epithelial cells[7]. In the same way, the parasite *Trypanosoma cruzi* recognizes sialic acid on host glycoconjugates. After binding, they transfer host sialic acids to their own surface by the coordinated activity of a sialidase and sialyltransferase (enzyme which transferes sialic acid)[11,12,13]. This trans-sialylation activity protects the parasite from the host immune response and complement system.

2. Fertilization studies in mice have shown recognition of the egg by sperm is initiated by carbohydrate-protein interactions. In particular, a galactosyltransferase on the sperm head interacts specifically with a oligosaccharide containing a galactose residue in the egg coat or zona pellucida[10]. By comparison, mannose ligands on the human zona pellucida have been suggested to be involved in sperm-egg recognition[11,12].

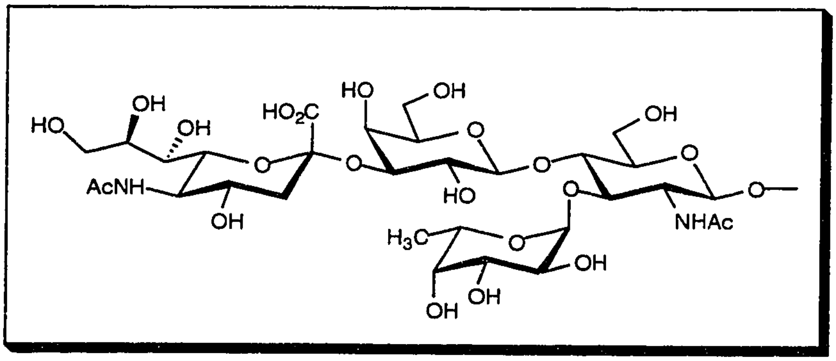
3. Carbohydrates have been implicated in embryonic development. Deletion of a transferase gene in mice using gene knockout experiments resulted in embryonic lethality at about 10 days of gestation[13,14].

4. Oligosaccharide residues can be important in the modulation of protein activity. Functional diversification can be achieved by altering the number and type of oligosaccharides on the protein surface. These modifications allow for the protein to expand their functional roles and modulate their activity without having to modify their amino acid sequence[2].

5. Aberrant glycosylation is a common feature of many transformed cells including cancer cells. Alteration of cell-surface carbohydrates not only allows the tumor to migrate away from the primary tumor (metastasis), but may also aid their colonisation at remote tissues[15-19].

6. Carbohydrates interact with a group of proteins referred to as selectins[20,21] which are included in the control of the inflammation process. During an inflammatory response, lymphocytes pass from the blood stream through the vascular endothelium to the site of inflammation. The process occurs by a slowing down or "rolling" of the white blood cells followed by a stronger "sticking" process and eventually passage through the endothelium to the inflamed site[22,23]. It is in the "rolling" stage where selectin-carbohydrate interaction occurs to initiate the process. There are three types of selectins namely L-selectins[24,25], P-selectins[26,27] and E-selectins[28,29]. The L-selectins are glycoproteins found on the lymphocyte surface that bind to carbohydrates on the endothelial cell. The P and E selectins are found on the endothelial cells and interact with carbohydrates on the lymphocytes. Both P and E selectins interact specifically with a tetrasaccharide called Sialyl Lewis^X (Figure 2)[28,29,30].

Figure 2: Sialyl Lewis^X:



These examples represent a fraction of the postulated carbohydrate mediated functions. One can see that the scope of the biological function of carbohydrates is very large and can be crucial for life processes. It is for these reasons that a tremendous volume of research is being directed towards understanding the role of complex carbohydrates in biological systems. In order to do so an appreciation of the biological roles of carbohydrates is needed.

B. Biological Occurrence of Carbohydrates:

1. Energy and Metabolism:

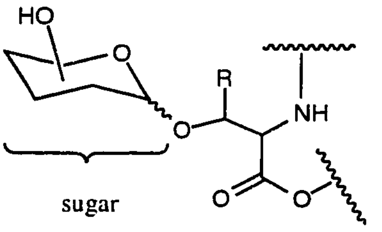
The classical role attributed to carbohydrates is that of a medium for energy storage and transport. Ingestion of substances such as starch, lactose and sucrose results in the absorption of their constituent monosaccharides (sugars, mainly glucose) into the bloodstream as a result of the action of enzymes present in the digestive system. Glucose is stored as a highly branched polymer (glycogen) in tissues such as muscle and liver, and released back into the bloodstream when there is demand for energy. Following transport across the cell membranes of target tissues, glucose is converted by the enzyme hexokinase into its 6-phosphate derivative, a reactant in several cellular pathways including the production of ATP (adenosine triphosphate) and acetyl-CoA, both essential metabolic intermediates.

2. Glycoproteins and Glycolipids:

Carbohydrates of biological relevance usually consist of several covalently-linked monosaccharide units and are referred to as oligosaccharides or glycans. There are ten monosaccharides found in mammalian systems (Figure 1) which may be additionally modified, typically by acylation or sulphation. Oligosaccharides are in most cases associated with other biomolecules such as lipids or proteins. These hybrids are known as glycoconjugates and can be classified as glycoproteins, glycolipids and proteoglycans. Glycoproteins are by far the most complex glycoconjugates and account for functions such as the determination of blood type. There are two major subtypes of glycoproteins: *N*-linked structures, where the sugar is covalently attached to the protein via the amide nitrogen of asparagine, and *O*-linked glycoproteins, where the sugar is covalently attached to the protein via the hydroxyl of either serine or threonine (Figure 3). The oligosaccharide chains themselves are often branched and a large number of subtypes exist.

Figure 3: General Structure of *O*- and *N*-linked Glycoproteins:

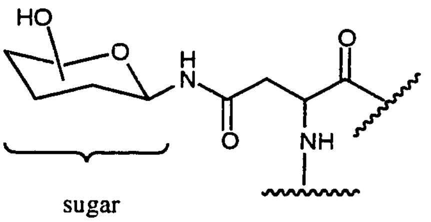
O-linked glycopeptides:



sugar : α-Fuc, α-Gal, β-Gal, α-GalNAc, α-Glc, β-Glc, β-GlcNAc, α-Man, β-Xyl

R = H, Me

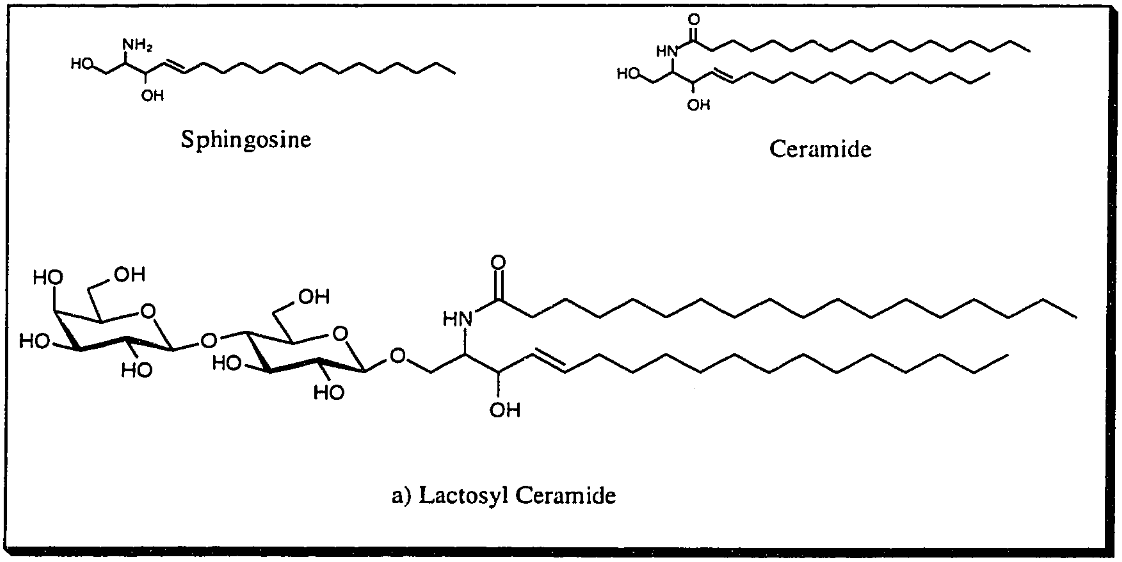
N-linked glycopeptides:



sugar : β-GlcNAc, β-Glc, β-GalNAc

Glycolipids are made up of an oligosaccharide linked covalently to a fatty acid portion by means of a sphingosine or an inositiol moiety. To the amino-group of sphingosine, fatty acids of varying length are attached via amide bonds, forming the glycolipid's hydrophobic tail, called ceramide. The carbohydrate units are bound to the primary hydroxyl groups of ceramide via glycosidic linkages and form glycosphingolipids (Figure 4a). Gangliosides are a special class of acidic glycosphingolipids bearing sialic acid residue(s) attached to the carbohydrate moiety. The association of the non-polar function with the cell membranes effectively anchors these molecules to the extracellular surface. A different class of glycolipids known as the glycophosphatidyl inositol anchors act as sites of attachment for proteins to the cell membrane (Figure 4b). The carbohydrate portion of glycoproteins and glycolipids often acts as a site for the binding of other large biomolecules such as cell-surface proteins (called lectins or adhesins), bacterial toxins, hormones and antibodies[1]. As such, glycoconjugates mediate many cell-cell interactions and are responsible not only for the defence of an organism against pathogens but also, paradoxically, often facilitate infection.

Figure 4a: Components of Glycosphingolipids:

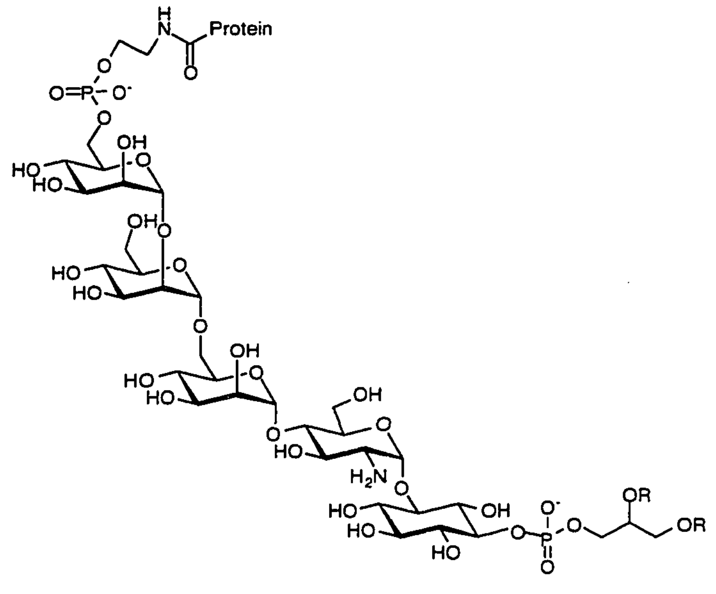


Sphingosine

Ceramide

a) Lactosyl Ceramide

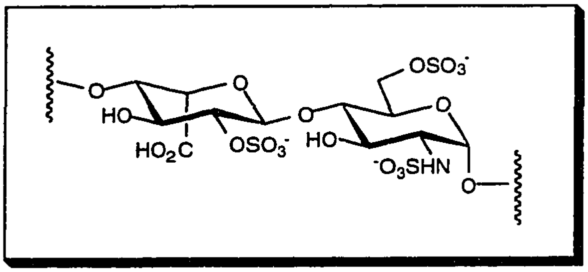
Figure 4b: Glycosyl Phosphatidyl Inositol (GPI) Anchor:



3. Proteoglycans:

A third class of glycoconjugates, the proteoglycans[31], are composed of a protein and a high molecular weight carbohydrate component. These substances are responsible for the integrity of connective tissues and also act as storage sites for biologically active compounds. The carbohydrate portion of the proteoglycans, called glycosylaminoglycans, are polysaccharides with molecular weights up to 30,000 daltons. They consist of repeating disaccharide units, themselves made up of unusual monosaccharides such as L-idouronic acid (Figure 1). A characteristic feature of the glycosylaminoglycans is the presence of sulphate groups on many of the sugar residues. Glycosylaminoglycans such as heparin (the repeating unit is shown in Figure 5) bind to a number of different proteins and thereby mediate a number of biological functions including blood clotting, formation of new blood vessels (angiogenesis), attachment of cells to extracellular matrix proteins (such as collagen in cartilage) and even regulation of gene expression[1,32,33,34]. Though the polymers are large, the binding sites of proteins can recognize structures as small as pentasaccharides.

Figure 5: Structure of Heparin:

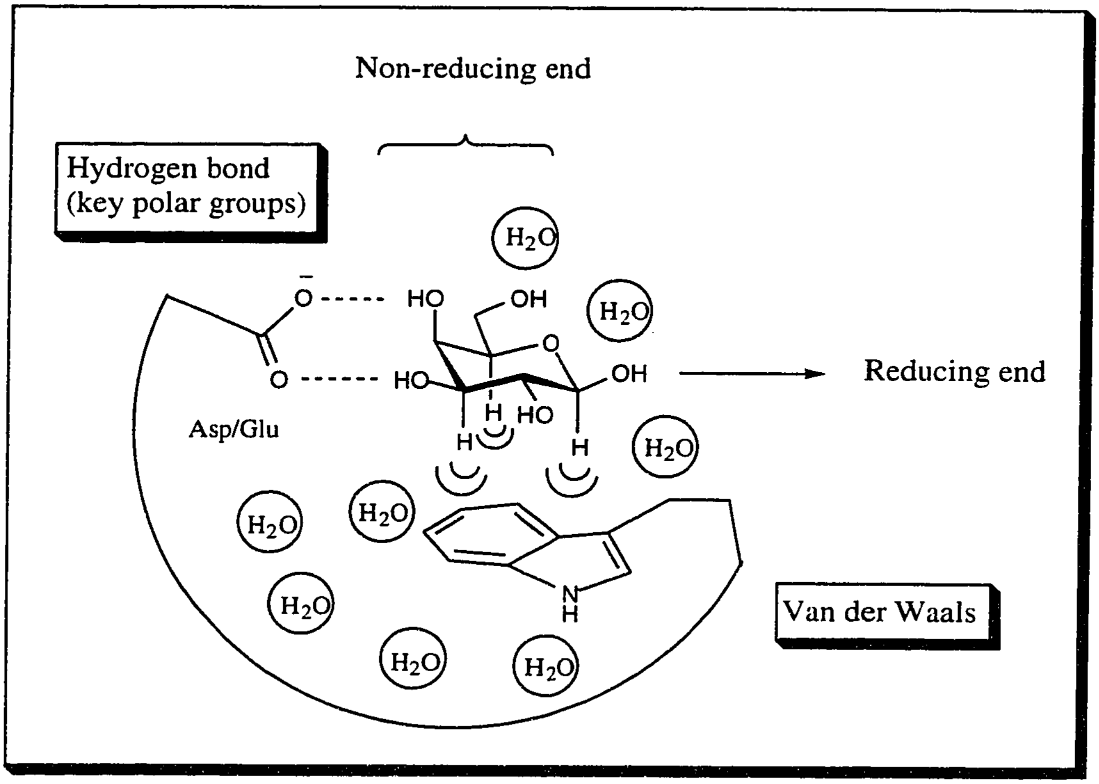


C. Carbohydrate-Protein Interactions:

Recognition of carbohydrates by proteins plays a central role in many intracellular and intercellular processes such as the trafficking of proteins and cells, bacterial and viral infection, normal cell differentiation and development, tumor progression and metastasis[35]. The information contained in an enormous variety of complex carbohydrate structures (glycoproteins, glycolipids and proteoglycans) is decoded by complementary sites present on carbohydrate binding proteins. A high fidelity in the protein-carbohydrate interaction is essential in these recognition processes.

At the atomic level, the specificity and affinity in these interactions is achieved through hydrogen bonds, with added contributions from van der Waals contacts and stacking interactions of aromatic residues against the hydrophobic region of the sugars (Figure 6)[36]. X-Ray crystallographic studies of several carbohydrate-protein complexes have shown that usually a small number of crucial hydroxyl groups (key-polar groups) in the carbohydrate molecule are involved in essential hydrogen bonds with the protein, whereas other hydroxyl groups at the periphery of the binding site or exposed to solvent are not essential for the formation of the complex[37,38]. Replacement of the key polar groups by hydrogen, halogen, amino or methoxy groups often leads to less active compounds[39] and has not generally been applied to improve carbohydrate-protein interactions[40].

Figure 6: Carbohydrate-Protein Interactions:



1. Thermodynamic Parameters Describing the Binding Constant in Carbohydrate-Protein Complexes:

The free energy of binding (ΔG) can be calculated on the basis of measurements of the binding and/or dissociation constant K according to equation (1)

$$(1)\ \Delta G^\circ = -RT\ln K$$

The free energy of binding comprises an enthalpic (ΔH) and an entropic contribution ($T\Delta S$) as shown in equation (2).

$$(2)\ \Delta G = \Delta H - T\Delta S$$

The change in free energy must be negative for complex formation. All systems tend to increase their entropy by a decrease in order. A high level of molecular flexibility and thus a large number of possible conformers of protein or carbohydrate will necessarily establish a rather large amount of entropy if no kinetic barrier for the interconversion of the different conformers exists. Binding of a carbohydrate ligand to a protein receptor immediately reduces conformational flexibility of both partners and consequently results in a loss of entropy. Hence, it is hardly conceivable that specific recognition can be achieved with two completely flexible molecules, unless pronounced enthalpic forces occur that compensate for the drastic loss of spatial flexibility and thus the entropy. Inspection of the types of molecular interaction between carbohydrate and protein shows that polar interaction (hydrogen bonds) between the hydroxyl function of carbohydrates with the charged or hydrophilic amino acid side chain of aspartate, asparagine, glutamate, threonine and serine predominate[36,41,42]. Strong ionic interactions will not be found very often in glycobiology since saccharides are usually uncharged molecules, with the exception of uronic acids, sialic acids, carbohydrate sulfates or phosphates and ketodeoxynonulosonic acid (KDN), as well as 3-deoxyoctulosonic acid (KDO) in the bacterial endotoxins. In contrast to a superficial view of carbohydrates as entirely polar substances, a more detailed inspection reveals that hydrophobic as well as van der Waals interactions between carbohydrate and protein can add to the binding energy. L-Fucose with its 6-deoxy function displays a strongly decreased capacity for hydrophilic interactions but is a common part of biologically important structures such as Lewis blood group epitopes[43]. Similarly, abequose (a 3,6-dideoxyhexopyranose) as part of a *Salmonella* antigen, has been found to interact with tryptophan, histidine and phenylalanine residues of a monoclonal antibody[44]. Besides this special case of deoxy sugars, one has to take into account that each carbon atom not only carries a hydroxyl function but also a hydrogen atom of low polarity. Depending on their orientation within a given monosaccharide, an area with hydrophobic character may be formed (see the underface of β-Gal in Figure 6). Finally, we must also consider the participation of the solvent, which is water for all biological systems, in the association process. Solvated protein receptors and solvated carbohydrate ligands carry water molecules attached to them by hydrogen bonding. Due to the strong polar interactions between, for example, the saccharide's hydroxyl functions and the water molecules, there will be a gain in enthalpy, but a concomitant loss in entropy for both: for the carbohydrate forming a larger ordered and thus less flexible structure than without water, and for the water molecules, which would have a higher degree of freedom in bulk water than when associated with the saccharide. The formation of such a solvation sphere for the ligand, which is equally conceivable at least for parts of the combining site of the receptor, will strongly be influenced by their individual functionalities. The hydrophobic areas of carbohydrates, for example are hydrated to a significantly lesser extent than the hydrophilic areas. What would be expected to happen if a hydrated saccharide approaches a hydrated protein? On the basis of the required specificity of interaction resulting from a great number of hydrophilic, hydrophobic and van der Waals contacts, the majority of the water molecules in the vicinity of the interacting areas must leave the hydration sphere, so the two species can contact. Consequently, the entropy of this complex may decrease, because its mobility may be lower than that of the solvated individual binding partners. For the water molecules, however being released from the ordered hydration sphere to the less ordered bulk water, a significant gain in entropy can be expected[37,40,43,45,46,47]. A further important role of the solvent may be that a distinct, spatially fixed water molecule can be used as a molecular bridge between a receptor and a ligand[37,43,46].

2. Analysis of Carbohydrate-Protein Interaction Using Engineered Ligands:

Although the number of available high resolution X-ray or ^{1}H-NMR structures has grown substantially in recent years, the glycobiologist is still most likely confronted with the fact of not having sufficient 3D structural data for his investigation. In such a case, binding studies with synthetic carbohydrate analogs are a powerful tool to elucidate the hydrogen bonding pattern. Replacement of individual hydroxyl groups by hydrogen or fluorine can be used to identify hydroxyl groups directly involved in hydrogen bonding to the protein and even to dissect their energetic contribution to the binding. Replacement with hydrogen will result in the abrogation of any hydrogen-bond at that position, whereas an electronegative fluorine atom cannot act as a hydrogen-bond donor but it can be a hydrogen-bond acceptor, albeit a weak one[48,49]. Thus, a significant reduction in the binding affinity upon deoxygenation at a given position reveals the involvement of that particular hydroxyl group in hydrogen bonding to the protein. The overall free energy contribution of the hydrogen bond can be estimated from the loss in affinity of the deoxy derivative. In addition, the behavior of the corresponding fluorodeoxy derivative indicates the participation of the hydroxyl group as a hydrogen-bond donor or as an acceptor. However, the use of deoxyfluoro analogs for the detection of hydrogen bonds has been criticized. It has been suggested that a fluoro substituent may destabilize a complex by repulsion between the fluorine atom and electronegative side-chains that accept important hydrogen bonds from the ligand, and that a water molecule may donate a hydrogen bond in place of the removed hydroxyl group[50].

In contrast, replacement of a hydroxyl group with an O-alkyl side-chain (methyl, ethyl or propyl) provides not only information about the hydrogen-bonding relationships at that position but also on the flexibility of the combining site to steric demands for complex formation[51]. This kind of mapping should allow the localization of solvent exposed hydroxyl groups in the carbohydrate-protein complex[52], which are usually unaffected by simple monomethylation[40]. The information obtained by these binding studies could then be used to design new ligands which are able to interact with hydrophobic side chains of the protein located at the periphery of the carbohydrate-protein combining site as hypothesized and shown by Lemieux[40,53].

Binding studies using other synthetic derivatives, such as epimeric and amino or C-methyl analogs can provide additional insights into the hydrogen-bonding and steric requirements, as well as information on the distribution of polar and non-polar regions within the combining site. Other synthetic saccharides, including pseudo sugars (compounds in which the ring oxygen of a monosaccharide has been replaced by a different atom such as C, S or N) and heteroatom glycosides (C-, S-, N- glycosides, anomerically linked via an atom other than oxygen) have also been used in the analysis of carbohydrate-protein interactions[54,55]. In addition, these compounds can be valuable as inhibitors of enzymes and other carbohydrate-binding proteins and as hydrolytically or enzymatically stable analogs.

The conformational properties of the ligands in solution also play an important role in recognition[56]. It is commonly assumed that a single conformation of the sugar is selected out of the range of possible conformations existing in solution for binding to a protein.

Therefore, the study of the recognition phenomenon based on engineered ligands must include the analysis of the possible changes in their conformational preferences. Only when the functional group replacement does not modify substantially the distribution of low energy conformers can the relative affinity of the protein for the engineered ligand be correlated with the presence or the absence of a certain group, and with the size and shape of the hydrophilic and the hydrophobic surfaces of the saccharide molecule. On the other hand, binding studies using conformationally restricted saccharides can provide information on the conformational requirements of the combining site for efficient recognition. Unfortunately, methods for conformational restriction of glycosidic linkages are rather limited and rely on the introduction of extra rings or substituents that can cause steric hindrance[55].

3. Combinatorial Carbohydrate Chemistry as an Efficient Tool to Probe the Binding Site of Carbohydrate-Binding Proteins:

Modern organic/medicinal chemistry is undergoing a "cultural revolution" in the way drugs are discovered and developed. Instead of discrete synthesis and biological screening of individual compounds, which often takes years to identify and optimize, the currently developing technology called "combinatorial chemistry"[57-61] can rapidly provide large numbers of chemicals (libraries) in a short time. In conjunction with these new synthetic methods, high-throughput screening can rapidly screen the libraries produced and in so doing, can provide information for optimizing lead compounds.

In contrast to the remarkable progress on small molecule libraries and biopolymers, the development of Combinatorial Carbohydrate Chemistry[62] has been slow. Reasons for this arise mostly from the inherent chemical difficulties presented by this class of compounds.

Firstly, there is no general glycosylation methodology available comparable to the coupling approach of peptide and phosphodiester formation, where the yields are essentially quantitative. Secondly the process of glycosylation creates a new stereocentre at the anomeric carbon (which can be either α or β) and very few reactions can give a glycosidic bond with absolute stereospecificity, a problem not encountered with the amide or phosphate linkages in nucleotide and peptide chemistry. Moreover, each of the naturally occurring sugars (Figure 1) carries at least three hydroxyl groups of various activities towards glycosylation. Extensive branching or other functionalization such as sulphation or phosphorylation further complicates the chemistry. Nevertheless, methods for solution phase[62] and solid phase combinatorial carbohydrate synthesis[62] have been developed. The first approach to oligosaccharide libraries applied a "random glycosylation" strategy[63] (Figure 7). In this strategy, less than 1 equiv. of protected donor per free acceptor hydroxyl group, is reacted with an unprotected acceptor attached to a hydrophobic aglycon. The increased solubility of the unprotected sugar provided by the hydrophobic aglycon in organic solvents facilitated the glycosylation and product isolation. Under appropriate conditions of promoter, solvent and reaction temperature, all possible disaccharides, including positional isomers and anomers could be obtained in a single reaction. As acceptor size increases the glycosylation products obtained in the library increase exponentially. Nevertheless, the low conversion of acceptor and need for extensive product purification remain serious impediments to its widespread use. Liang et al.[64] were able to produce an encoded library containing 1300 compounds using a polyethylene glycol-grafted polystyrene (TentaGel) as solid support (beads) and anomeric sulfoxides as donors (Figure 8). First, six glycosides with carboxylic acid aglycons were attached to TentaGel separately, and the resulting beads were encoded individually. After deacetylation, the resulting intermediates were pooled and then split into twelve parts and then glycosylated with twelve donors individually. Once more, the products were separately encoded with 12 new tags. Since the acceptor was immobilized on TentaGel, excess donor can be utilized to assure that the reaction goes to completion, without causing any difficulties in purification as in solid phase synthesis. After the construction of a

Figure 7: "Random Glycosylation Strategy" for the Synthesis of Oligosaccharide Libraries:

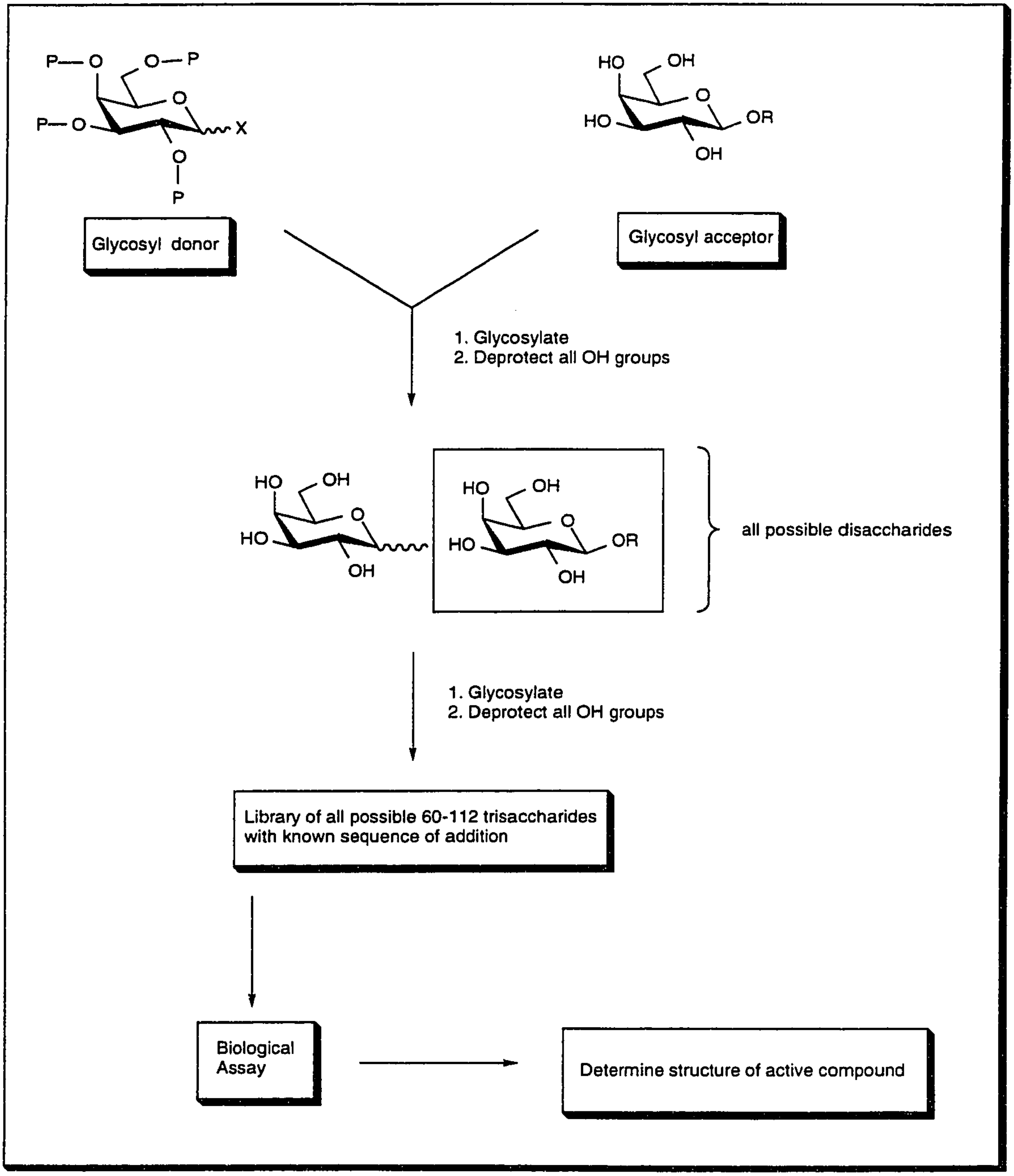
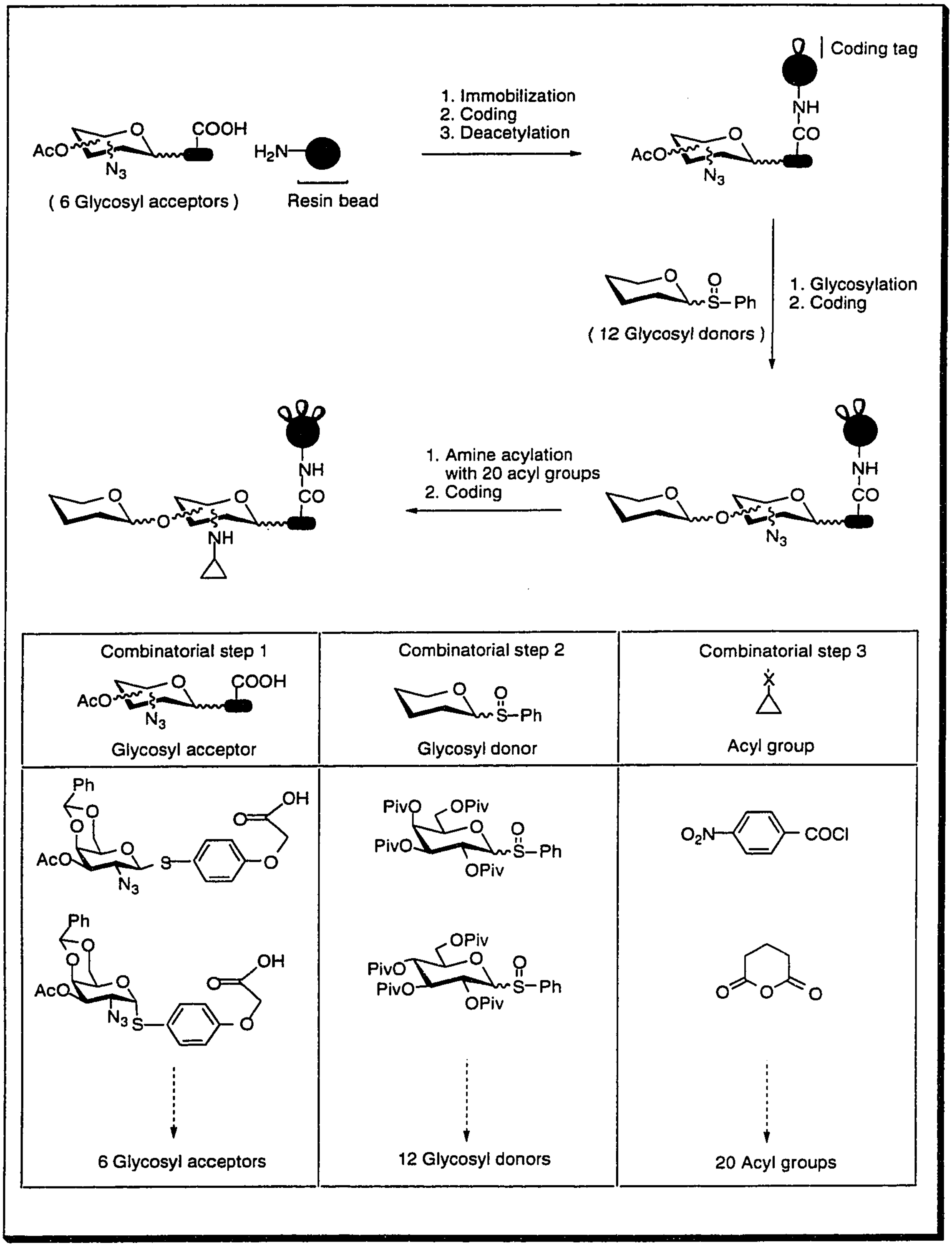


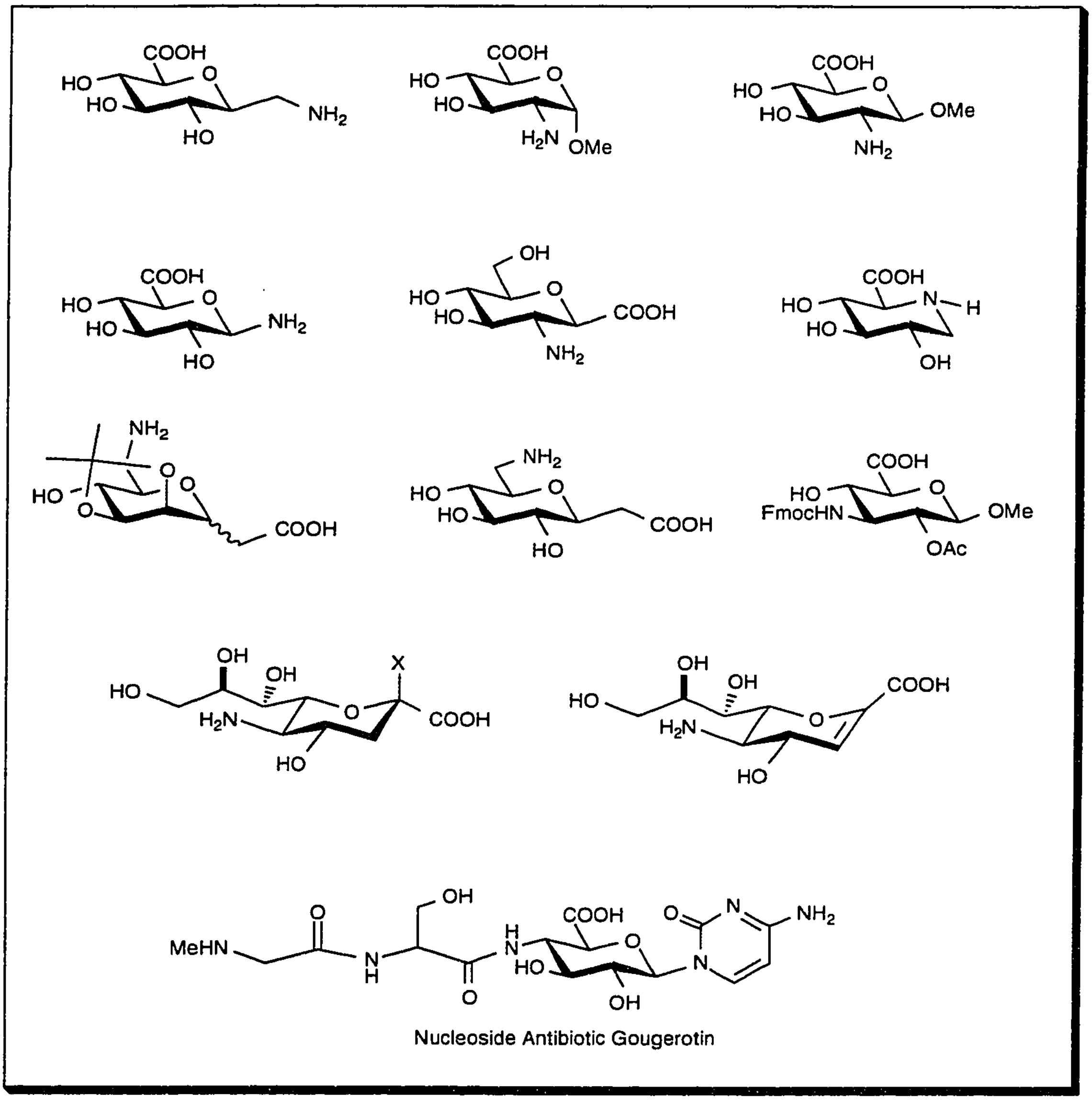
Figure 8: Synthesis of a Coded Oligosaccharide Library Using Sulfoxides as Donors:



disaccharide or trisaccharide library, the azide function was transformed into an amine, which could be further acylated with 20 different acyl groups into a large number of oligosaccharide derivatives. Coding of these derivatives attached on the TentaGel and subsequent removal of protecting groups provided libraries on the polymer for direct bioassay with carbohydrate-binding proteins such as *Bauhinia purpurea* lectin. Importantly, the protein can discriminate very well between beads containing the hit ligand and beads containing related ligands in spite of the inhibitory concentrations being so similar. A similar but solution approach towards oligosaccharide libraries employed allyl glycosides[65] which can be readily converted into vinyl glycosyl donors and acceptors. Thus the product (disaccharide) of a glycosylation reaction is again able to be converted into a new acceptor.

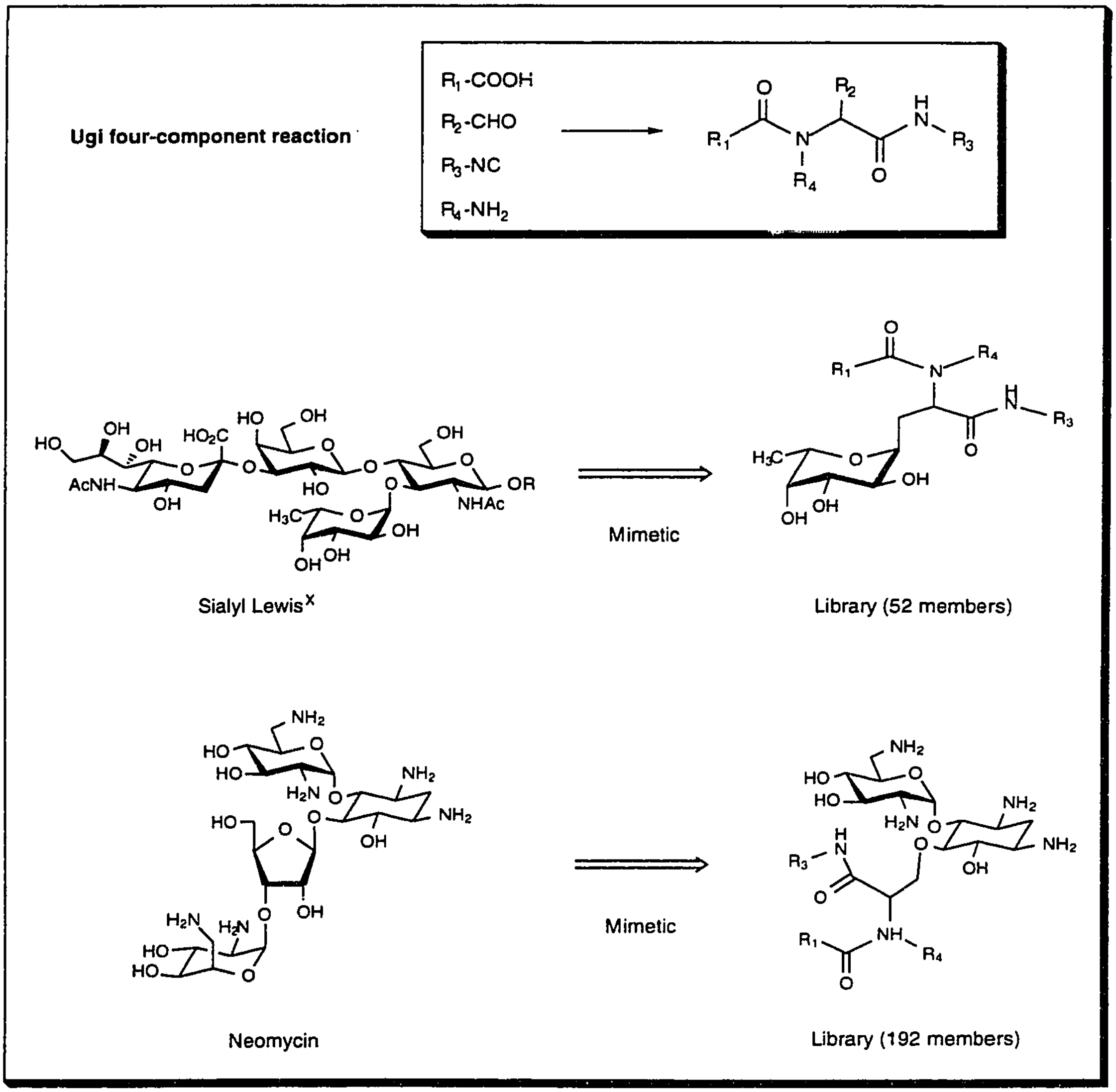
Despite the significant progress in Combinatorial Carbohydrate Chemistry over the last years, combinatorial carbohydrate synthesis has not become routine. Even if the above mentioned obstacles could be overcome, an oligosaccharide library may not produce drug candidates due to the low bioavailabilities (susceptibility to glycosidases and poor cellular uptake) of its components. New approaches designed to circumvent these problems have emerged and have resulted in a class of compounds termed glycomimetics (carbohydrate mimics). Particularly the introduction of an amino and a carboxylic acid function into the carbohydrate, leading to sugar amino acids (SAAs)[66-75] has been employed (Figure 9). SAAs then can easily undergo oligomerization[70,71,75,76-90] to saccharide peptide hybrids[80], glycopeptoids[89,90] and glycotides[75], amide linked sugars[87] and saccharopeptides[87] via well-developed peptide chemistry and represent ideal building blocks for combinatorial synthesis[57-61]. The resultant oligomers may be useful drug candidates, since they would not be susceptible to glycosidases and may be resistant to proteases due to the altered backbone relative to natural peptide substrates. Functional group modifications, in particular hydroxyl protection, could increase the lipophilicity of the molecules and render them more likely to permeate cell membranes provided that the particular hydroxyl group is not involved in key polar interactions to the protein. Several glycomimetic libraries have recently appeared.

Figure 9: List of Previously Published Sugar Amino Acids (SAAs):



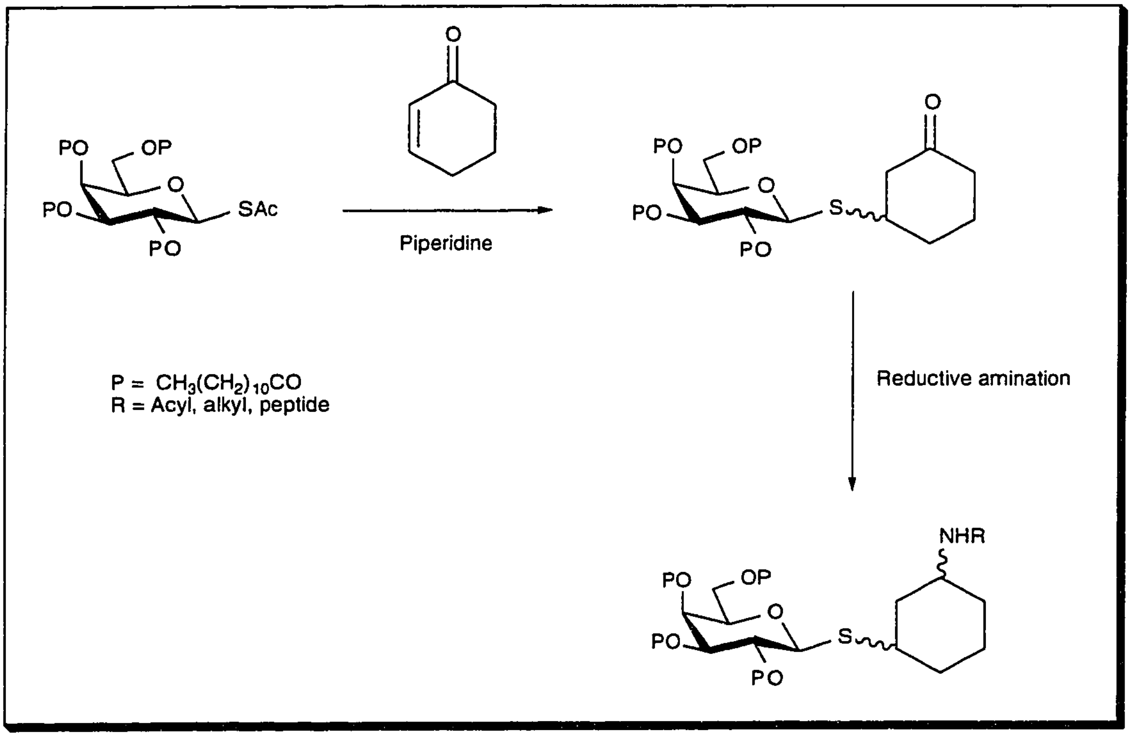
The Ugi four component condensation[91] (aldehyde, amine, isocyanate, carboxylic acid) has been used to construct libraries mimicking sialyl Lewis^X [92] and the aminoglycoside antibiotic neomycin[93] (Figure 10). In both cases a modified sugar (monosaccharide or disaccharide) was chosen as the aldehyde component. The amine part was immobilized on insoluble or soluble polymer.

Figure 10: Ugi Four-Component Condensation Used in the Synthesis of Glycomimetic Libraries of Sialyl Lewis^X and Neomycin:



Recently an aglycon-functionalized β-Gal library[94] utilizing 1-thio-β-D-Gal derivative in Michael addition reactions of carbonyl compounds followed by reductive amination of the carbonyl group has been described. Each hydroxyl group in the galactose residue was present as its lauroyl ester, which facilitated the isolation of products by reversed-phase chromatography (Figure 11).

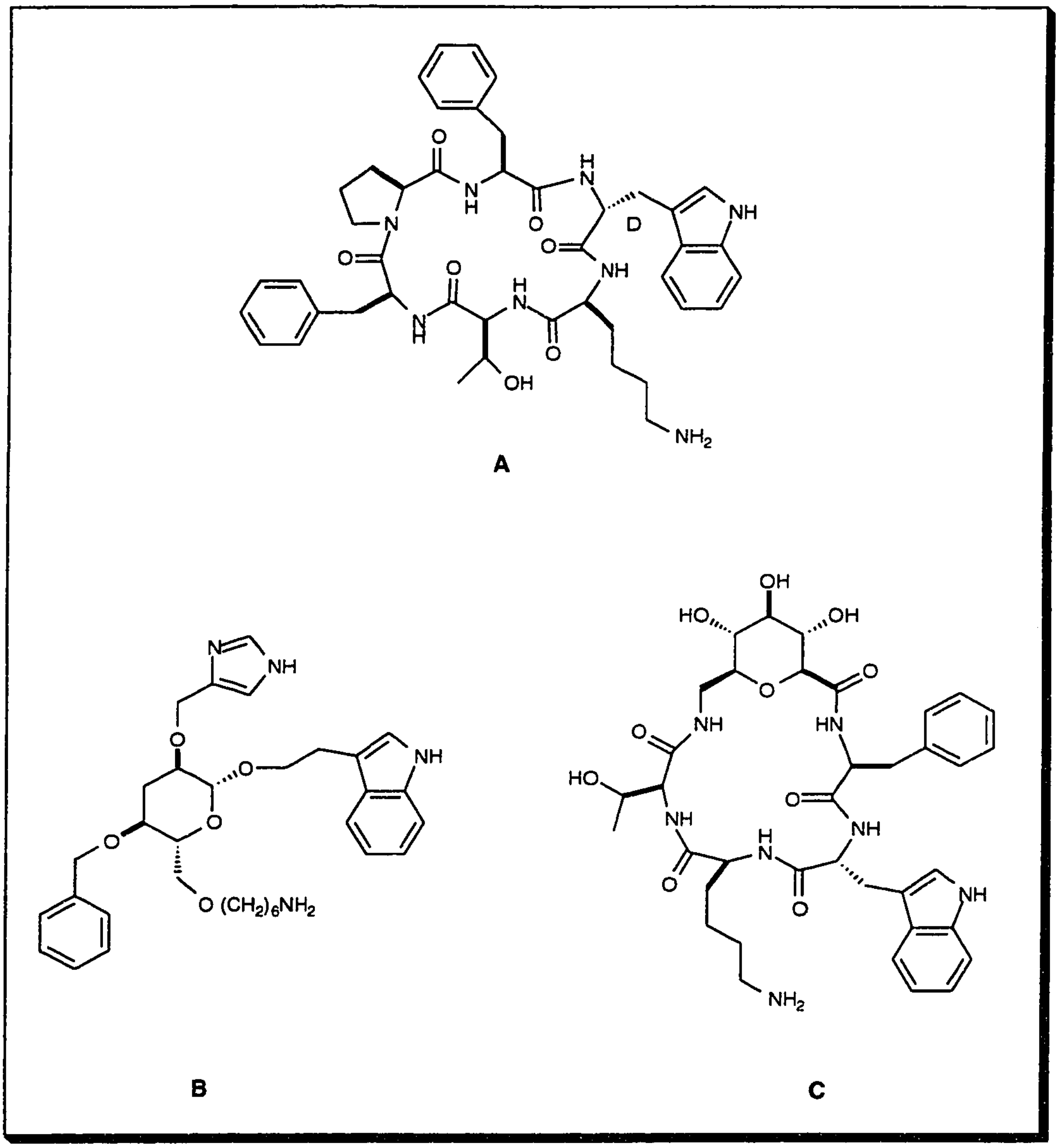
Figure 11: Aglycon Functionalized β-D-Gal Library:



D. Carbohydrate Based Peptidomimetics:

In addition to the earlier mentioned essential biological functions of oligosaccharides, carbohydrates have also been recognized as highly functionalized and rigid synthetic scaffolds which if modified can display interesting properties. In particular the use of sugar derived skeletons as somatostatin peptidomimetics has been pursued independently by two groups[74,95]. The peptide hormone somatostatin (SRIF) is a cyclic tetradecapeptide which inhibits the release of several hormones including growth hormone (GH) and has attracted attention as a therapeutic agent[96]. Its very short biological half life led to the design and synthesis of longer acting peptidal peptidomimetics but to date no SRIF analog possessing adequate oral bioavailability has been described[95]. SRIF contains a β-turn composed of the tetrapeptide sequence Phe-Trp-Lys-Thr[97-99]. This tetrapeptide retains the ability to elicit SRIF-like biological effects as long as the side chains of these four amino acids are constrained in nearly the same orientation as in the bioactive conformation of SRIF. For example, the cyclic hexapeptide **A** (Figure 12) with the sequence c-(Phe-D-Trp-Lys-The-Phe-Pro) is a potent agonist[100] but still contains the typical problems associated with peptides such as poor oral bioavailability (susceptibility to peptidases, poor solubility, etc.). The design of non-peptide peptidomimetics represents a new approach to this problem. Carbohydrates, like glucose, with its well defined conformation, the ability to position the required side chains in an equatorial orientation around the pyran ring, the requisite enantiomeric purity of a starting material and the vast array of synthetic knowledge are attractive options. For instance, modifications on the D-glycoside skeleton resulted in an active (IC_{50} = 1.9 μM) somatostatin peptidomimetic **B** which mimics the hexapeptide **A** (Figure 12)[95]. In addition the dipeptide (Phe-Pro) of the cyclic hexapeptide **A** was successfully replaced by a sugar amino acid leading to a highly active (IC_{50} = 0.15 μM) somatostatin mimetic **C** (Figure 12)[95].

Figure 12: Somatostatin Peptidomimetics:

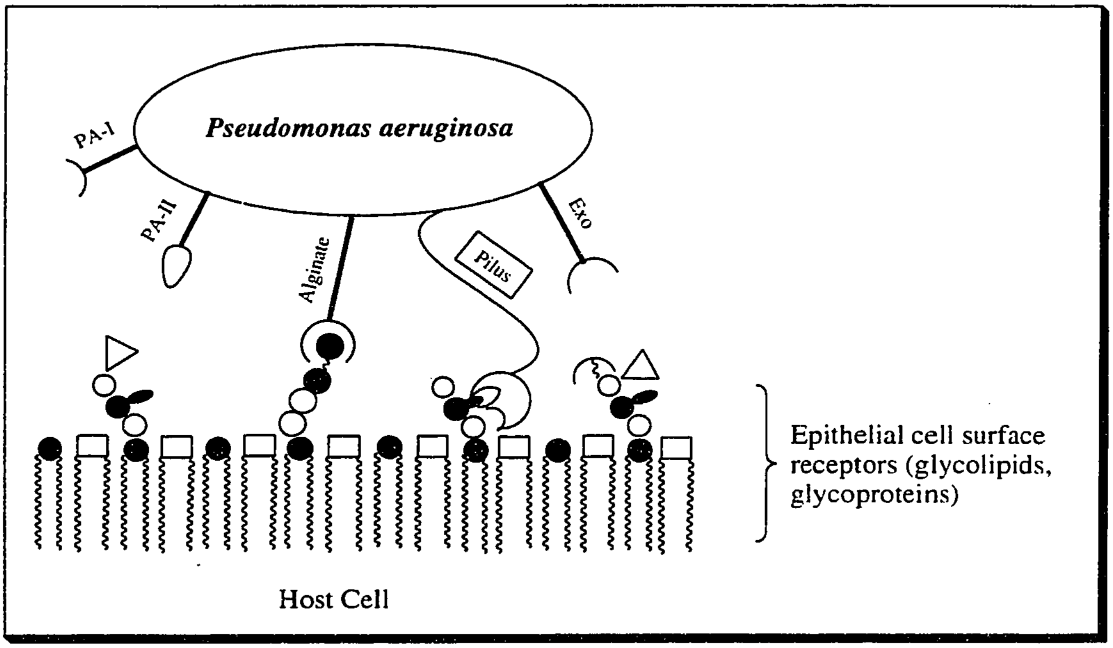


Chapter 2

Interaction Between the Pili of *Pseudomonas aeruginosa* PAK and its Carbohydrate Receptor β-D-GalNAc-(1→4)-β-D-Gal Analogs:

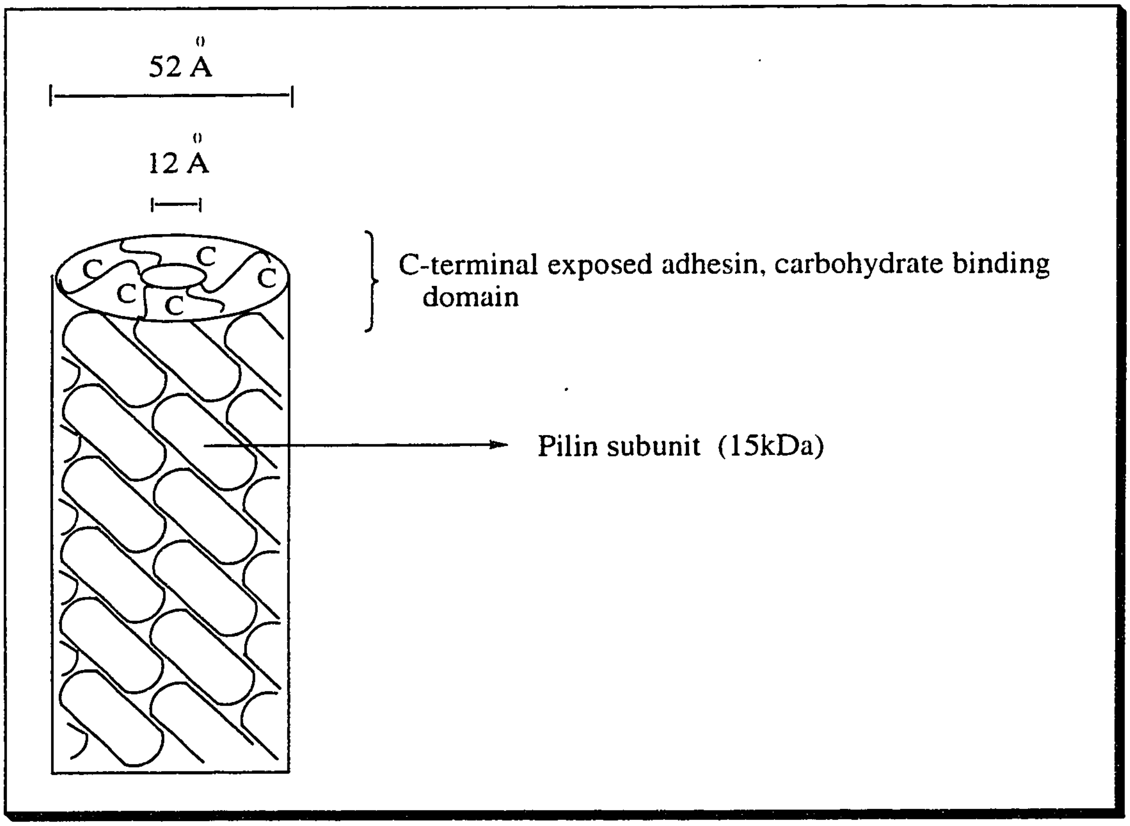
A. Pilus Adhesin of *P. aeruginosa*:

Figure 13: Proposed Model of the Adherence of *P. aeruginosa* to Epithelial Cell Surfaces:



Pseudomonas aeruginosa infections are a major concern for patients in intensive care units[101], cystic fibrosis patients[102], burn injury-victims, and leukemia patients[103-106]. In the initiation of an infection, the adherence of a bacterium to an epithelial cell plays a critical role[107]. So far at least four different adhesins that mediate the adherence of the pathogen to the host tissue have been identified. These include the pili[108,109], alginate (heteropolymer of D- and L-guluronic acids[110,111,112], exoenzyme S[113,114] and the outer membrane proteins[115-117] like *P. aeruginosa* lectins PAI[118] and PAII[118] (Figure 13). Analysis of binding properties[112,119,120] and competition studies[121] indicate that the pilus is the dominant adhesin responsible for initiating infection[120]. In particular, the pilus adhesin is a significant virulence factor in animal infection models[122] and antibodies that inactivate the pilus adhesin confer protection from subsequent challenge[123].

Figure 14: Model of the Pilus Adhesin:



The pilus of *P. aeruginosa* (Figure 14) is a proteinacous, filamentous surface appendage which resembles a hollow tube of 5.2 nm in outer diameter, 1.2 nm in central channel diameter and an average length of 2.5 µm[124,125,126]. Unlike the type I pili or Pap pili that consist of multi-components, *P. aeruginosa* pili are composed of a single protein subunit termed pilin. The pilus is assembled by pilin subunits into a helical array of five subunits per turn with a pitch of 4.1 nm[125,127]. The *P. aeruginosa* strain PAK has an amino acid sequence of 144 residues with a molecular weight of 15 kD[127,128]. It has been suggested that the pilin monomer can be roughly divided into three regions, a highly conserved N-terminal sequence, a semi-conserved C-terminal sequence with an intrachain disulfide bridge and a variable central region sequence[129,130]. The N-terminal sequence contains the modified amino acid N-methyl-phenylalanine as the first residue of the pilins and are referred to as the N-methylphenylalanine (NMePhe) pili. This region is highly homologous to the pilin sequences found in *Neisserie gonorrhoea*, *N. meningitidis*, *Dichelobacter nodosus*, *Moraxella bovis*, *M. nonliquefaciens*, and *Vibrio cholerae*[131,132]. The semi-conserved C-terminal region of the pilin monomer has been shown to contain the binding domain of *P. aeruginosa* to host epithelial cells[109,112,126,133-136]. The amino acid sequences of four various strains (PAK, KB7, PAO and P1) of *P. aeruginosa* at the C-terminus are shown in Figure 15. Despite their limited homology at the C-terminus, all pilin proteins have a disulfide loop in common. Interestingly, synthetic peptides containing the sequence of the last 17 amino acids as in the pilin wild types (referred to as PAK 128-144, PAO 128-144, KB7 128-144 and the 21 amino acid sequence in P1 126-148) contain the adherence binding domain and bind to a common glycosphingolipid cell surface receptor[123,137].

Figure 15: C-Terminal Peptide Sequences of PAK, KB7, PAO and P1:

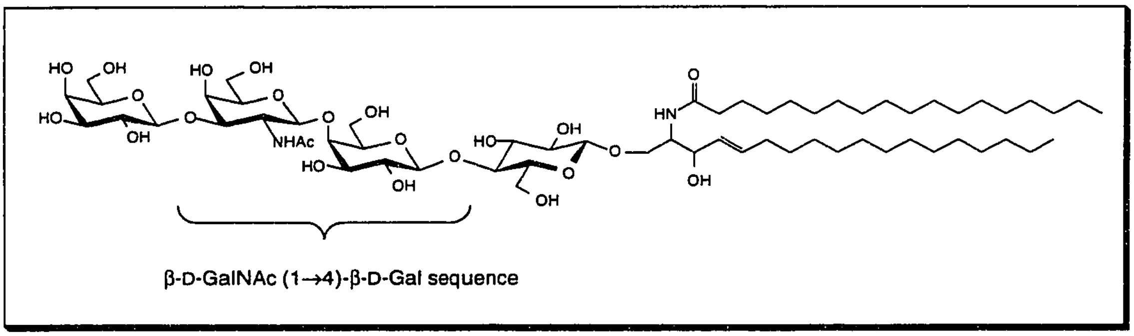
Strain	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148
PAK			K	C	T	S	D	Q	D	E	Q	F	I	P	K	G	C	S	K				
KB7			S	C	A	T	T	V	D	A	K	F	R	P	N	G	C	T	D				
PAO			A	C	K	S	T	Q	D	P	M	F	T	P	K	G	C	D	N				
P1	N	C	K	I	T	K	T	P	T	A	W	K	P	N	Y	A	P	A	N	C	P	K	S

In an effort to understand the structural basis for the cross-reactivity with the receptor, the solution structures of PAK 128-144, PAO 128-144 and KB7 128-144 and P1 126-148 peptides have been determined and a structural motif of two sequential β-turns have been revealed[138,139,140]. The three shorter peptides, PAK, PAO and KB7, display a type I β-turn in the conserved sequence Asp134-X-X-Phe137 and a type II β-turn in the conserved Pro139-X-Gly-Cys142. The longer P1 peptide displays a type I β-turn in the region spanned by residues Thr-134-Ala135-Trp136-Lys137, followed by another turn of undefined conformation in the region spanned by Pro138-Asn139-Tyr140-Ala141. Interestingly, the position (residues 134 to 137) and conformation (type I) of the first turn appears to be conserved across all four strains, regardless of the fact that there is no sequence homology between the smaller 17-residue peptides and the longer P1 peptide in this region. Beyond the structurally conserved β-turn regions, the four pilin peptides show little structural homology in solution, as expected from their primary sequences. Differences in the tertiary folds of the four peptides are largely determined by differences in the arrangements of their hydrophobic pockets[139,140]. The PAK peptide shows a hydrophobic pocket composed of the side-chains of Phe137 and Ile138 and Pro139, the PAO peptide shows a pocket composed of the side-chains of Phe137 and Pro139 and the KB7 peptide shows a hydrophobic "face" comprised of the side-chains of Val133 and Phe137. The hydrophobic pocket in the larger P1 peptide is comprised of the side-chains Ile129, Trp136, Pro138, Tyr140 and Pro142.

B. *P. aeruginosa* Pilus Receptors:

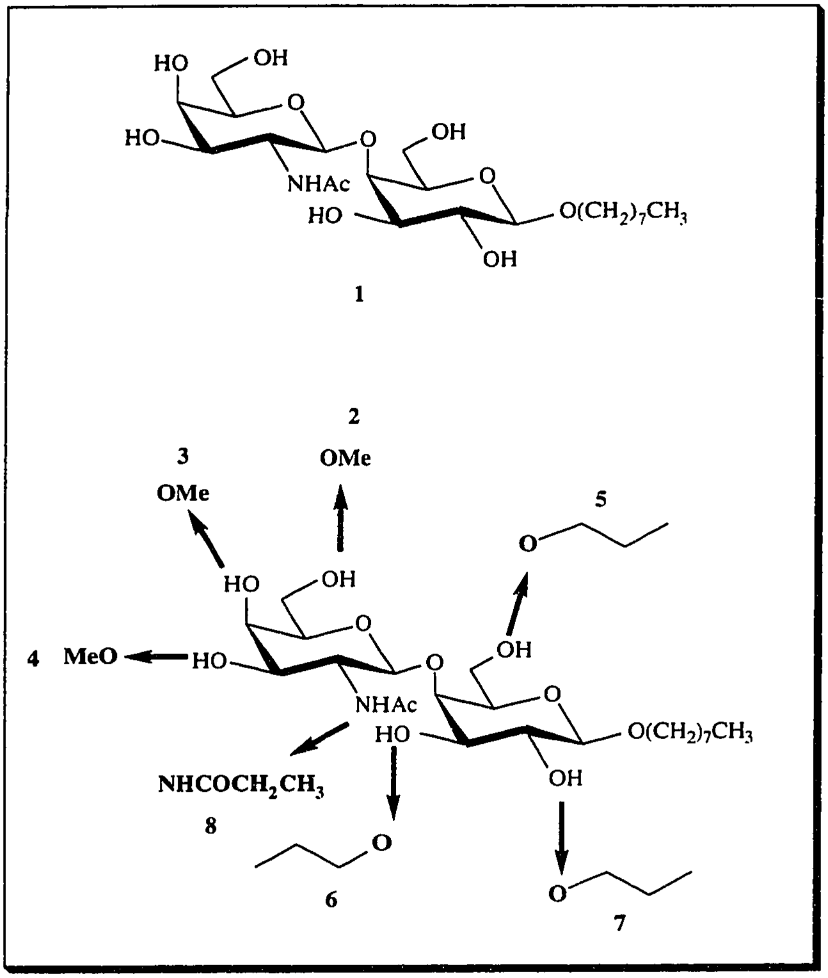
Several investigations have identified potential receptor candidates for the pilus adhesin including the glycosphingolipids asialo-GM$_1$ and asialo-GM$_2$[141] (Figure 16), sialic acid containing glycosphingolipids[141], lactosyl-ceramide[141] and glycoproteins[136,142]. The importance of the carbohydrates in binding was demonstrated by the loss of pilus binding upon treatment with periodate[136,142]. The glycosphingolipid asialo-GM$_1$ has been strongly implicated in the pulmonary infection of cystic fibrosis suffering patients which show a 430% superficial overexpression of asialo-GM$_1$ compared to normal subjects[143]. Lee et al.[137] showed that *P. aeruginosa* pili bind to asialo-GM$_1$ at the tip of the pili. Further studies showed that *P. aeruginosa* binds to the internal carbohydrate sequence β-D-GalNAc-(1→4)-β-D-Gal found in the glycosphingolipids asialo-GM$_1$ and asialo-GM$_2$[123] (Figure 16). The same sequence appears to be the recognition element for a variety of other pathogens including *E. coli*, *Salmonella*, *Staphylococcus*, *Streptococcus pneumoniae* and *Haemophilus influenzae*[144].

Figure 16: The Glycosphingolipid Asialo-GM$_1$:



C. Mapping of the Carbohydrate (*P. aeruginosa* PAK)-Pilus Combining Site with β-D-GalNAc-(1→4)-β-D-Gal Receptor Analogs:

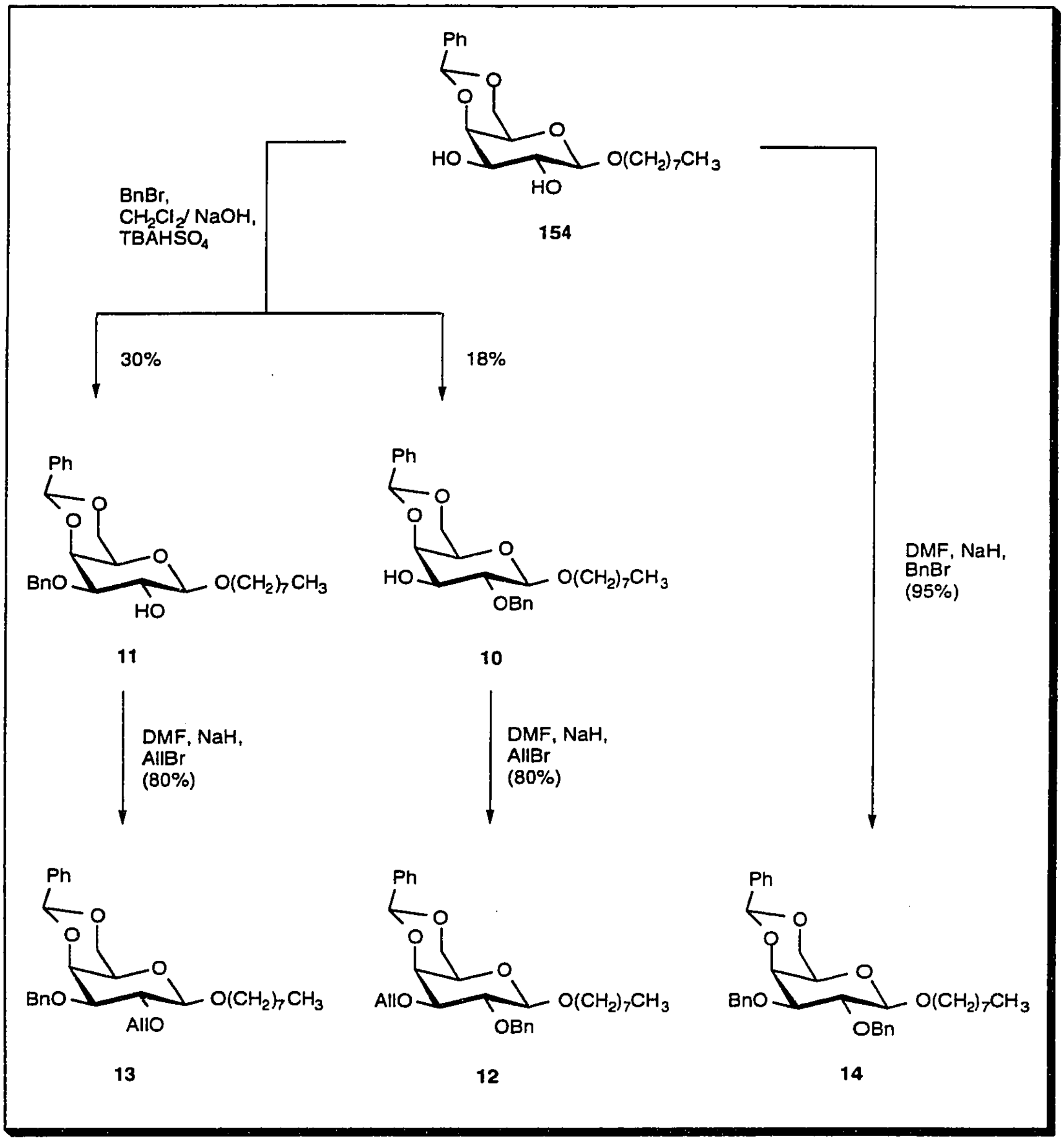
Figure 17: Mapping Approach of the Sugar-Pilus Combining Site:



In order to gain a more detailed understanding of the PAK pilus-carbohydrate interaction, we chose a chemical mapping approach employing single *O*-modified and *N*-modified sugar analogs[38,145] (Figure 17). The replacement of sugar hydroxyl groups by hydrogen[38], fluorine[146,147] or *O*-alkylation, such as methylation[51,145] are established methods for probing the molecular basis of carbohydrate-protein recognition. *O*-Alkylated derivatives were used in this study as the synthesis of alkyl derivatives is less problematic than a deoxygenation approach to producing structural analogs. Furthermore, *O*-alkylation may allow the localization of the partially or completely solvent-exposed hydroxyl groups at the carbohydrate-protein binding site. For instance, it was expected that *O*-alkylation of completely solvent-exposed hydroxyl groups would have little effect on the stability of the carbohydrate-protein complex[53]. In comparison, alkylation of partially solvent-exposed hydroxyl groups at the periphery of the combining site have been shown to increase the affinity to the protein in some cases[38,51]. The enhancements were explained by van der Waals contacts of the *O*-alkylated side chain of the carbohydrate with hydrophobic amino acid residues at the binding site[51]. This result is in agreement with Monte Carlo simulations of the hydration sphere around the combining site of the lectin IV of *Griffonia simplicifolia* which demonstrated that the water order around the combining site prior to complex formation is perturbed and shows the presence of voids[40]. Water molecules in close proximity (< 8Å) to the protein combining site possess considerably more energy and less entropy than their counterparts in bulk water. The filling of the so-created voids with polyamphiphilic surfaces like carbohydrates may provide an important driving force for association[40]. Similar conclusions were derived by another study which concluded that solvent reorganization around the ligand and the receptor contributes up to 80% of the reaction enthalpy of the overall enthalpy[148]. In this respect mono-*O*-methylation (compounds 2-4) and *O*-propylation (compounds **5**-**7**), as well as exchange of the acetamido group to a propionamido group (compound **8**) seemed an appropriate tool to map the apron of the combining site (Figure 17). *O*-Propylation of the galactose moiety was chosen since the precursors are synthetically derived from the *O*-allyl group which can be further functionalized at a later stage to a variety of structurally diverse analogs. Furthermore, the galactose moiety was propylated since it was expected that the GalNAc fraction of the disaccharide would be buried deeper in the binding site compared to the galactose half. The octyl group was chosen as aglycon since it facilitates the purification and isolation of the final unprotected disaccharides[149].

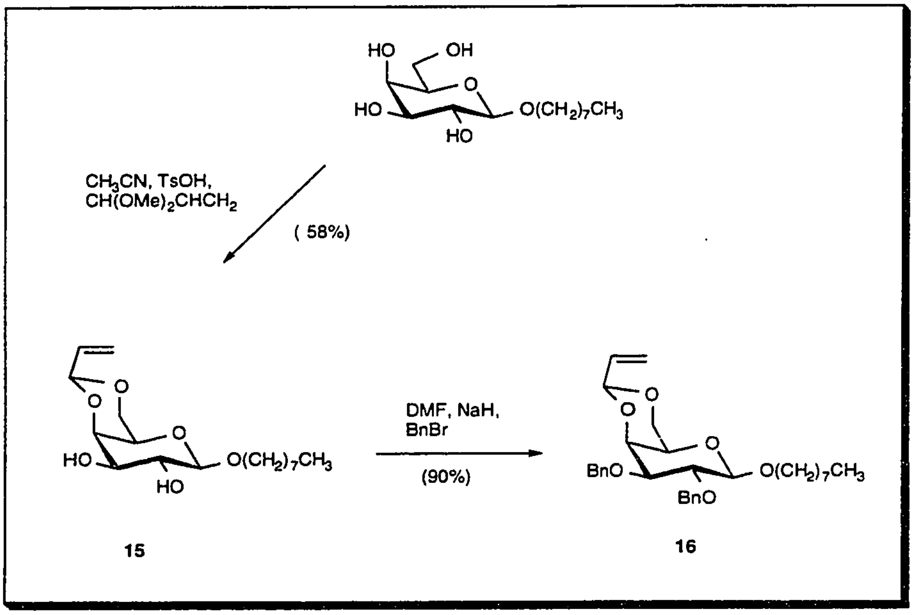
D. Synthesis of β-D-GalNAc-(1→4)-β-D-Gal Receptor Analogs:

Figure 18: Synthesis of Compounds **12-14**:



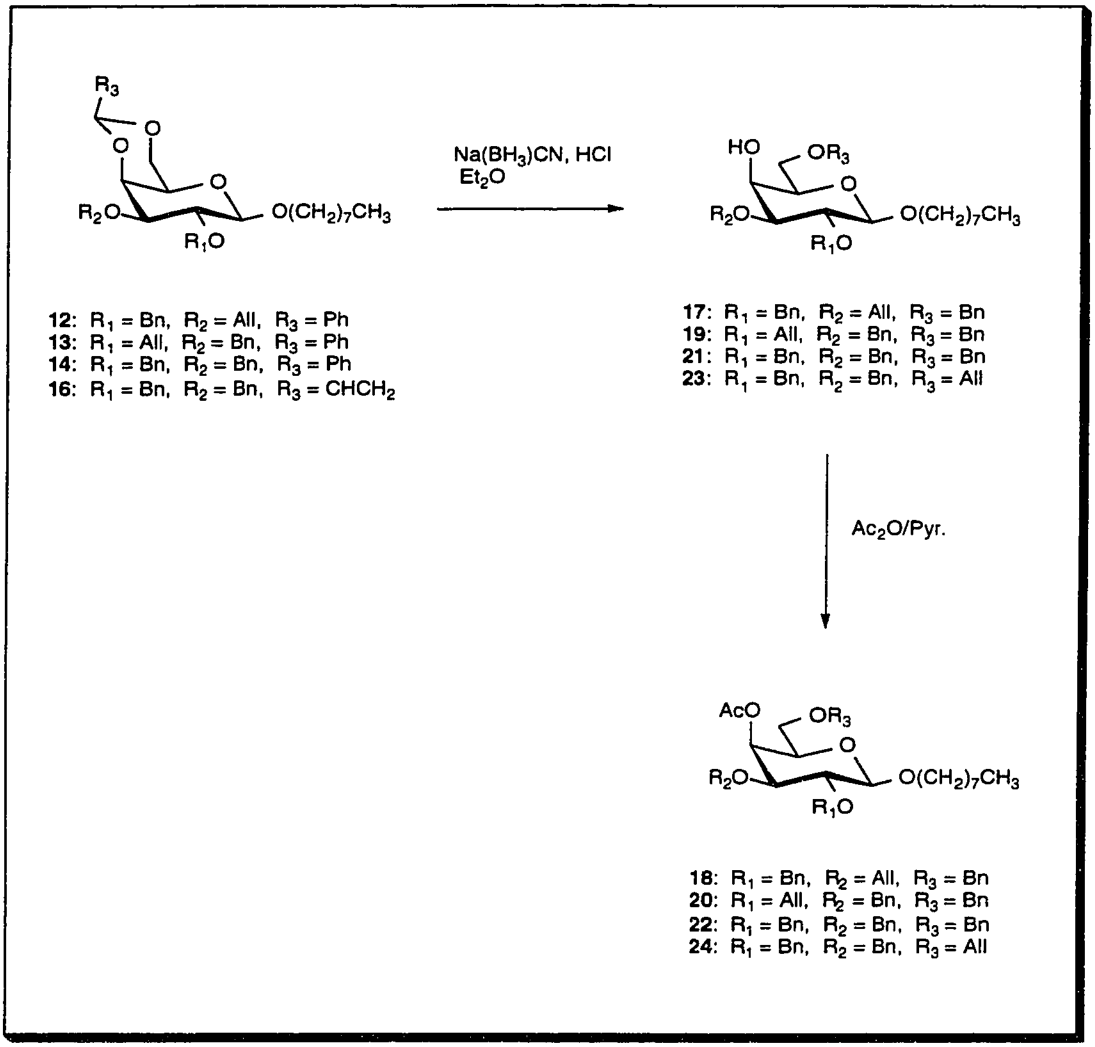
The synthesis of the native disaccharide **1**, the *O*-propyl derivatized disaccharides **5-7** and the *N*-propionamido modified disaccharide **8** started from the acetal **154** which was subjected to phase transfer alkylation conditions[150] using tetrabutylammonium hydrogen sulfate ($TBAHSO_4$) as catalyst. Depending on the concentration of the alkylating reagent and time, various amounts of the compounds **10**, **11** and **14** were obtained. Longer reaction times favored the formation of the dialkylated product **14**. Short reaction times led to significant amounts of **10** and **11** and unreacted starting material. Under these conditions compound **11** was usually isolated as the major product. The identities of **10** and **11** were established via acetylation giving rise to significant low field shifts (~1.5 ppm) of the ring proton on the carbon bearing the acetate group. Further alkylation of **10** and **11** gave the allyl ethers **12** and **13** in good yields. Similarly, the dibenzylated ether **14** was obtained from the starting acetal **154** in 95% yield (Figure 18). Figure 19 outlines the synthesis of the allyl acetal **16** a precursor for the synthesis of disaccharide **5** bearing a propyl side-chain at the 6-position of galactose.

Figure 19: Synthesis of the Allyl Acetal **16**:



Octyl β-D-galactopyranoside was treated with acrolein dimethyl acetal under acid catalyzed conditions to afford **15** which was further benzylated to give the allyl acetal **16** in 90 % yield. The regioselective ring opening of acetals **12, 13, 14**, and **16** was achieved by reduction with sodium cyanoborohydride under acidic conditions in ether as shown in Figure 20.

Figure 20: Synthesis of the Acceptors **17, 19, 21** and **23**:



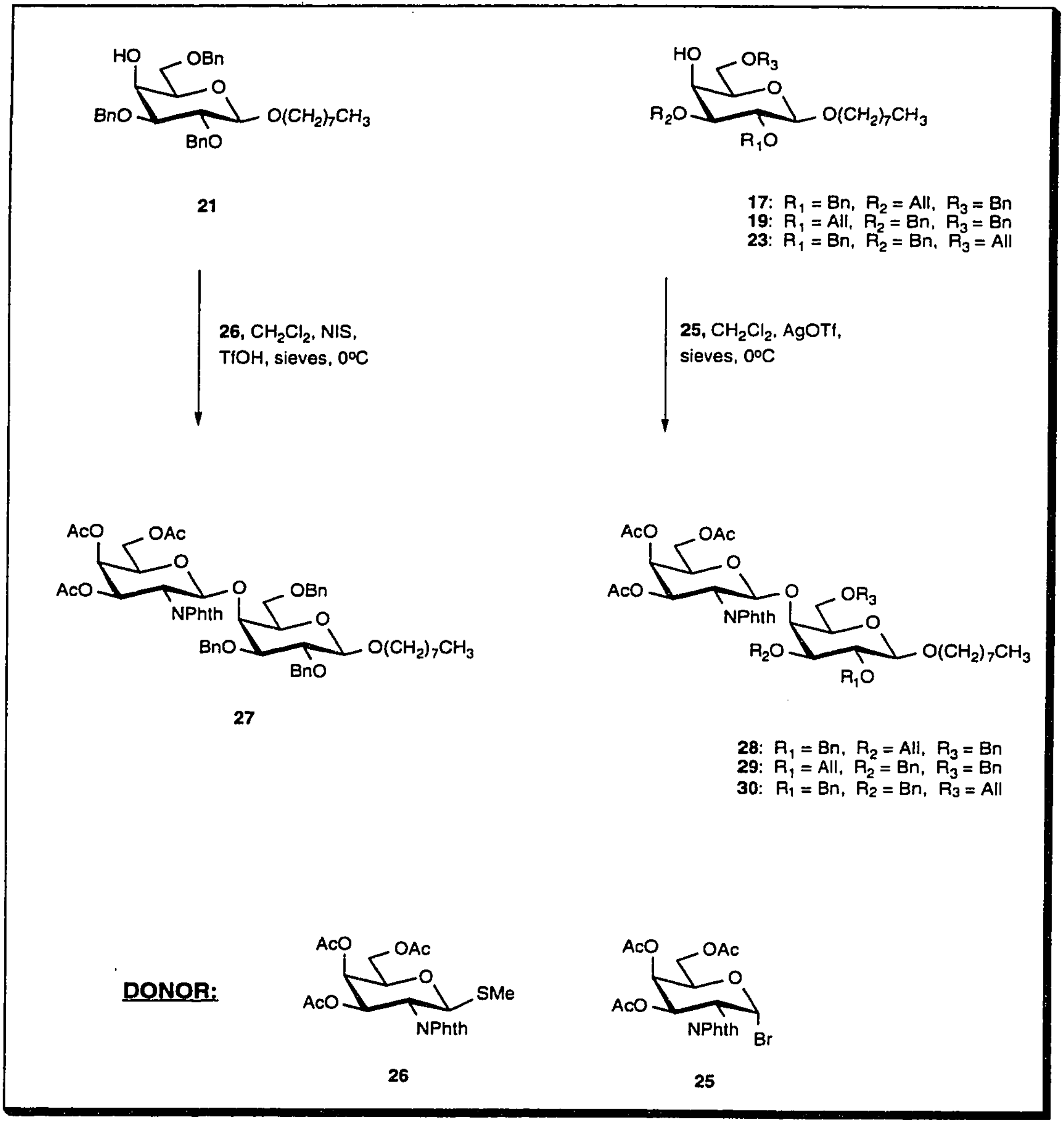
12: R_1 = Bn, R_2 = All, R_3 = Ph
13: R_1 = All, R_2 = Bn, R_3 = Ph
14: R_1 = Bn, R_2 = Bn, R_3 = Ph
16: R_1 = Bn, R_2 = Bn, R_3 = $CHCH_2$

17: R_1 = Bn, R_2 = All, R_3 = Bn
19: R_1 = All, R_2 = Bn, R_3 = Bn
21: R_1 = Bn, R_2 = Bn, R_3 = Bn
23: R_1 = Bn, R_2 = Bn, R_3 = All

18: R_1 = Bn, R_2 = All, R_3 = Bn
20: R_1 = All, R_2 = Bn, R_3 = Bn
22: R_1 = Bn, R_2 = Bn, R_3 = Bn
24: R_1 = Bn, R_2 = Bn, R_3 = All

In all cases the alcohols **17**, **19**, **21** and **23** bearing a hydroxyl function at the 4-position were obtained on average in 70% yield for all 4 species. The regioselective opening of the acetals was confirmed by acylation of the alcohols **17**, **19**, **21** and **23** to give the acetates **18**, **20**, **22** and **24**, whose ^{1}H NMR spectra were easily interpreted. The alcohols **17**, **19**, **21** and **23** were then glycosylated with the donors **25** and **26** shown in Figure 21.

Figure 21: Glycosylation of the Alcohols **17**, **19**, **21** and **23** with Donor **25** and **26**:



The alcohol **21** was glycosylated with the thio-donor **26** using *N*-iodosuccinimide (NIS)[152] as promoter to yield **27** (64%). Attempts to glycosylate the alcohols **17**, **19** and **23** with the thio-donor **26** failed and did not yield any desired disaccharides **28-30**. Presumably the presence of an allyl group in **17**, **19** and **23** interferes with the thioglycoside activation via formation of iodonium species. In contrast the use of the galactosyl bromide **25** gave the desired disaccharides **28-30** under silver triflate (AgOTf) promoted conditions in 45-78% yield. Deblocking of each of the disaccharides **27-30** proceeded in four steps, starting with Zemplén deacetylation (NaOMe/MeOH)[153], dephthaloylation using a mixture of butanol and ethylenediamine heated at 90°C for several hours[154] and selective *N*-acylation using acetic anhydride or propionic anhydride in methanol. Finally, the benzyl ethers were removed by hydrogenation with palladium on charcoal (Pd/C). These procedures gave the desired deblocked disaccharides **1**, **5**, **6**, **7** and **8** in 60-70% yield from the blocked disaccharides **27-30** (Figure 22).

The synthesis of the disaccharides **2-4** started from the three available methyl ethers **36-38**[#] (Figure 23). The removal of the trimethylsilylethyl group in **36-38** was achieved with a 50% solution of trifluoroacetic acid (TFA) in CH_2Cl_2. The resulting hemiacetals were then acylated with a mixture of pyridine and acetic anhydride to afford α/β mixtures of **39**, **41** and **43**. Treatment of the acetates **39**, **41** and **43** with trimethylsilyl trifluoromethanesulfonate and methylthiotrimethylsilane in CH_2Cl_2 gave the desired thiomethyl glycosides **40**, **42** and **44** in ~80-90% yield. The thiomethylglycosides were then used as donors in the glycosylation of alcohol **21** (Figure 24). In all cases NIS/ TfOH promoted conditions[152] were used to synthesize disaccharides **45-47**. The disaccharides **45** and **47** were obtained in good yields (~70%), whereas the disaccharide **46** was obtained in an excellent 88% yield.

[#] The methyl ethers **36-38** were synthesized by Mr. H. Jiao at the Department of Chemistry, University of Alberta

Figure 22: Deblocking of the Disaccharides **27**, **28**, **29** and **30**:

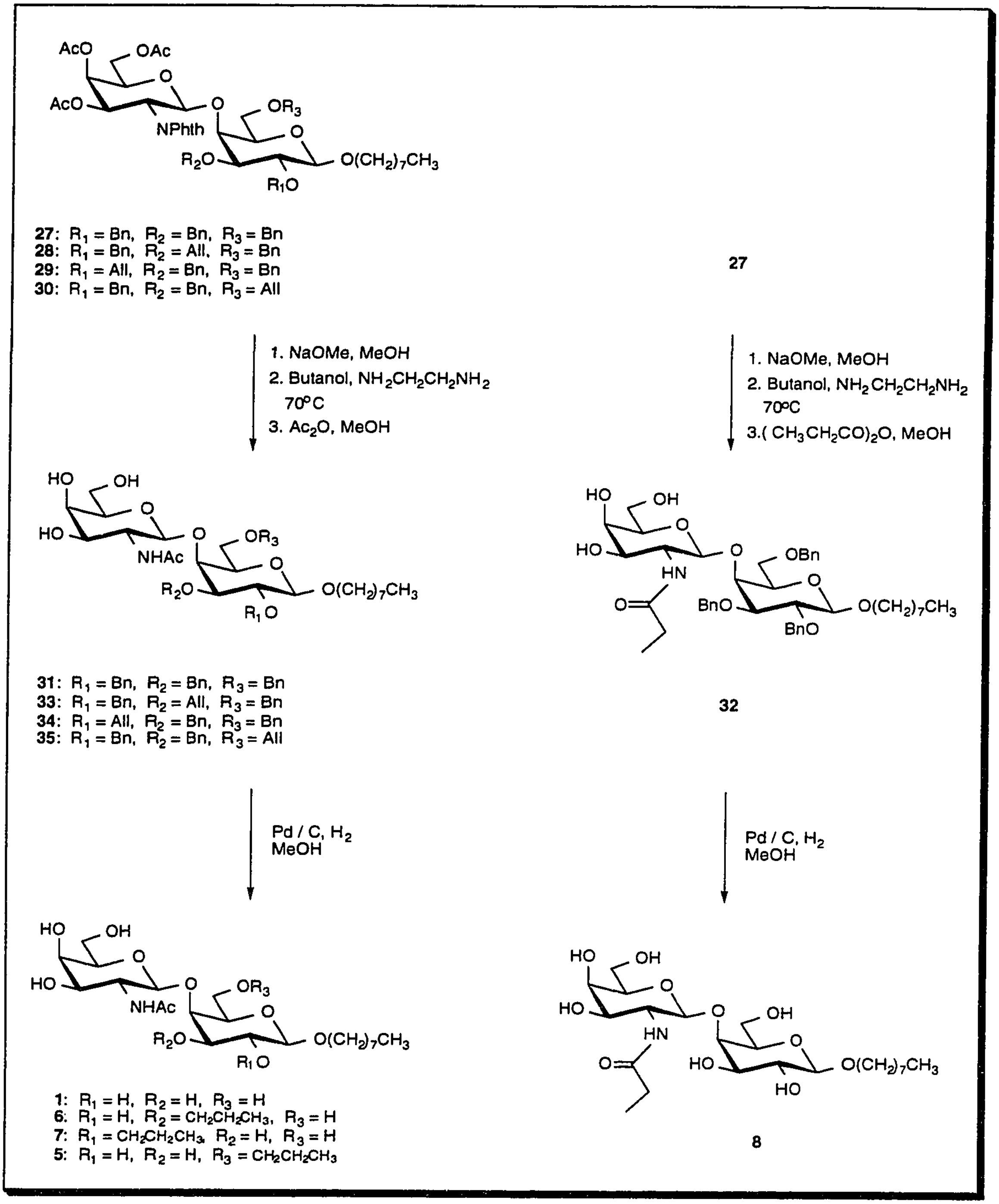


Figure 23: Synthesis of the Donors **40**, **42** and **44**:

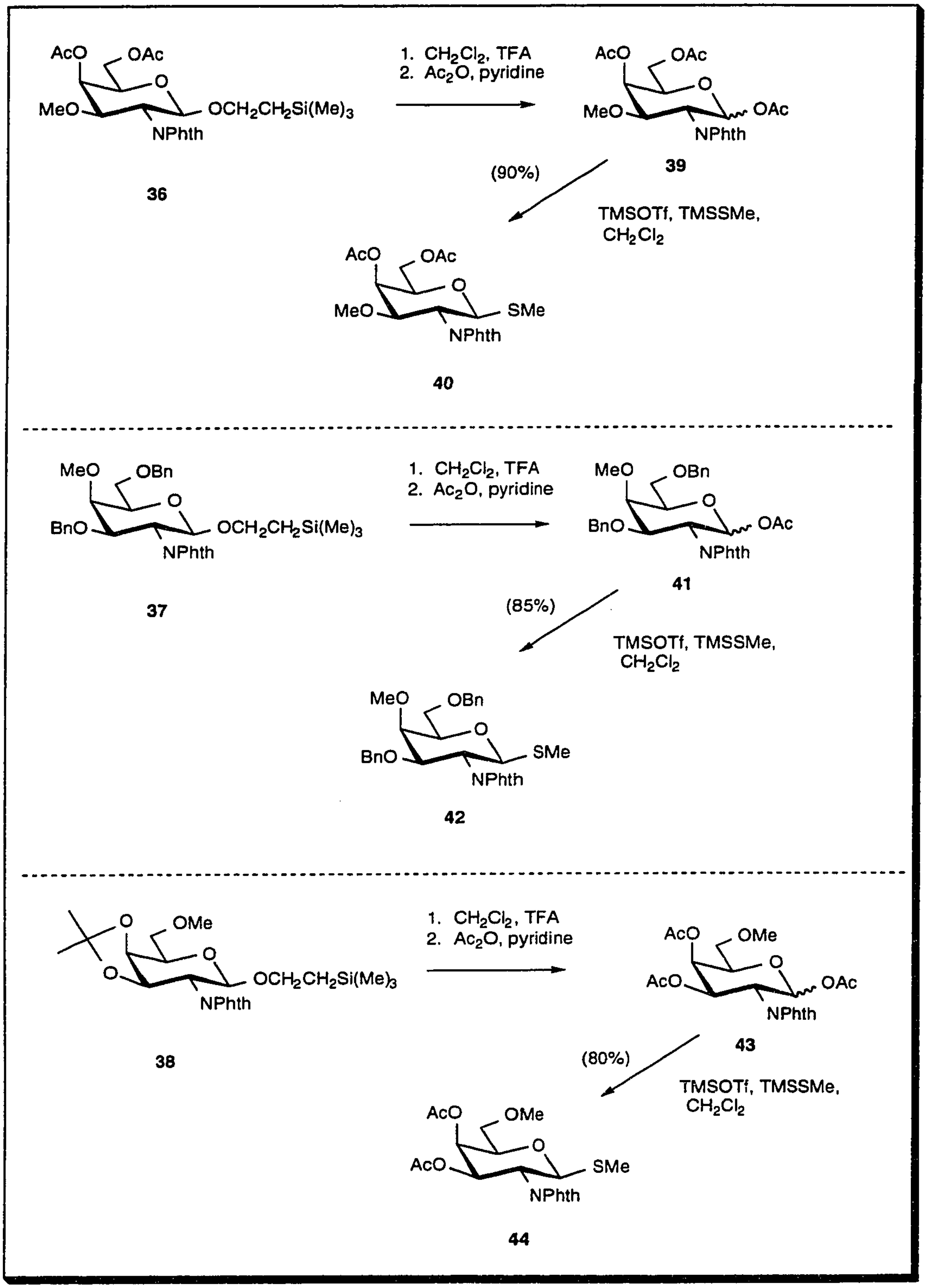
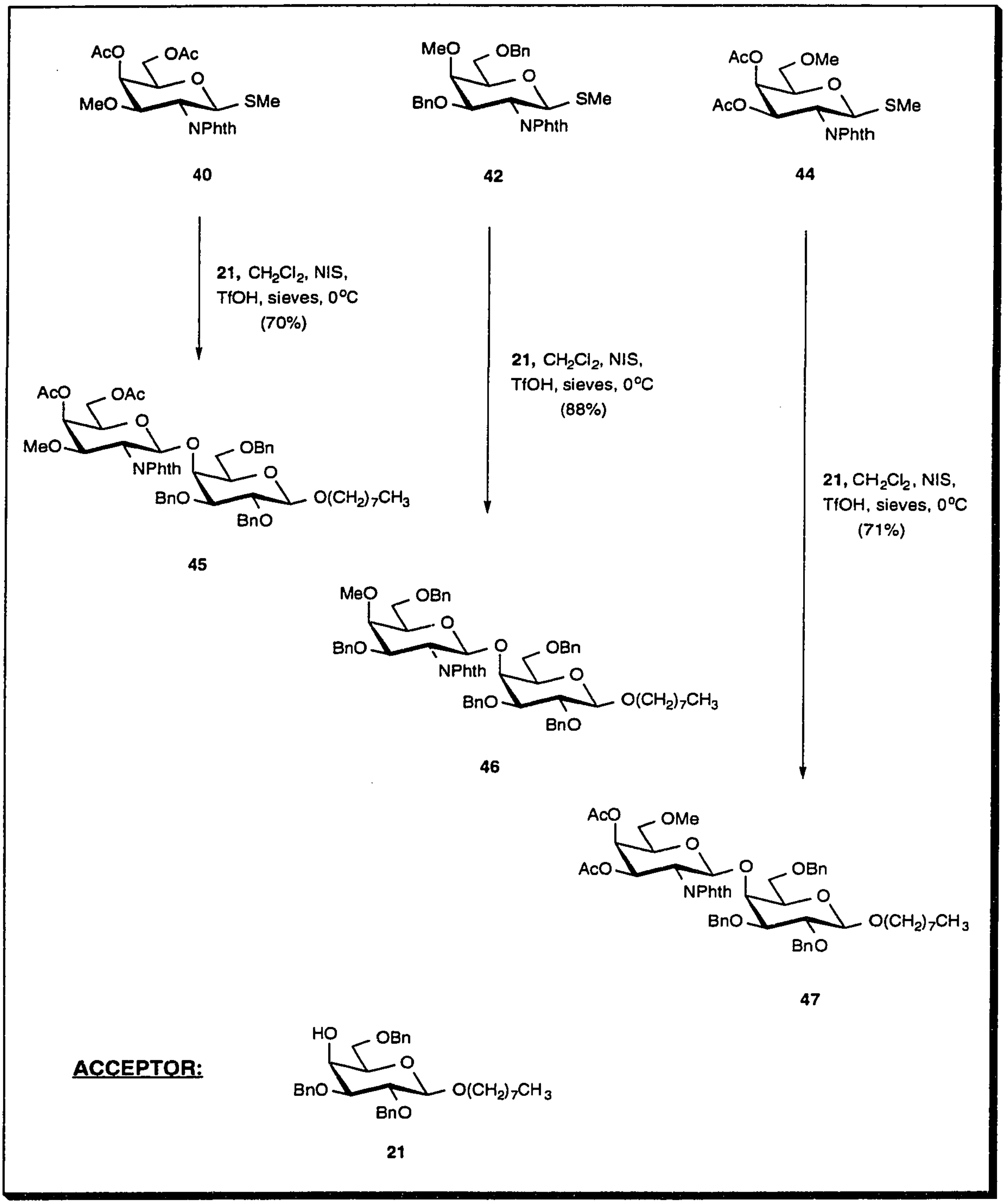
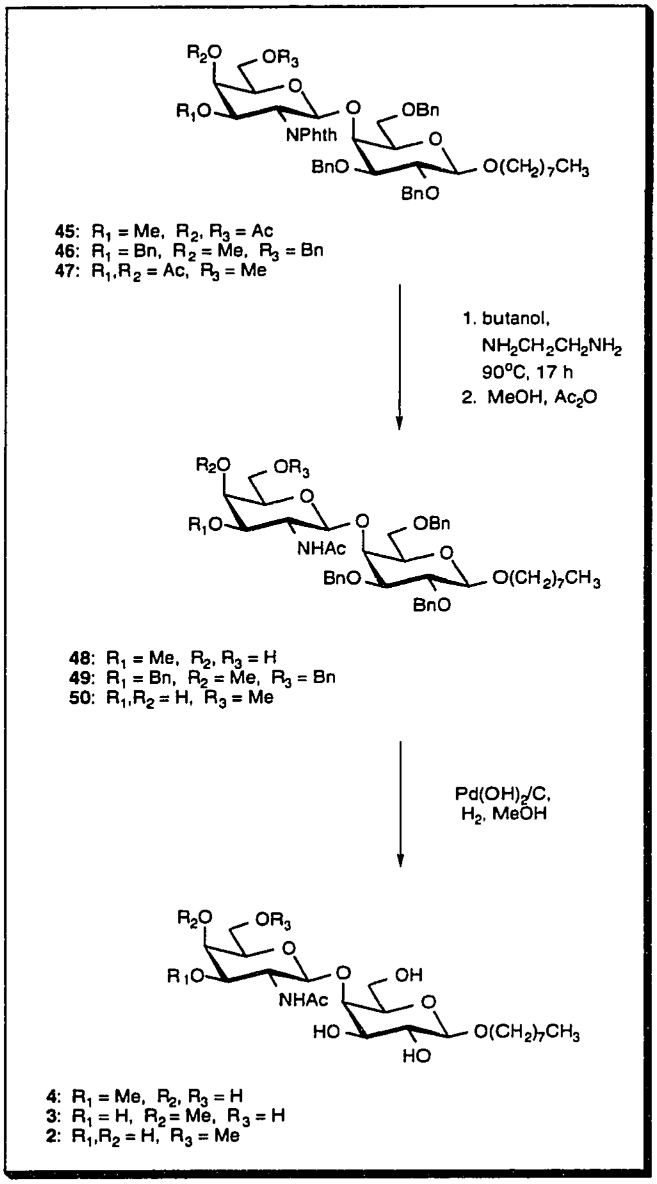


Figure 24: Synthesis of the Disaccharides **45**, **46** and **47**:



Deblocking of the disaccharides **45**-**47** (Figure 25) was achieved as previously described using a mixture of butanol and ethylenediamine for dephthaloylation[154] and *O*-deacetylation followed by selective *N*-acetylation with acetic anhydride in methanol. The benzyl ethers were removed by hydrogenation using Pearlman's catalyst[155].

Figure 25: Deblocking of the Disaccharides **45**-**47**:



E. Competitive Inhibition of *P. aeruginosa* PAK Pili Binding to Asialo-GM$_1$ with β-D-GalNAc-(1→4)-β-D-Gal Receptor Analogs:

The synthetic disaccharides **1-8** were evaluated using the competitive solid phase assay outlined in Figure 27. Microtitre plates were coated with the glycosphingolipid asialo-GM$_1$ which was used as a model cell surface receptor. Non-specific binding sites on the polystyrene microtitre plate were blocked with a high concentration of the protein bovine serum albumin (BSA). After a washing step, biotinylated *P. aeruginosa* PAK pili are incubated together with various concentrations of the synthetic disaccharide. Under these conditions the water soluble sugar analog competes with the immobilized asialo-GM$_1$ for the binding sites of PAK pili. The incubation is followed by a washing step before the conjugate streptavidine-alkaline phosphatase is added. The assay concludes with a third washing step and the addition of p-nitrophenylphosphate, a chromogenic substrate for the phosphatase. The release of p-nitrophenol is observed spectrophotometrically at 405 nm and leads to the determination of an IC$_{50}$ (concentration where 50% inhibition of PAK pili binding to asialo-GM$_1$ is observed). The direct binding of biotinylated *P. aeruginosa* PAK pili to asialo-GM$_1$ is concentration dependent as shown in Figure 26.

Figure 26: Direct Binding of *P. aeruginosa* PAK Pili to Asialo-GM$_1$:

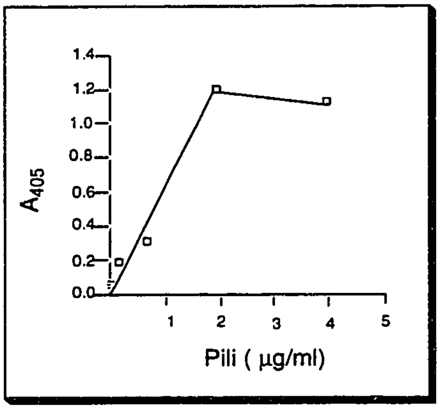
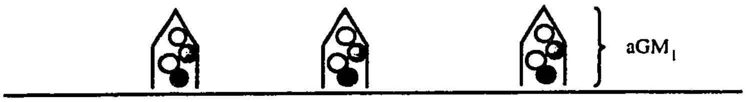
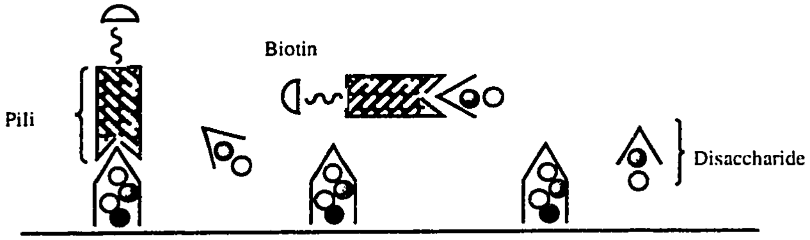


Figure 27: Competitive Solid Phase Assay Used to Determine IC_{50}-Values:

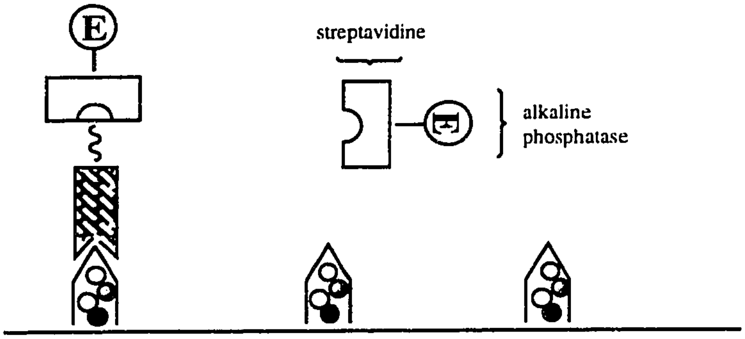
1. Adsorption of asialo-GM_1 on solid phase



2. Blocking with 5% BSA
3. Washing (4x) with 0.05% BSA in PBS buffer
4. Addition of *Pseudomonas aeruginosa* PAK pili (biotinylated) and β-D-GalNAc(1→4)β-D-Gal analog (various concentrations)



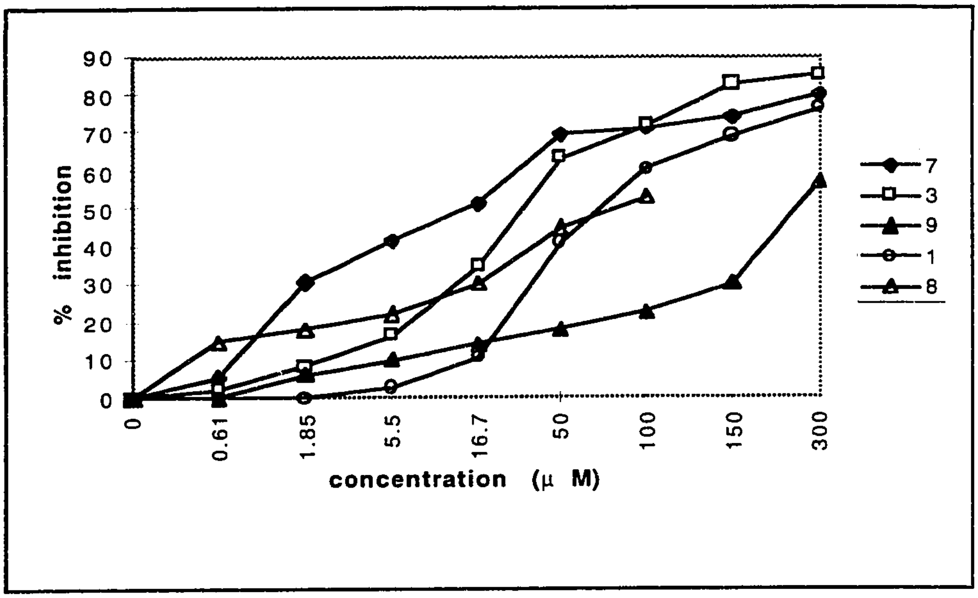
5. Washing (4x) with 0.05% BSA in PBS buffer
6. Addition of streptavidine-alkaline phosphatase conjugate



7. Washing (4x) with 0.05% BSA in PBS buffer
8. Addition of p-nitrophenyl phosphate
9. Determination of optical density (OD) at 405 nm
10. Comparison of OD (inhibited) with OD (non inhibited) leads to IC_{50}

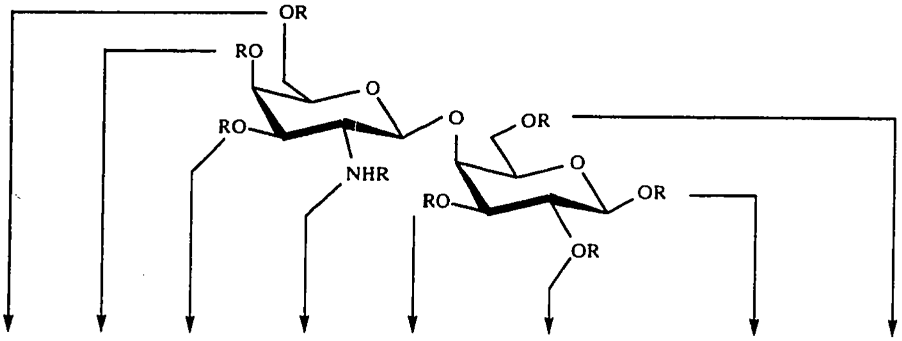
The disaccharides **1-9** as well as the monosaccharides **152** (the terminal monosaccharide of the natural ligand) and **153** (an unrelated negative control having an octyl aglycon) were used in the assay (Table 1). Typical inhibition curves for some of the compounds are shown in Figure 28. The IC_{50} values, relative inhibitory power and ΔΔG values are presented in Table 1.

Figure 28: Inhibition of *P. aeruginosa* Pili Binding to Asialo-GM$_1$ Using Varying Concentrations of Disaccharides **1**, **3**, **7**, **8** and **9**. The Results Are Expressed as the % Inhibition Resulting from the Addition of Compounds **1**, **3**, **7**, **8** and **9**:

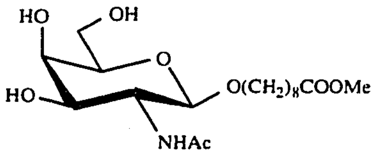


Very surprisingly, the data obtained demonstrate that all of the mono-*O*-alkylated sugar analogs bind with a similar order of magnitude (IC_{50} 60-130 μM). Substitution at the 6-position of Gal (**5**) and modification of the N-acetamido group (**8**) decreases the affinity (in both cases, ΔΔG = 1.2 kJ mol^{-1}). Most *O* -substituted analogs (**2**, **3**, **4** and **7**) are better inhibitors than the native disaccharide **1**, suggesting the presence of hydrophobic interactions in the carbohydrate-pilus complex. Compounds **7** (ΔΔG = - 5.7 kJ mol^{-1}) and

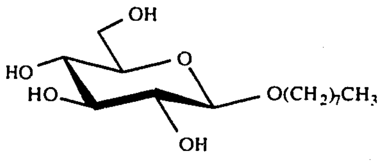
Table 1: Inhibition Results of the Compounds **1-9**, **152** and **153**:



1	R = H	H	H	$COCH_3$	H	H	$O(CH_2)_7CH_3$	H
2	R = OCH_3	H	H	$COCH_3$	H	H	$O(CH_2)_7CH_3$	H
3	R = H	OCH_3	H	$COCH_3$	H	H	$O(CH_2)_7CH_3$	H
4	R = H	H	OCH_3	$COCH_3$	H	H	$O(CH_2)_7CH_3$	H
5	R = H	H	H	$COCH_3$	H	H	$O(CH_2)_7CH_3$	$O(CH_2)_2CH_3$
6	R = H	H	H	$COCH_3$	$O(CH_2)_2CH_3$	H	$O(CH_2)_7CH_3$	H
7	R = H	H	H	$COCH_3$	H	$O(CH_2)_2CH_3$	$O(CH_2)_7CH_3$	H
8	R = H	H	H	$COCH_2CH_3$	H	H	$O(CH_2)_7CH_3$	H
9	R = H	H	H	$COCH_3$	H	H	$O(CH_2)_8COOCH_3$	H



152



153

cmp no.	IC $_{50}$ (μ M)	Inhib. Power	ΔΔG (kJ mol^{-1})
1	79 ± 18	100 %	0.0
2	56 ± 13	140 %	- 0.9
3	30 ± 7	260 %	- 2.4
4	65 ± 7	120 %	- 0.5
5	130 ± 36	60 %	1.2
6	98 ± 11	81 %	0.5
7	8 ± 4	990 %	- 5.7
8	129 ± 30	61 %	1.2
9	217 ± 32	35 %	2.5
152	246 ± 14	32 %	2.8
153	-	-	-

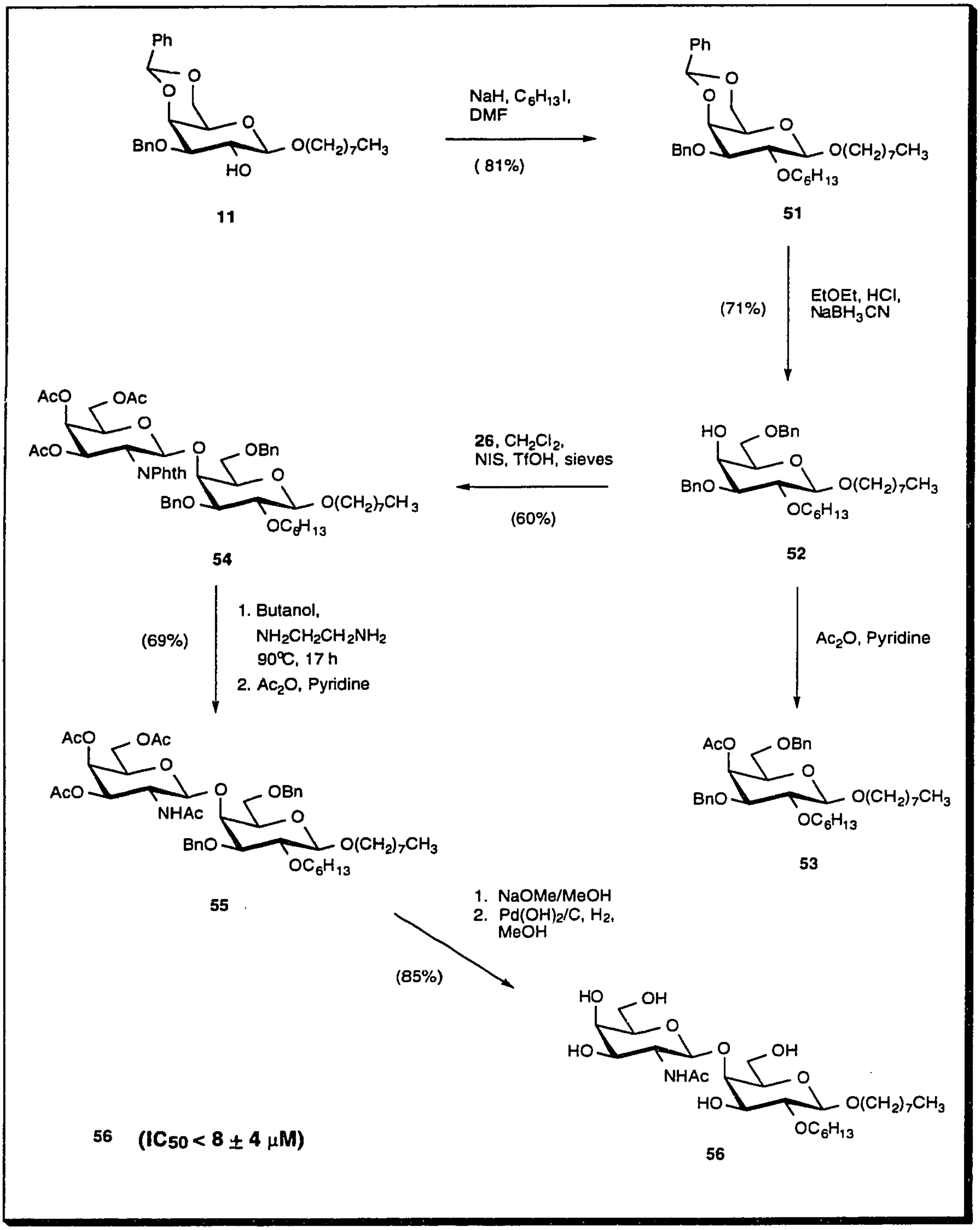
3 ($\Delta\Delta G = -2.4$ kJ mol^{-1}) are particularly noteworthy in that they show substantial binding enhancements.

The nature of the aglycon was also found to have a surprising effect on the binding. Compound **9**, carrying the 8-methoxycarbonyloctyl aglycon instead of the octyl group, is almost three fold less inhibitory than **1** ($\Delta\Delta G = 2.5$ kJ mol^{-1}), suggesting that even minor changes in the aglycon can perturb the interaction with the pili. This result is reminiscent of the work of Kihlberg et al .[145] who showed that structural modifications of the aglycon of galabiose changed significantly the affinity to the PapG adhesin of uropathogenic *E. coli* .

F. Synthesis of β-D-GalNAc-(1→4)-2-OR-β-D-Gal Analogs:

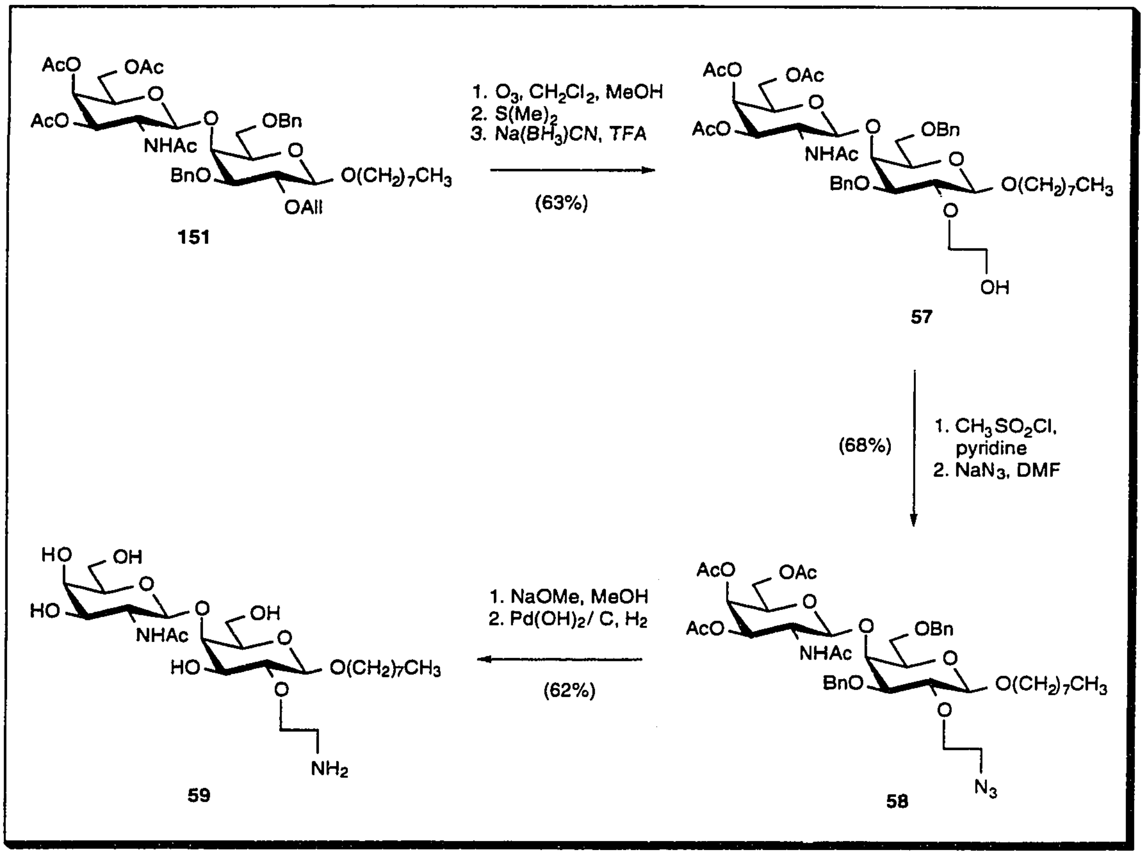
The inhibition results (part E, chapter 2) suggested that improved *P. aeruginosa* PAK pili inhibitors of adhesion may be prepared by introducing a hydrophobic side chain at the 2-position of galactose in the β-D-GalNAc-(1→4)-β-D-Gal receptor analog. In order to verify the results obtained with the 2-*O*-propyl modified disaccharide **7**, the disaccharide **56**, carrying a more hydrophobic hexyl side chain was synthesized and tested (Figure 29). The synthesis started with the previously synthesized alcohol **11** which was alkylated with 1-iodohexane in good yield. Regioselective reductive ring opening of **51** using $Na(BH_3)CN$ in HCl/ether gave **52** in 71% yield which was then glycosylated using NIS/TfOH[152] promoted conditions with the thioglycoside donor **26**. The disaccharide **54** was obtained in 60% yield. Dephthaloylation and acylation of **54** was achieved with ethylenediamine in butanol followed by treatment with acetic anhydride and pyridine. Finally, Zemplén deacetylation[153] and hydrogenation using Pearlman's catalyst[155] afforded the unblocked disaccharide **56**. Inhibition results of **56** showed an $IC_{50} < 8 \pm 4$ µM. Exact IC_{50} determinations were difficult to obtain due to the poor water solubility of **56**.

Figure 29: Synthesis of the Disaccharide **56**:



Other analogs with different side chains at the 2-position of galactose were synthesized from the allyl ether **151** which was available via acylation of the previously described disaccharide **34**. The allyl group was converted into an ethylamino group via a three step sequence outlined in Figure 30.

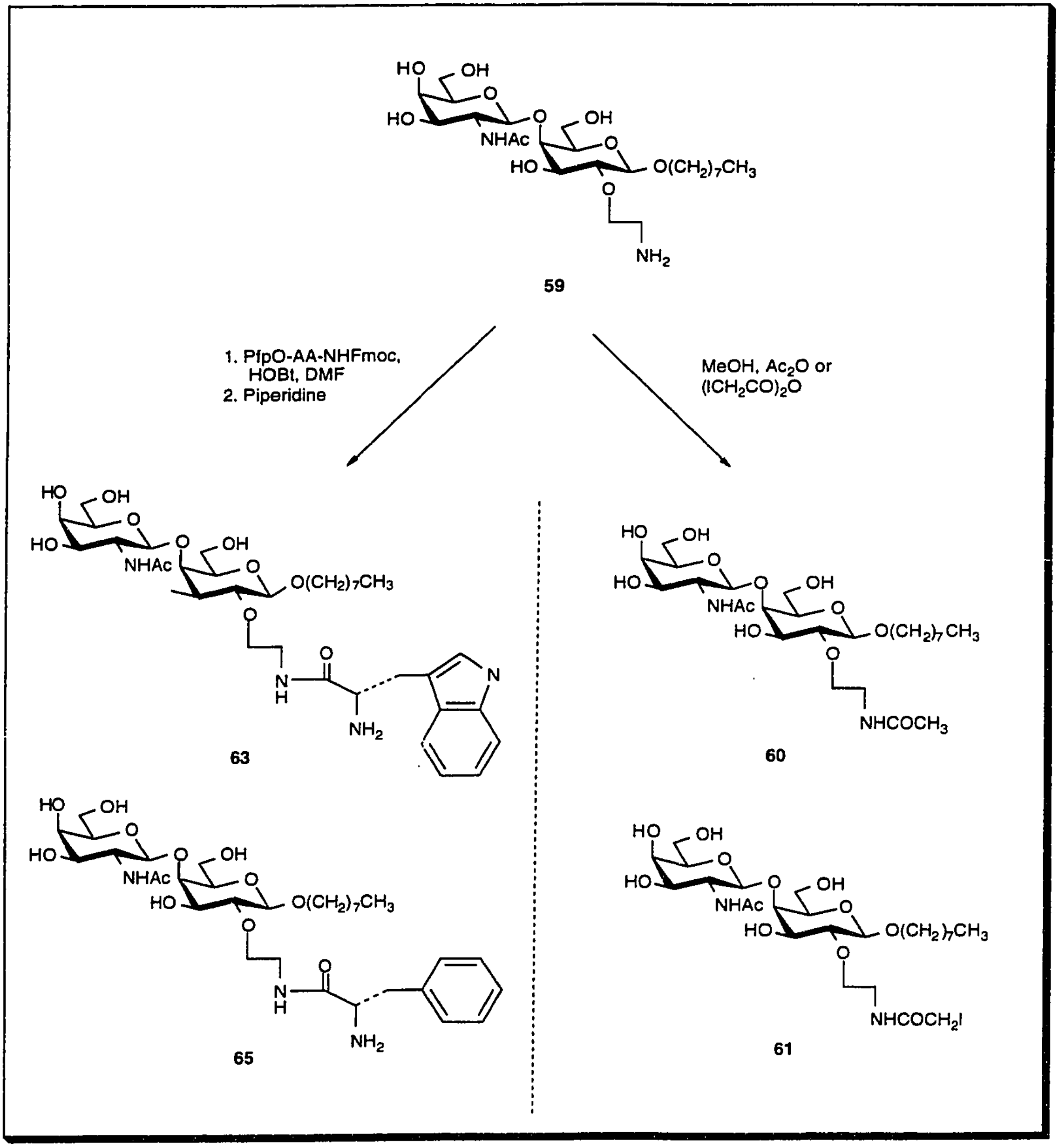
Figure 30: Synthesis of the Amino Functionalized Unblocked Disaccharide **59**:



Ozonolysis of the allyl ether **151** followed by reductive work up with dimethyl sulfide and reduction of the aldehyde with sodium cyanoborohydride under acidic conditions (TFA)[156] gave the alcohol **57** in 63% yield. Mesylation of the alcohol **56** in pyridine followed by nucleophilic displacement of the mesylate with sodium azide (NaN_3) produced the azido compound **58** in 68% yield. Zemplén deacetylation[153] and hydrogenation in the presence of Pearlman's catalyst[155] afforded the deblocked disaccharide **59** in 62% yield.

The amine **59** was acylated with a variety of reagents including the pentafluorophenyl (Pfp) activated and *N*-(9-fluorenylmethoxycarbonyl) (Fmoc) protected hydrophobic amino acid L-tryptophan and L-phenylalanine to produce, after removal of the Fmoc group, **63** and **65**, respectively[157]. Additionally, the amine **59** was acylated with acetic anhydride and iodoacetic anhydride to afford compounds **60** and **61**, respectively (Figure 31). Although compound **61** is a potential alkylating agent no (irreversible) inhibition was observed during the pili-binding assays. The hydrophobic amines **63** and **65** did not show any inhibition but rather showed high level of "negative inhibition" during the assay. That means strongly increased optical density readings were observed relative to the negative control. The reason for this is not clear but it can be speculated that **61** and **62** lead to the disassembly of the pili[158] into its pilin subunits which then adhere non-specifically to the microtitre surface. The increased readings in optical density would then be the result of more superficially exposed biotin units.

Figure 31: Acylation of the Amine **59** with Various Acylating Reagents:

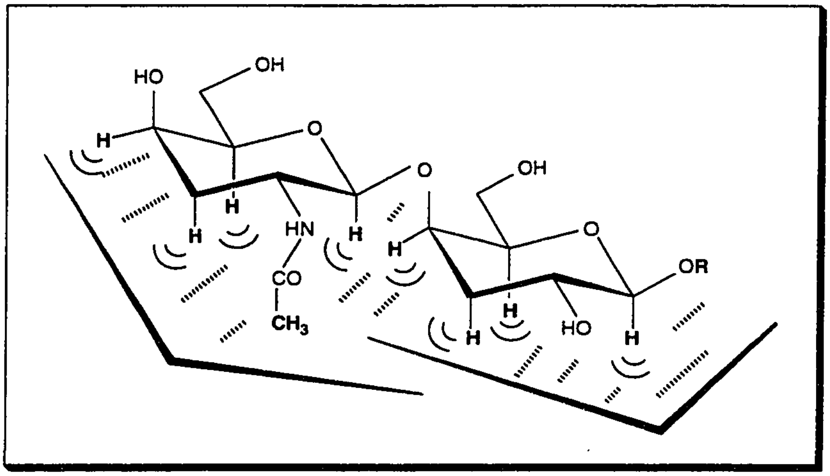


D. Conclusions:

The inhibition results indicate that improved inhibitors of adhesion may be prepared by attaching hydrophobic side chains on the disaccharide skeleton. In particular, the 4-position of GalNAc (**3**) and the 2-position of Gal (**7**, **56**) are noteworthy in that they show substantial binding enhancements. Rather surprising is the fact that most mono-*O*-alkylated sugar analogs bind with a similar order of magnitude ($IC_{50} = 60\text{-}130\ \mu M$) suggesting the absence of any "key-polar groups" that are deeply buried in the protein combining site. Only substitution at the 6-position of Gal (**5**) and modification of the *N*-acetamido group (**8**) decreases the affinity (in both cases, $\Delta\Delta G = 1.2$ kJ mol^{-1}). The relative loss of binding affinity, however is too weak to correlate with the loss of one uncharged hydrogen bond, expected to be in the range of 2.1 - 6.3 kJ mol^{-1} [159].

It can be speculated that the higher binding affinity of **7** might be related to the small distance between the hydrophobic side-chain and the hydrophobic octyl aglycon, forming a strong lipophilic region in the disaccharide. On the basis of these results, it seems likely that the reducing-end Gal-residue is involved in hydrophobic interactions with the pili. The fact that all *O*- and *N*-substituted analogs are accepted with similar affinities suggests the absence of a tight binding pocket. It therefore seems likely that water molecules are involved to modulate the interaction between sugar and protein. Substitution of a hydroxyl group with a more bulky methoxy or propoxy group would not cause a dramatic change on the binding affinity and would only affect the water order around the sugar-protein interface. The structural key recognition element would therefore be the hydrophobic backbone of the disaccharide receptor, contributing more to the affinity and specificity than those of the individual hydroxyl groups. A scheme of the proposed recognition of β-D-GalNAc-(1→4)-β-D-Gal in the pilus combining site is shown in Figure 32.

Figure 32: Hydrophobic Face of the Sugar Receptor that May Interact with the PAK Pili of *P. aeruginosa*. Some of the Hydroxyl Groups Are Omitted:



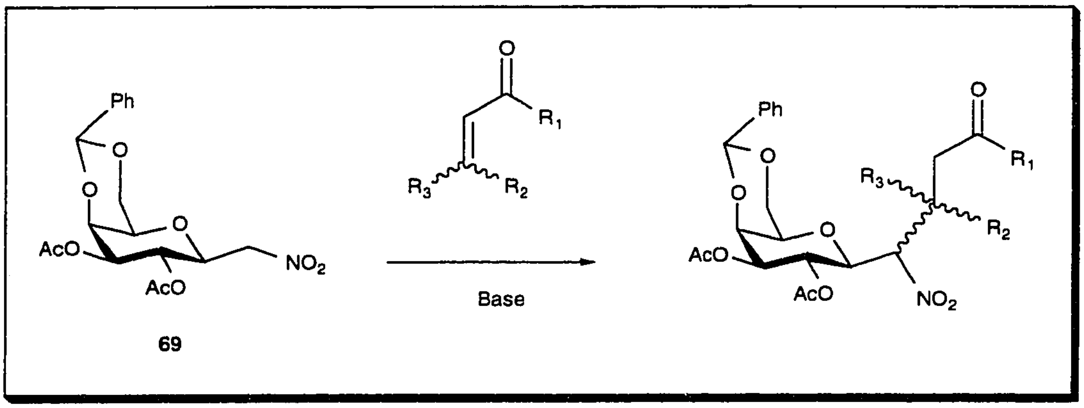
CHAPTER 3

Synthesis of Glycomimetic Probes for the Study of Galactose Binding Proteins:

Molecular recognition of carbohydrates by proteins plays an important role in many intracellular and intercellular processes, such as the trafficking of proteins, bacterial and viral infection, normal cell differentiation, tumor progression and metastasis[1]. The specificity and affinity of these interactions are usually achieved through hydrogen bonds with added contributions from van der Waals contacts and stacking interactions of aromatic residues against the hydrophobic regions of the sugar[36]. The typical affinity of carbohydrates binding to proteins is generally weak in the range of 10^3 - 10^6 M^{-1} [45] which greatly reduces their potential as drug candidates. X-Ray crystallographic studies of several carbohydrate-protein complexes have shown that usually one to three sugar units and a small number of hydroxyl groups (key polar groups) in the carbohydrate molecule are involved in essential hydrogen bonds with the protein[36]. Consequently, a significant proportion of the sugar molecule may be found at the interface of protein and solvent. Most monosaccharides bind to proteins via the non-reducing end, allowing the protein to distinguish, for example, between the C-4 epimers galactose and glucose[160]. Replacement of the "key polar groups" by hydrogen, halogen, amino or methoxy groups often leads to less active compounds and does not appear to be a promising route for drug design[37]. The remaining hydroxyl groups at the periphery of the binding site are normally solvent exposed and have little effect on the stability of the complex[51]. However, hydrophobic interactions at the periphery of the carbohydrate-protein binding site have been suggested to lead to substantial (10 to 1000-fold) binding enhancements[53,160,161]. C-Glycosides with increased hydrophobic side chains might therefore be promising target molecules to enhance the binding affinities for carbohydrate-binding proteins. In order to generate a collection of highly diverse C-glycosides, C-glycosyl nitroalkanes were chosen as Michael donors for 1,4 conjugate additions (Michael addition).

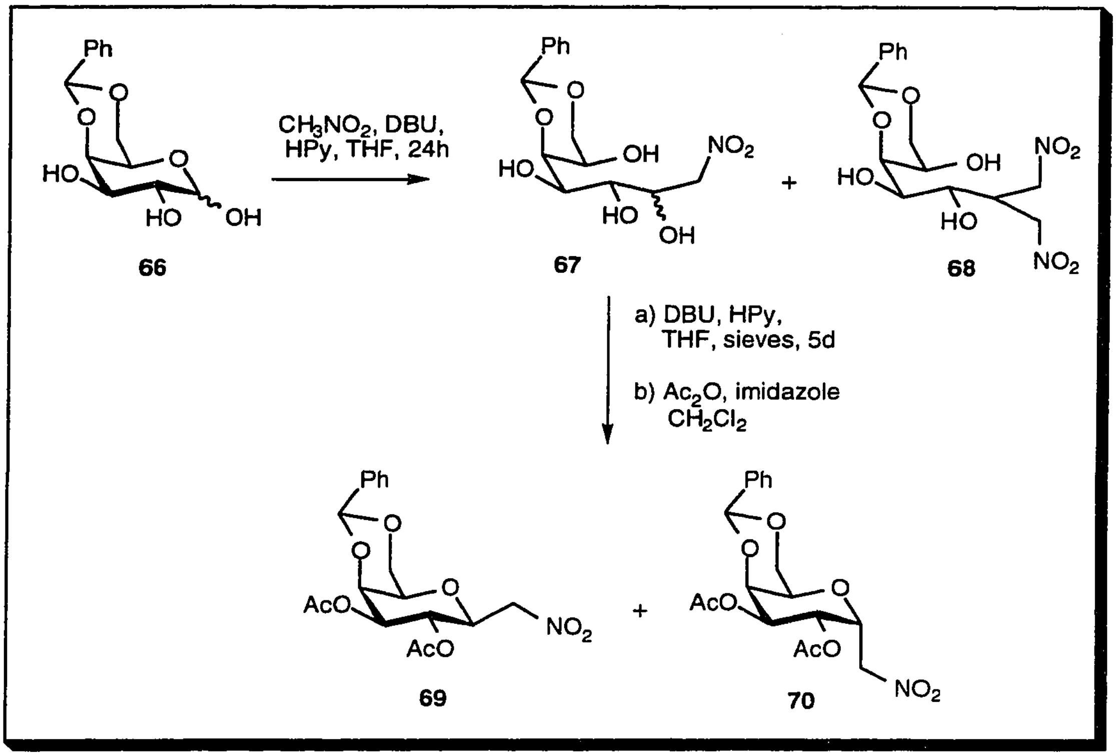
A. Use of 3,4-di-*O*-Acetyl-2,6-anhydro-1,3-benzylidine-7-deoxy-7-nitro-D-*glycero*-L-*Manno*-heptitol (69) in Michael Additions and Alkylation Reactions

Figure 33: C-Glycosyl Nitroalkanes as Potential Nucleophiles in Michael Additions:



Nitroalkanes have been widely used as highly stabilized carbanions in 1,4-conjugate additions[162,163]. In carbohydrate chemistry, addition of nitromethane ($pK_a = 10.2$) to aldoses has been used as a method for extending the carbon chain of aldoses[164,165]. It was envisaged that **69** could be used as a carbanion precursor to react with a variety of Michael acceptors to generate C-glycosyl compounds (Figure 33). The synthesis of **69** and its isomer **70** is shown in Figure 34.

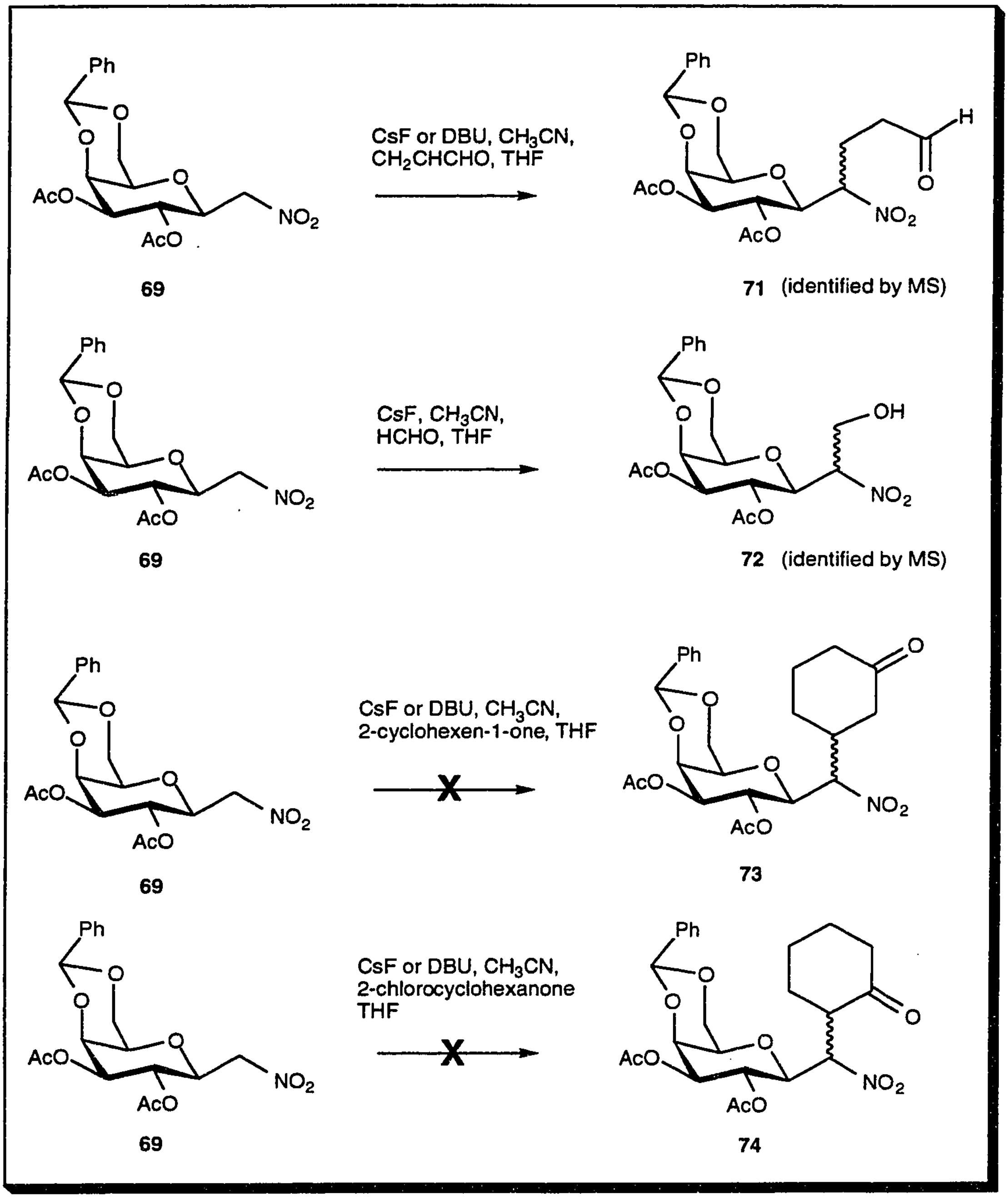
Figure 34: Synthesis of the Nitro Compounds **69** and **70**:



The synthesis of the nitro compound **69** and **70** started from the acetal **66**[166]. Addition of nitromethane to **66** catalyzed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 2-hydroxypyridine (HPy), previously applied to glucose[167] gave the nitro adducts **67** and **68** in 62% and 15% yield, respectively after 24h. Prolonged reaction time gave substantially increased amounts of the dinitro adduct **68**. Removal of nitromethane and extended exposure to HPy, DBU and molecular sieves for 5 days followed by acetylation using a mixture of acetic anhydride and imidazole produced the C-Glycosides **69** and **70** in 45% yield (ratio **69/70** : 5/1). Attempts to use nitroacetate, nitroethanol or THP protected nitroethanol instead of nitromethane failed to furnish any C-Glycoside. The deprotonated C-glycoside **69** (CsF or DBU) was then treated with a variety of Michael acceptors like methyl vinylketone, 2-cyclohexen-1-one or the simple electrophile formaldehyde and an alkylating reagent (α-haloketone: 2-chlorocyclohexan-1-one) (Figure 35). Only in the case of methylvinylketone and formaldehyde were products identified which corresponded (MS) to the desired products **71** and **72**. In both cases complex mixtures were obtained resulting from dialkylation and aldol condensations. More sterically demanding Michael acceptors like 2-cyclohexenone did not react at all even after extended time and large excess of reactants. Only the starting material **69** was isolated after chromatographic purification. Similarly, alkylation of **69** with of 2-chlorocyclohexanone did not occur.

The intrinsic low reactivity of the nitro Michael donor **69** does not allow Michael additions to sterically demanding Michael acceptors. In the case of unhindered Michael acceptors, complex mixtures (dialkylation, aldol condensations) were obtained. As a result, the use of the nitro C-glycosyl compound was not further pursued.

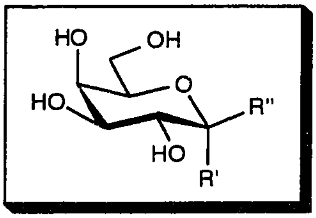
Figure 35: Attempted Conjugate Addition and Alkylation of **69**:



B. Synthesis of α,α-Disubstituted Pyrans:

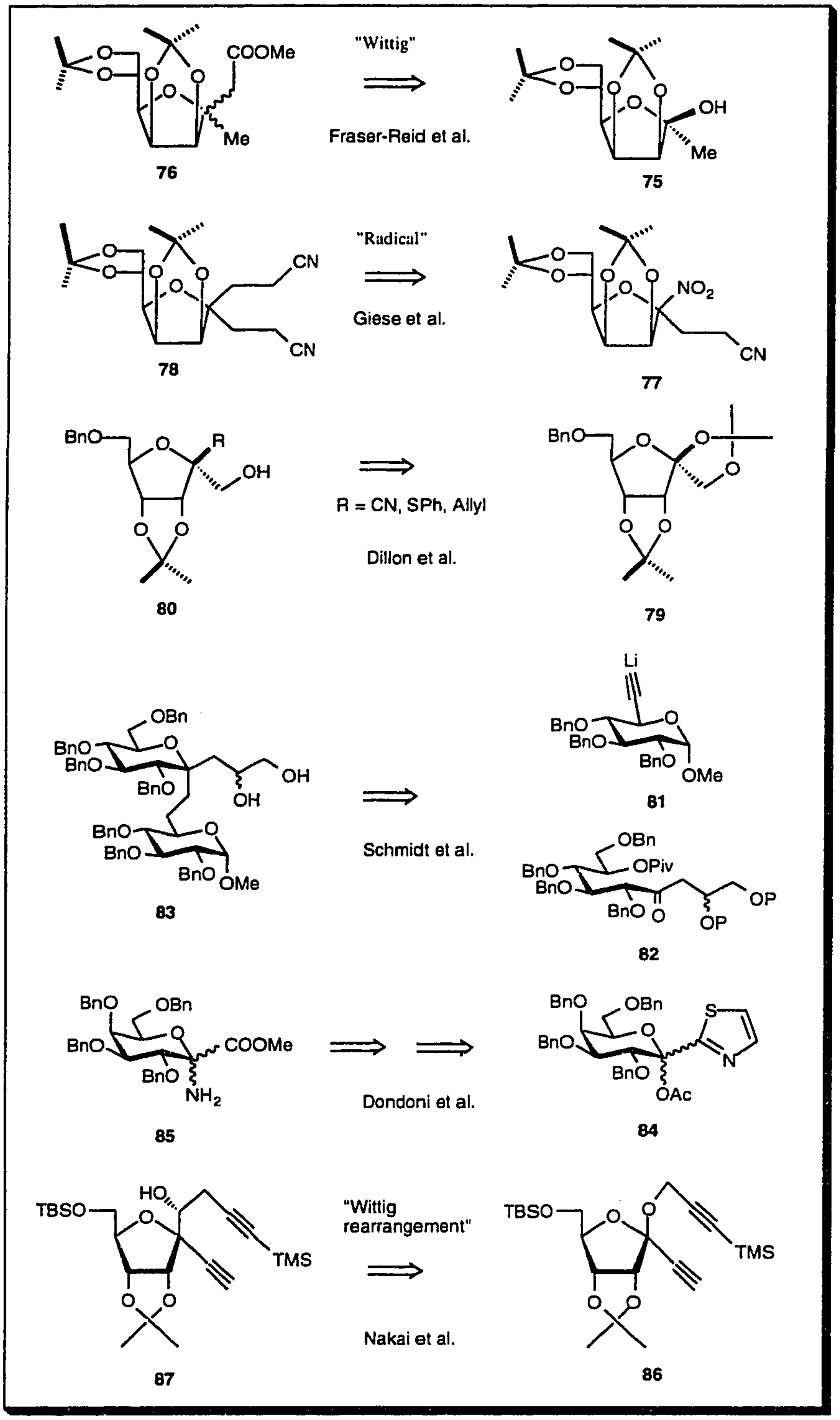
Disubstituted α,α-pyrans (Figure 36) project functional groups in both α-(R') and β-(R") directions and, thus display unique glycomimetic features. Provided that the side chains are appropriately functionalized, a variety of novel glycomimetics can be synthesized and used as molecular probes to study the combining site of carbohydrate-binding proteins. Attachment of hydrophobic side chains on both side chains could lead to increased van der Waals contacts at the periphery of the combining site while enhancing the overall affinity. This is of particular interest since many sugar binding proteins (with the exception of glycosidases) recognize monosaccharidic α- or β-glycosides equally[160].

Figure 36: Novel α,α-Disubstituted Pyrans as Molecular Probes for Carbohydrate Binding Proteins. R' and R" Represent Side Chains which May Enhance the Binding Affinity with a Protein at the Periphery of the Sugar-Protein Combining Site:



Several attempts to synthesize α,α-disubstituted pyrans and furans have been reported in the literature and are summarized in Figure 37. The first sugar derived α,α-disubstituted pyran **76** was described by Fraser Reid[168] in 1980 who used a Wittig reaction to introduce a second side chain on the ketone **75**. The nitro compound **77** was used by Giese[169] as a radical precursor which was successfully added to the Michael acceptor acrylonitrile to give the α,α-disubstituted pyran **78**. Dillon[170] succeeded to open the spiroacetal **79** with various tin based electrophiles under trimethylsilyl trifluoromethanesulfonate (TMSOTf) catalyzed conditions to produce **80**. The "1,6"-linked C-disaccharide **83** was synthesized

Figure 37: Known Syntheses of α,α-Disubstituted Pyrans and Furans:



by Schmidt[171] employing the acetylide **81** and the ketone **82**. Dondoni[172] used the thiazole based C-glycoside **84** as a precursor in the synthesis of the double branched amino pyran **85**. The amino group was introduced with trimethylsilylazide ($TMSN_3$) under TMSOTf catalyzed conditions. Recently, Nakai[173] described a novel [1,2]-Wittig rearrangement which yielded **87** starting from the O-Glycoside **86**.

B.1 Attempted Addition of Nitromethane to the Hemiacetals 89 and 91:

The hemiacetals **89** and **91** (Figure 39) were chosen as test compounds for the addition of nitromethane. Both compounds possess additional functionalities in their side chains (ester or aldehyde) which could potentially be used for further reactions. An added nitro group could in principle be reduced to an amine to permit further functionalization. The initial step for the nitromethane addition should be the ring opening of the hemiacetal to the ketone. It was expected that the ring opening of **89** would be complicated due to the presence of a potential 6-membered hydrogen bond between the hydroxyl group and the carbonyl group (shown in Figure 39). Compound **91** is devoid of this hydrogen bond and was used for comparison. Compound **89** was synthesized by addition of *tert-* butylacetate enolate to the known lactone **88**[172] in 84% yield. Analogously, the hemiacetal **91** was obtained via addition of the lithium salt of propiolaldehyde diethyl acetal to the lactone **88** in 75% yield (Figure 38). Unfortunately, attempts to add nitromethane to both acetals using the same conditions previously applied in the synthesis of the nitro compounds **69** and **70** did not yield any addition products even after extended reaction times (4 d) (Figure 39). In both cases, only unreacted starting material was recovered in nearly unchanged amounts. Interestingly, addition of thiocresolate (50 equiv.) to the double conjugated acetal **92** gave only mono addition products with a high regioselectivity. The major product **93** resulting from an attack of the sulphur nucleophile α to the aldehyde function, was isolated in 50% isolated yield (Figure 40). Presumably, product **93** is the thermodynamically favoured product assuming reversible Michael addition conditions.

Figure 38: Synthesis of Hemiacetals **89** and **91**:

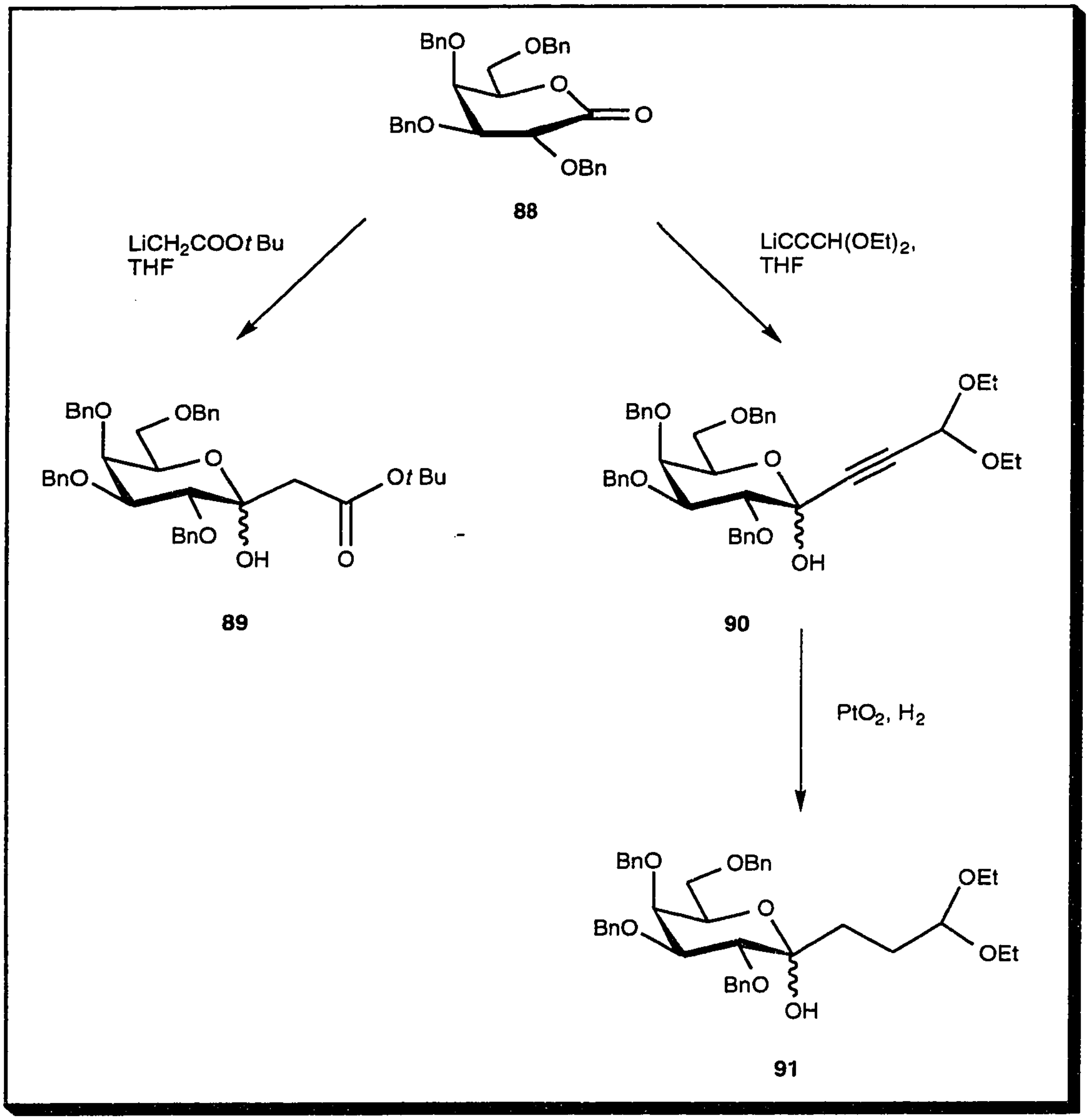


Figure 39: Attempted Nitromethane Addition to Compounds **89** and **91**:

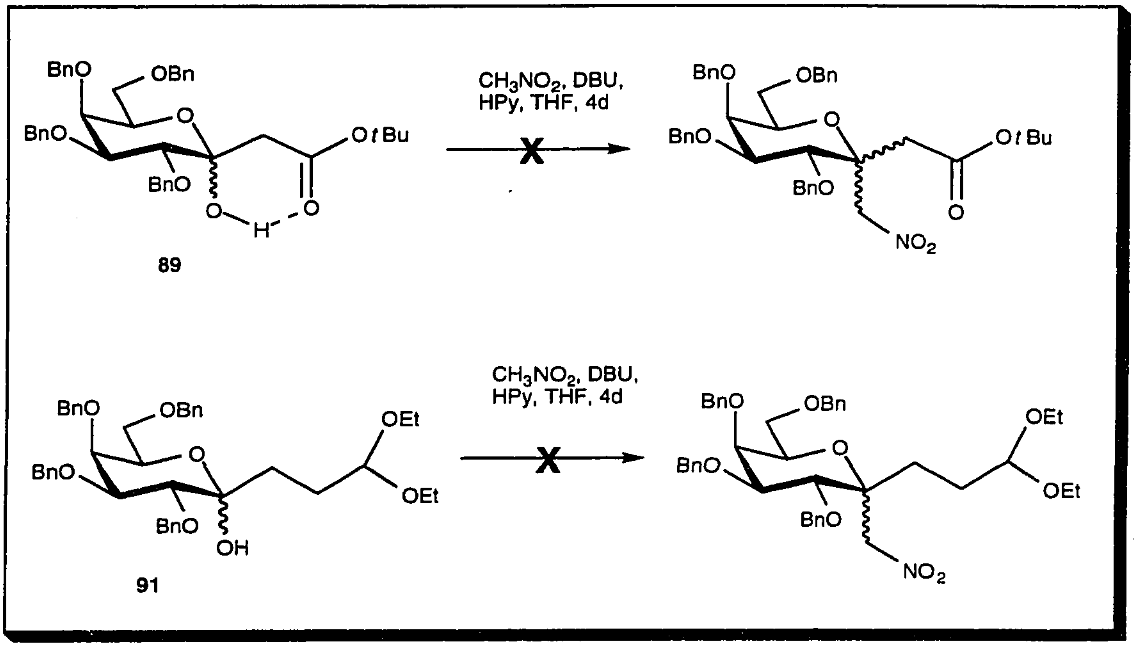
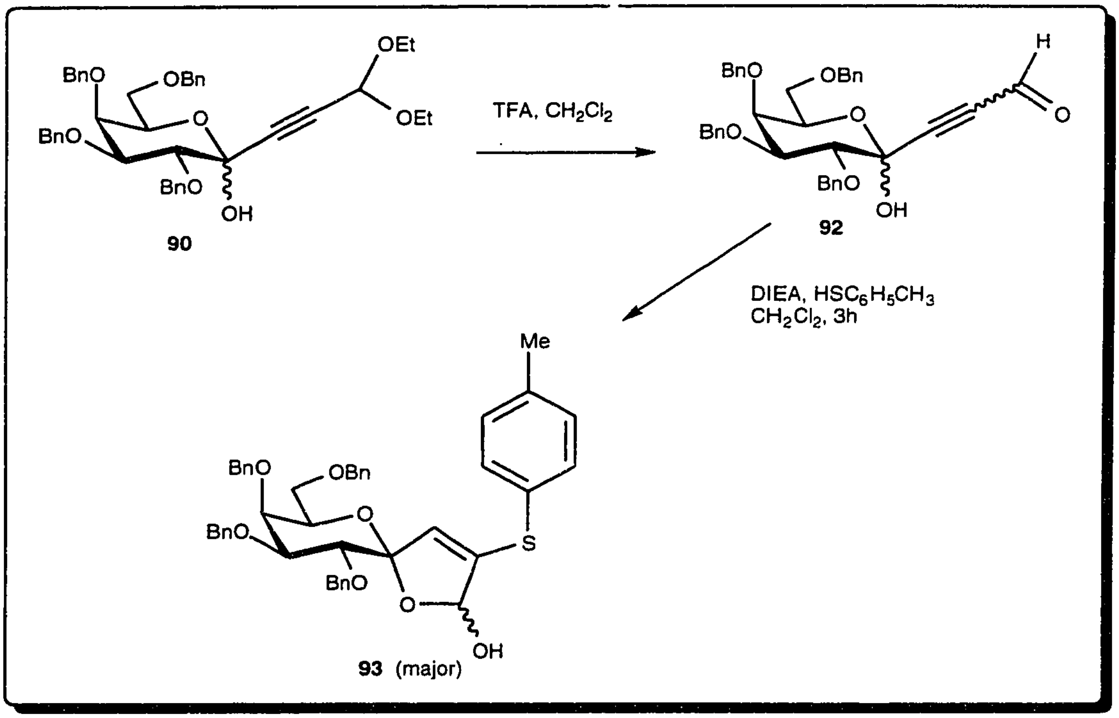


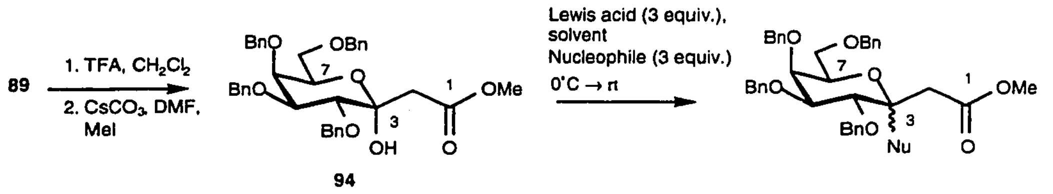
Figure 40: Attempted Addition of a Sulphur Nucleophile to the Michael System **92**:



B.2 C-, S- and O-Glycosylation of the Acetal **94**:

The previously synthesized acetal **89** with its ester function appeared to be a promising starting material for introducing the second side chain (R' in Figure 36). The protected acid function is linked through an one carbon tether to the pyran to ensure steric accessibility for further coupling. Due to the acidic instability of the *tert* -butyl group, **89** was converted into the methyl ester **94** which was then treated with a variety of nucleophiles (3 equiv.) including allyltrimethylsilane (TMSAll), trimethylsilyl azide ($TMSN_3$), trimethylsilyl cyanide (TMSCN), trimethylsilylurea ($TMSNHCONH_2$), trimethyl(trifluoromethyl)silane ($TMSCF_3$), trimethylsilylacetonitrile ($TMSCH_2CN$), methyl trimethylsilyl acetate ($TMSCH_2COOMe$), trimethylsilyl isocyanate (TMSNCO), thiophenol (HSPh), thiolacetic acid (CH_3COSH) and methanol in the presence of a Lewis acid (TMSOTf or BF_3 etherate, 3 equiv.) as outlined in Table 2. Decreased amounts of Lewis acid resulted in prolonged reaction times but did not affect the stereochemistry of the products. Surprisingly, the silyl derived nucleophiles like allyl, cyano and azido as well the *S*-nucleophiles thiophenol and thioacetate and the *O*-nucleophile methanol could be directly introduced into the acetal **94** with high α-selectivity. Only the reactions with CH_3COSH and TMSCN led to observable amounts of β-product. The participation of the solvent[174] and the nature of the Lewis acid have little effect as the reactions with (TMSAll) in dichloromethane or acetonitrile under TMSOTf or BF_3 etherate promoted conditions to yield **95**, demonstrate (Table 2). The reactions with $TMSNHCONH_2$ and $TMSCF_3$ gave only the elimination product **98**. No reaction was observed with $TMSCH_2CN$, $TMSCH_2COOMe$ and TMSNCO under TMSOTf promoted conditions. Finally, the thioglycosides **99** and **100** as well as the methyl *O*-glycoside **101** were synthesized. The stereochemistry of the products were elucidated from the hetero coupling constants $^3J_{(H\text{-}4,C2)}$ and/or $^3J_{(H\text{-}4,C(Nuc))}$ (when accessible, Figure 38)[175].

Table 2: C-, S- and O-Glycosylation of **94**:



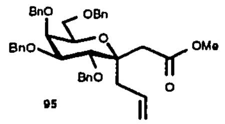
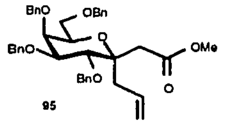
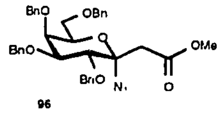
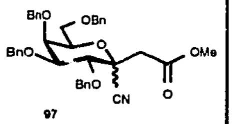
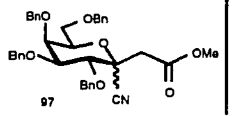
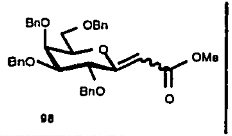
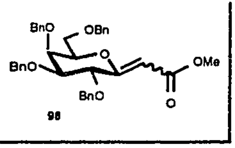
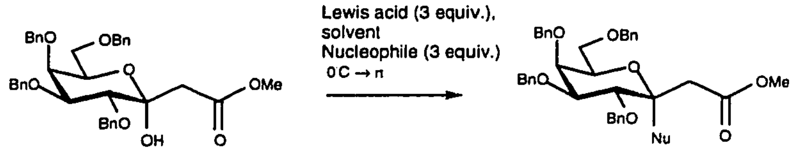
Lewis acid	Solvent	Time	Nucleophile	Yield	Roduct
TMSOTf or BF_3	CH_3CN	1h	TMSAll	65-72% (only α)	 95
TMSOTf	CH_2Cl_2	2h	TMSAll	65% (only α)	 95
TMSOTf	CH_3CN	4h	$TMSN_3$	75% (only α)	 96
TMSOTf	CH_3CN	8h	TMSCN	75% (α/β: 7/1)	 97
BF_3	CH_3CN	10h	TMSCN	67% (α/β: 6/1)	 97
TMSOTf	CH_3CN	8h	$TMSNHCONH_2$	55%	 98
TMSOTf	CH_3CN	1h	$TMSCF_3$	22%	 98

Table 2: Continued:



94

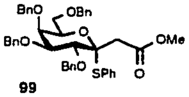
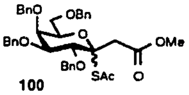
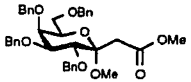
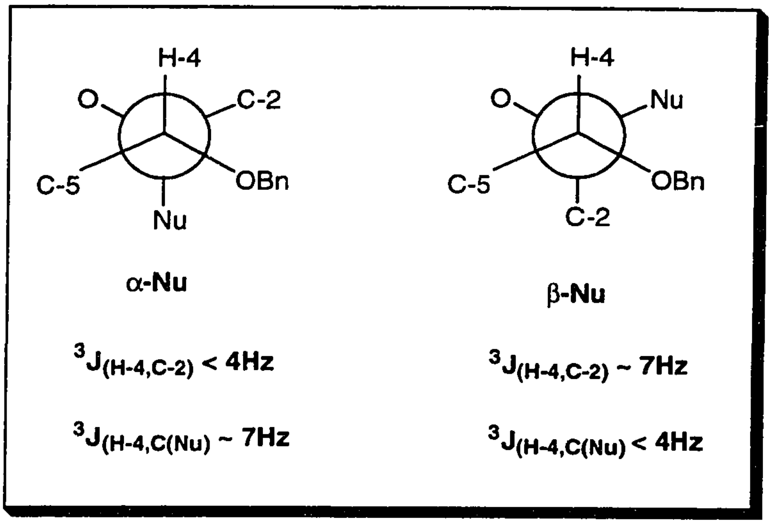
Lewis acid	Solvent	Time	Nucleophile	Yield	Product
TMSOTf	CH_3CN	5h	$TMSCH_2CN$	—	—
TMSOTf	CH_2Cl_2	5h	$TMSCH_2COOMe$	—	—
TMSOTf	CH_3CN	4h	TMSNCO	—	—
TMSOTf or BF_3	CH_3CN	2h	SPh	95% (only α)	 99
BF_3	CH_3CN	2h	CH_3COSH	68% (α/β: 6:1)	 100
BF_3	CH_2Cl_2	8h	MeOH	quant. (only α)	 101

Figure 41: Newman Projection Viewed along the C-$_3$ - C-$_4$ Bond in **95-97** and **99-101**. Small Hetero Coupling Constants $^3J_{(H\text{-}4,C\text{-}2)} < 4$ Hz and Large Coupling Constants $^3J_{(H\text{-}4,C(Nu)}$ ~ 7 Hz) Are Diagnostic of the Axial (α) while Large Hetero Coupling Constants $^3J_{(H\text{-}4,C\text{-}2)}$ ~ 4 Hz) and Small Coupling Constants $^3J(_{H\text{-}4,C(Nu)} < 4$ Hz) Are Diagnostic of the Equatorial (β) Orientation of Nu[175]:



B.3 C- and O-Glycosidation of the Donors **99** and **102**:

The fluoro donor **102** was prepared from the thiophenol glycoside **99** by *N*-bromosuccinimide (NBS) activation with a 20% solution of HF in pyridine using CH_2Cl_2 as solvent (Figure 42)[176]. Compounds **99** and **102** were reacted with a variety of nucleophiles including TMSCN, MeOH, cyclohexanol, the sugar alcohol (YH, Table 3)

Figure 42: Preparation of the Fluoro Donor **102**:

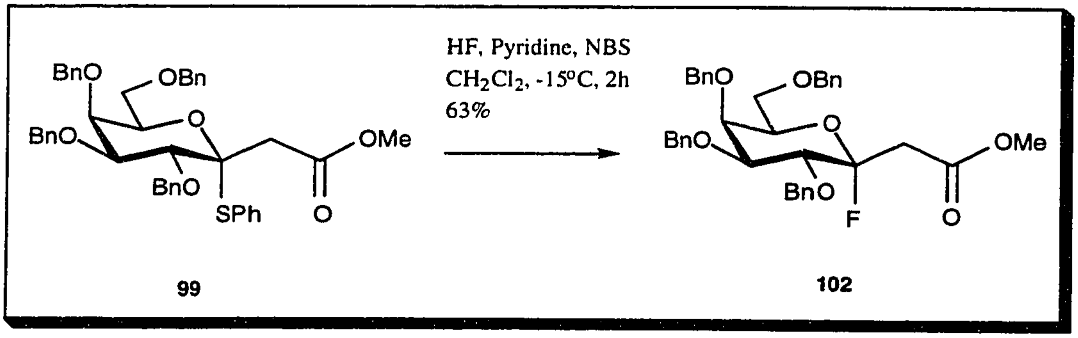
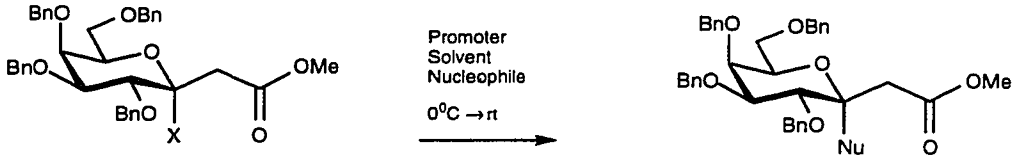
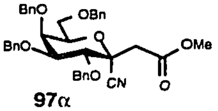
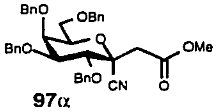
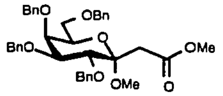
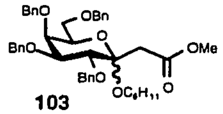
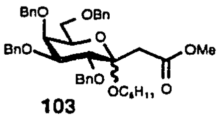
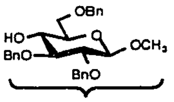
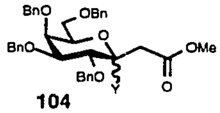
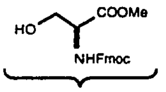
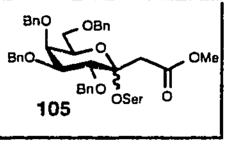


Table 3: *C*- and *O*-Glycosidation of **99** and **102**:



99 X = SPh
102 X = F

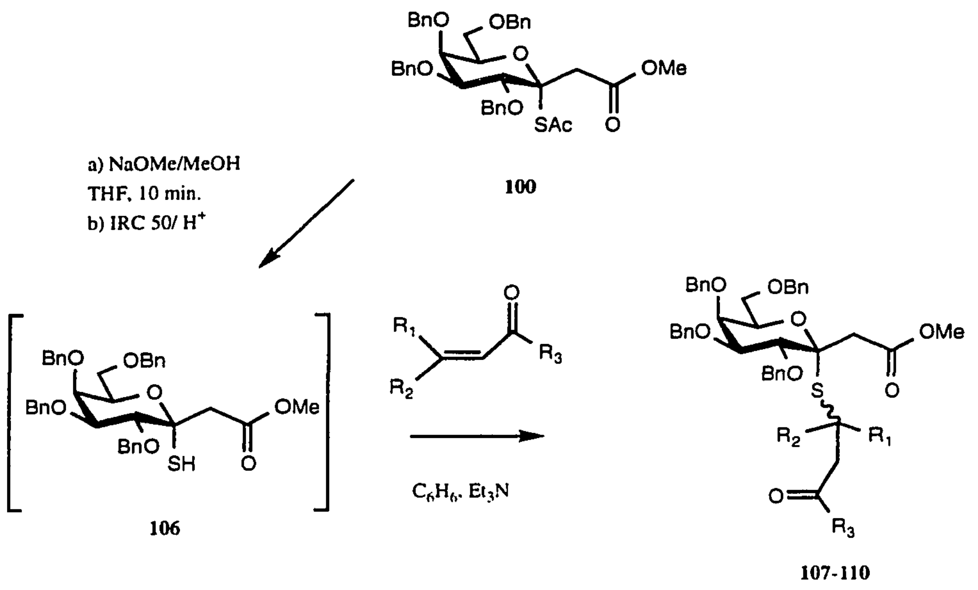
Promoter	Solvent	Time/Donor	Nucleophile	Yield	Product
BF_3 (3 equiv.)	CH_3CN	2h/102	TMSCN (3 equiv.)	75% (only α)	 **97α**
BF_3 (3 equiv.)	CH_2Cl_2	2h/102	TMSCN (3 equiv.)	73% (only α)	 **97α**
BF_3 (6 equiv.)	CH_2Cl_2	4h/102	MeOH (6 equiv.)	70% (only α)	 **101**
BF_3 (6 equiv.)	CH_2Cl_2	8h/102	Cyclohexanol (6 equiv.)	(traces)	 **103**
NBS (1.5 equiv.)	CH_2Cl_2	4h/99	Cyclohexanol (6 equiv.)	30%	 **103**
NBS (1.5 equiv.)	CH_2Cl_2	4h/99	 YH (1.5 equiv.)	(traces ?)	 **104**
NBS (1.5 equiv.)	CH_2Cl_2	4h/99	 SerOH (1.2 equiv.)	50%	 **105**

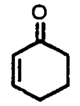
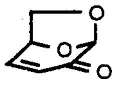
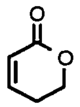
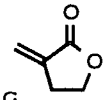
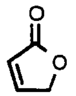
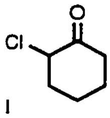
and the protected amino acid serine (SerOH) as shown in Table 3. Treatment of **102** with BF_3 and TMSCN in CH_2Cl_2 or CH_3CN gave only **97α** in 73% and 75% yield, respectively. *O*-Glycosylation with the unhindered primary alcohol MeOH yielded **101**. In contrast the more sterically hindered alcohol cyclohexanol gave only traces of the cyclohexyl glycoside **103**. Presumably, **103** is not stable in the presence of BF_3 dietherate. However, **103** was obtained in 30% yields starting from the thiophenyl glycoside **99** under neutral conditions (NBS promoted)[177] in CH_2Cl_2. Similarly, the protected alcohol SerOH was glycosylated with **99** to give **105** (30%) together with significant amounts of elimination product **98** (~ 40%), while the sterically more demanding sugar alcohol YH gave only traces of a new uncharacterized product.

B.4 Conjugate Additions and Nucleophilic Substitutions of the Thiol **106**:

In order to generate a highly diversified R' side chain (Figure 36), the tertiary thiol **106** was envisaged to react as a nucleophile in Michael additions to give stereoisomeric mixtures of Michael adducts (Table 4). Compound **106**, generated *in situ* from the thioacetate **100** via deacetylation, was treated directly with the Michael acceptors 2-cyclohexen-1-one (D), levoglucosenone (E), 5,6-dihydro-2H-pyran-2-one (F) and α-methylene-γ-butyrolactone (G). No reaction was observed with lactone (H) and α-haloketone (I). The reaction with levoglucosenone producing the Michael adduct **108** in 65% yield is particularly noteworthy. Presumably, the release of ring, steric and torsional strain is responsible for the high reactivity of E. The products **107**, **109** and **110** were obtained in about 20%, after prolonged reaction times. Side reactions such as elimination to give **98** or disulfide formation[178] of **106** contributed to the low isolated yields of **107**, **109** and **110**.

Table 4: Michael Addition with the Tertiary Thiol **106**:

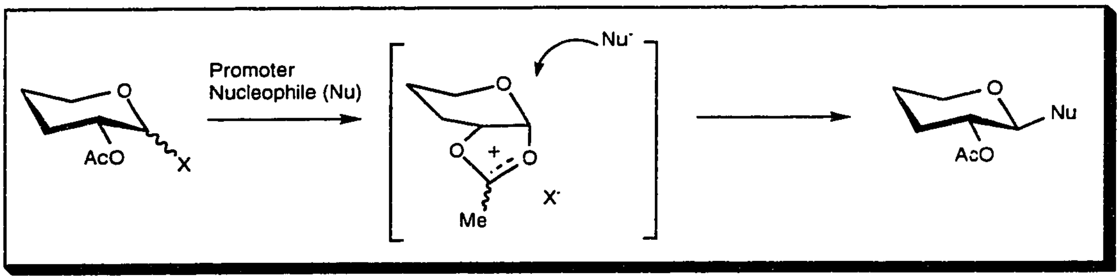


	 D	 E	 F	 G	 H	 I
observed product	+	+	+	+	–	–
reaction time	24 h	90 min.	4d	4d	6d	6d
yield / ratio of isomers	17% / 1 : 1.5	65% / only 1	20% / 1 : 1.5	22% / ~ 1 : 1	–	–
product no.	**107**	**108**	**109**	**110**	–	–

B.5 Influence of the Hydroxyl Protecting Groups on C-Glycosylations:

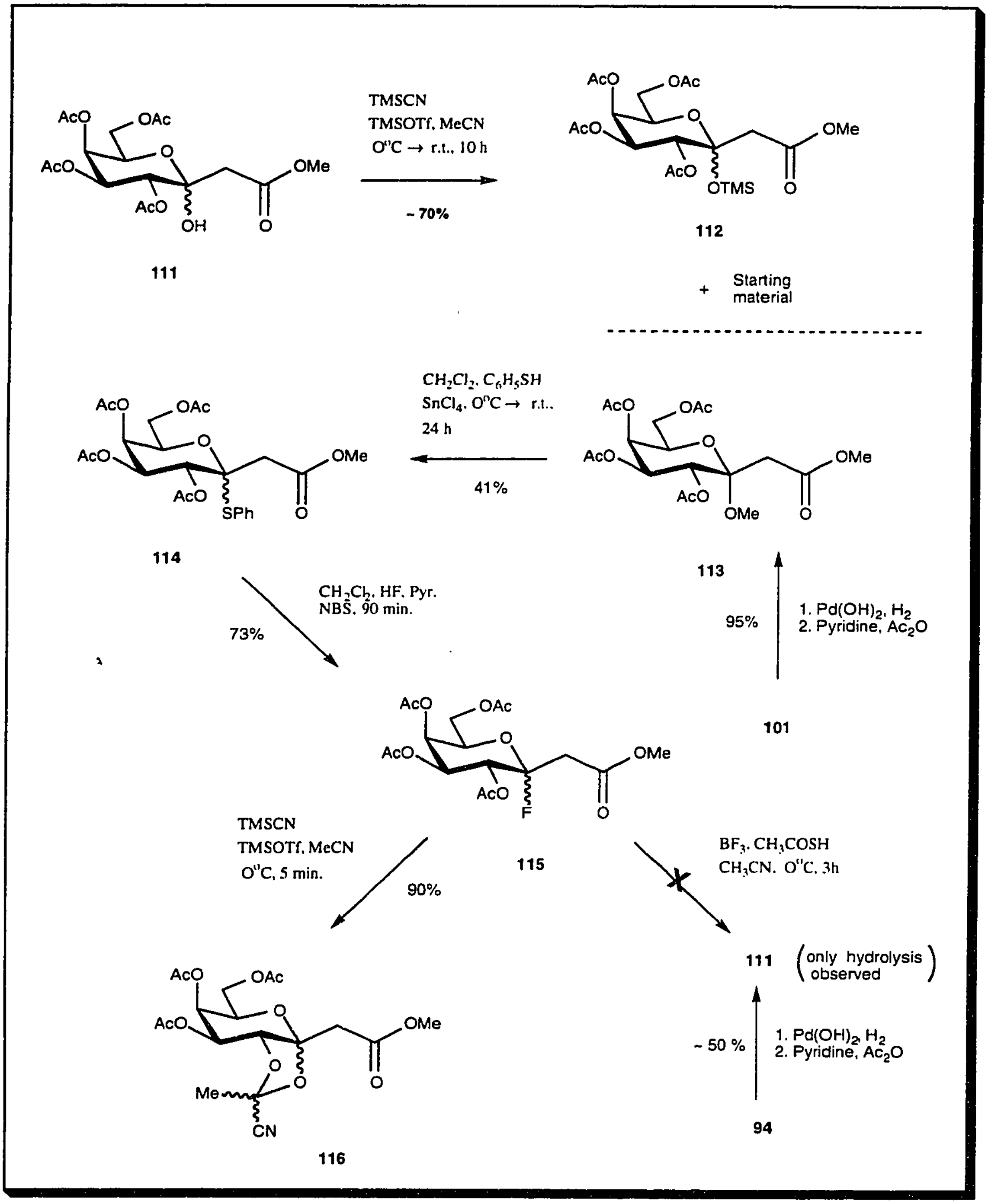
The previous C-, S- and O-Glycosylations (Table 2 and 3) were performed under Lewis acid catalyzed conditions using benzyl ethers as hydroxyl protecting groups. The reactions proceeded with high α selectivity independent of the nature of the Lewis acid or solvent used. It is well known that acetate protecting groups, in contrast to benzyl ethers give rise to high β-selectivity in glycosylation reactions[179]. The preferred equatorial β-attack is usually explained by the participating acetate group that blocks the α-face (Figure 43).

Figure 43: Participating Effect of an Acetate Protecting Group during the Glycosylation Reaction. The α-Face is Blocked for the Incoming Nucleophile (Nu) Resulting in High β-Selectivity:



It appeared therefore reasonable to investigate the effect of the acetate protecting group during the C-glycosylation reactions (Figure 44). The acetate protected acetal **111** was obtained from the benzylated acetal **94** via debenzylation (hydrogenation) and acetylation. Treatment of **111** with TMSCN under TMSOTf promoted conditions did not produce any C-glycoside, instead only the TMS ether **112** and unreacted starting material were isolated. It was reasoned that the acetal **111** might be to unreactive towards C-glycosylation and therefore the fluoro donor **115** was prepared from the methyl glycoside **101** in four steps. Compound **101** was debenzylated via hydrogenolysis using Pearlman's catalyst[155] and acetylated to afford **113** in 95% yield. The acetate protected methylglycoside donor **113** was then glycosylated with thiophenol under tin tetrachloride promoted conditions to give the thioglycoside **114** in 41% yield. The use of other Lewis acids (BF_3 or TMSOTf) gave significantly lower yields of **114**. The thio donor **114** was then converted with NBS and 20% HF in pyridine into the fluoro sugar **115**. Unfortunately, attempts to use **115** in *C*- and *S*-glycosylation reactions failed. For instance, treatment of **115** with TMSCN under TMSOTf catalyzed conditions gave only an inseparable mixture of the orthoesters **116**. Analogously, S-glycosylation of **115** with thiolacetic acid gave only hydrolyzed acetal **111** after work up.

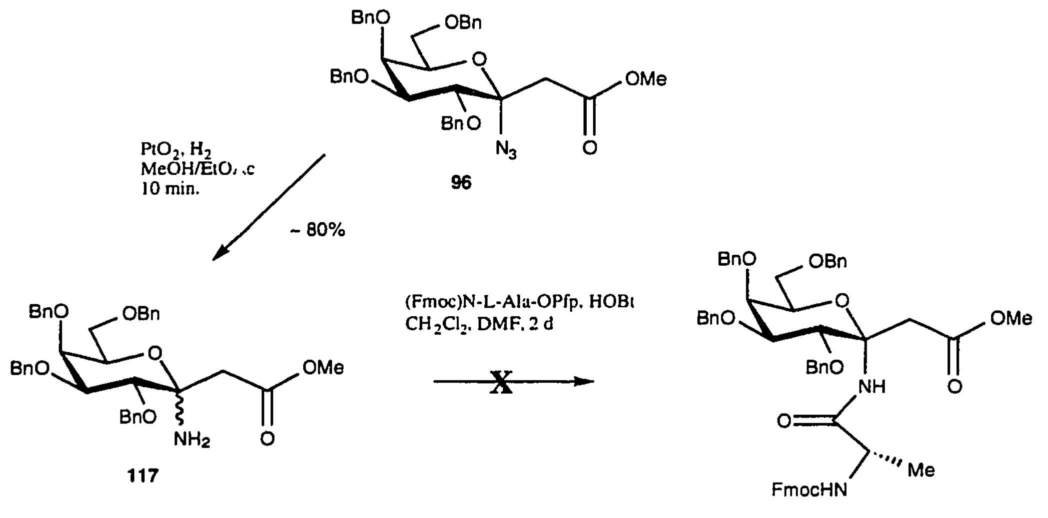
Figure 44: Influence of Acetate Protecting Groups on C- and S-Glycosylation:



B.6 Attempted Acylation of Glycosylamine **117** with Amino Acids:

The previously synthesized azide **96** was reduced to the glycosylamine **117** by hydrogenation using Platinium(IV)oxide as catalyst[180] for 10 min. (~80% yield, Figure 45). Attempted acylation of amine **117** with the (Fmoc)N-L-Ala-OPfp ester under HOBt activation conditions[157] did not produce the desired glycosylamide even after prolonged reaction times. Presumably the high steric hindrance of the tertiary glycosylamine **117** prevents the acylation (Figure 45).

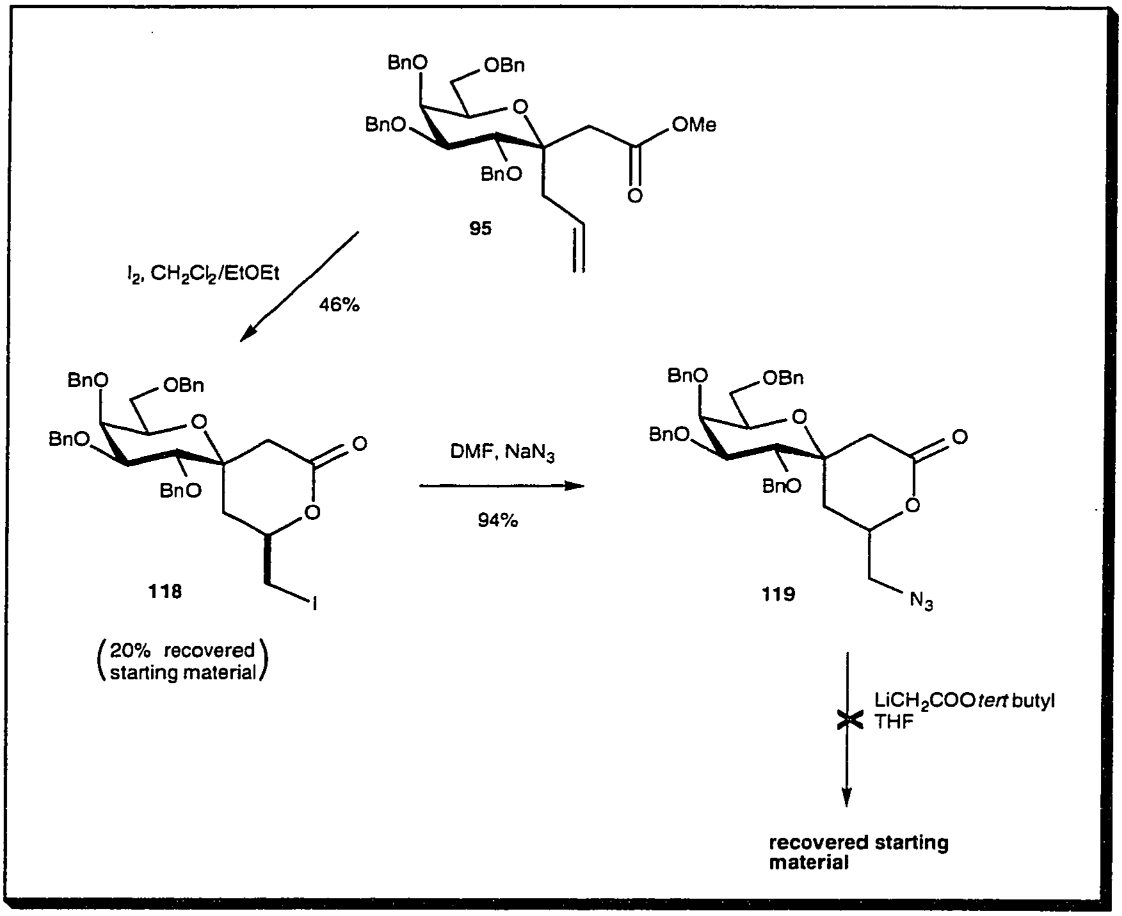
Figure 45: Attempted Acylation of Glycosylamine **117** with an Activated Amino Acid:



B.7 Functionalization of the C-Allyl Ketoside **95** via Iodolactonization:

The C-allyl ketoside **95** was treated with iodine in a mixture of CH_2Cl_2 and ether[18] to produce the iodolactone **118** in 46% yield together with 20% of unreacted starting material. Nucleophilic displacement of the iodine by azide gave compound **119** in 94% isolated yield. Attempted enolate addition of *tert*-butylacetate failed and only recovered starting material was obtained (Figure 46).

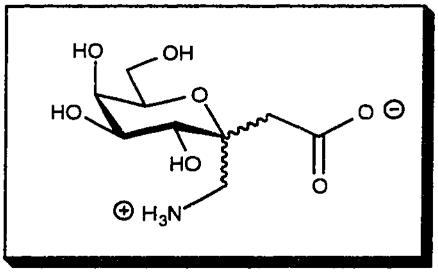
Figure 46: Iodolactonization of Allyl-C-Ketoside **95**:



B.8 Synthesis of a Novel Unprotected Sugar Amino Acid (SAA **122**) and Use in Peptide Coupling Reactions:

Sugar Amino Acids (SAAs) are ideal building blocks for oligomerization and thus have great potential for Combinatorial Synthesis. Although, several SAAs have been described (Figure 9) none of them contains a α,α-difunctionalized pyran skeleton (Figure 36). Based on the previous results with the glycosylamine **117** the following α,α-difunctionalized pyran derived SAAs (Figure 47) appeared to be ideal building blocks. The amino and carboxylic acid functions are linked through a methylene tether to the pyran ring to ensure steric accessibility for further coupling.

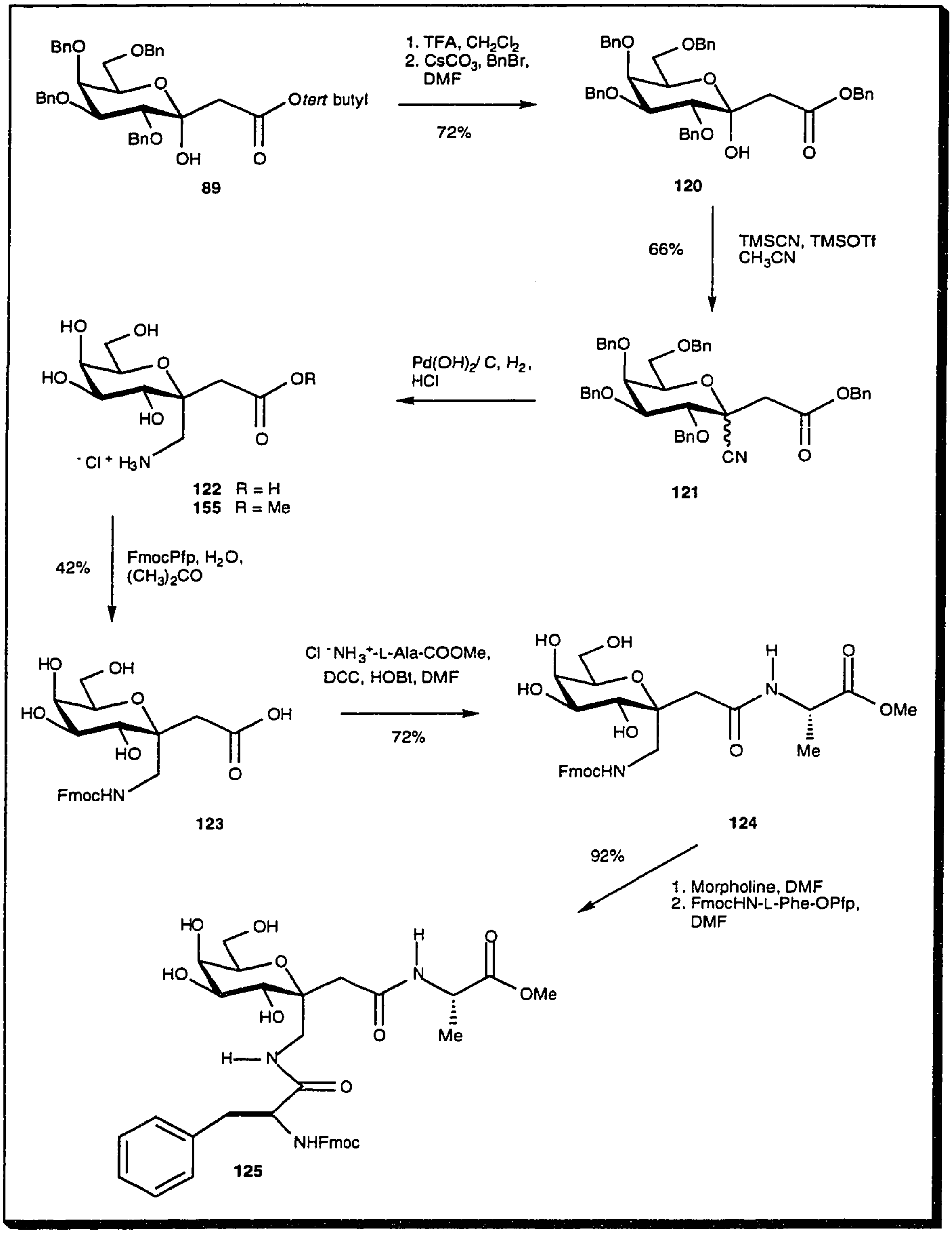
Figure 47: α,α-Difunctionalized Pyran Based SAA as Molecular Probes for Galactose-Binding Proteins:



The synthesis of the unblocked SAAs **122** and its methyl ester **155** bearing an equatorial carboxyl side chain and an axial amino side chain, started with the acetal **89** in a four step sequence (Figure 48). Removal of the *tert*-butyl group with TFA and subsequent benzylation of the free acid with benzyl bromide using cesium carbonate as base[182] gave the benzyl ester **120** in 72% yield. The cyanide function was introduced with TMSCN using TMSOTf as promoter and produced an α/β mixture of **121** (ratio α/β: 7/1). The mixture was easily separable by crystallization ($CHCl_3$). Hydrogenation of the major α-cyano isomer **121α** using Pearlman's catalyst[155] under acidic (HCl) conditions produced the unprotected SAA **122** in 66% yield. Compound **122** was contaminated with the methyl ester **155** (10-20%) resulting from an acid catalyzed esterfication during hydrogenation. The use of acetic acid instead of hydrochloric acid resulted in incomplete reduction of the cyano function.

In order to evaluate SAA **122** as a suitable building block for peptide synthesis the amine function was Fmoc-protected to afford the acid **123** in 42% yield. Compound **123** was then subjected to peptide coupling cycle (DCC, $Cl^{-}NH_3^{+}$-L-Ala-COOMe, HOBt, DMF) and the glycodipeptide **124** was isolated in 72% yield. Removal of the Fmoc group with morpholine[183] followed by additional peptide coupling (FmocHN-L-Phe-COOPfp, HOBt, DMF) afforded the glycotripeptide **125** in 92% yield (Figure 48). It is noteworthy that no intramolecular lactam formation was observed during this coupling procedure.

Figure 48: Synthesis and Use of SAA **122** as a Building Block for Unnatural Glycopeptide Synthesis:



B.9 Use of SAAs **125**, **129** and **130** in Solid Phase Glycopeptide Synthesis:

In order to facilitate and accelerate the synthesis of glycopeptide libraries, a solid phase approach would be desirable. A solid phase synthesis for the elongation of the peptide chain in the glycodipeptide **126** is shown in Figure 49. The previously described cyano compound **97α** was hydrogenated with Pearlman's catalyst under acidic (HCl) conditions to give the hydrochloride **155**. Compound **155** was directly acylated to give the glycopeptide **126** with the activated ester of phenylalanine (FmocHN-L-Phe-COOPfp) under mild basic ($NaHCO_3$) conditions[184] in a 65% overall yield, starting from **97α**. The immobilization of the dipeptide **126** was done on an excess Merrifield tritylchloride resin[185] (presumably through the primary OH-group) and was easily monitored by thin layer chromatography (disappearance of the UV active peptide **126**). The immobilized dipeptide **127** was then subjected to a peptide coupling cycle (1. morpholine, 2. FmocOGly-OPfp, HOBt). The resin cleavage was carried out with 0.5% TFA in CH_2Cl_2 producing the glycotripeptide **128** in 60% yield.

Attempts to use the immobilized protected SAAs **129** or **130** as building blocks for further peptide couplings failed (Figure 50). Removal of the Fmoc protecting group in **129** and **130** with morpholine and subsequent peptide coupling with the activated glycine ester (PfpO-Gly-NHFmoc) gave only the lactam **133** after resin cleavage (Figure 50).

In conclusion, a methodology for the synthesis of SAAs **122** and **155** has been developed. SAA **122** can be incorporated into peptides via the usual C→N fashion. The immobilization of the sugar dipeptide through the carbohydrate moiety further demonstrates the scope and use of this building block. Furthermore, the synthesis of cyclic peptides with an incorporated carbohydrate moiety may be envisaged. Finally, it is hoped that SAAs **122** and **155** may function as scaffolds for unnatural glycopeptide libraries.

Figure 49: Elongation of the Peptide Chain of the Glycodipeptide **126** by Solid Phase Synthesis:

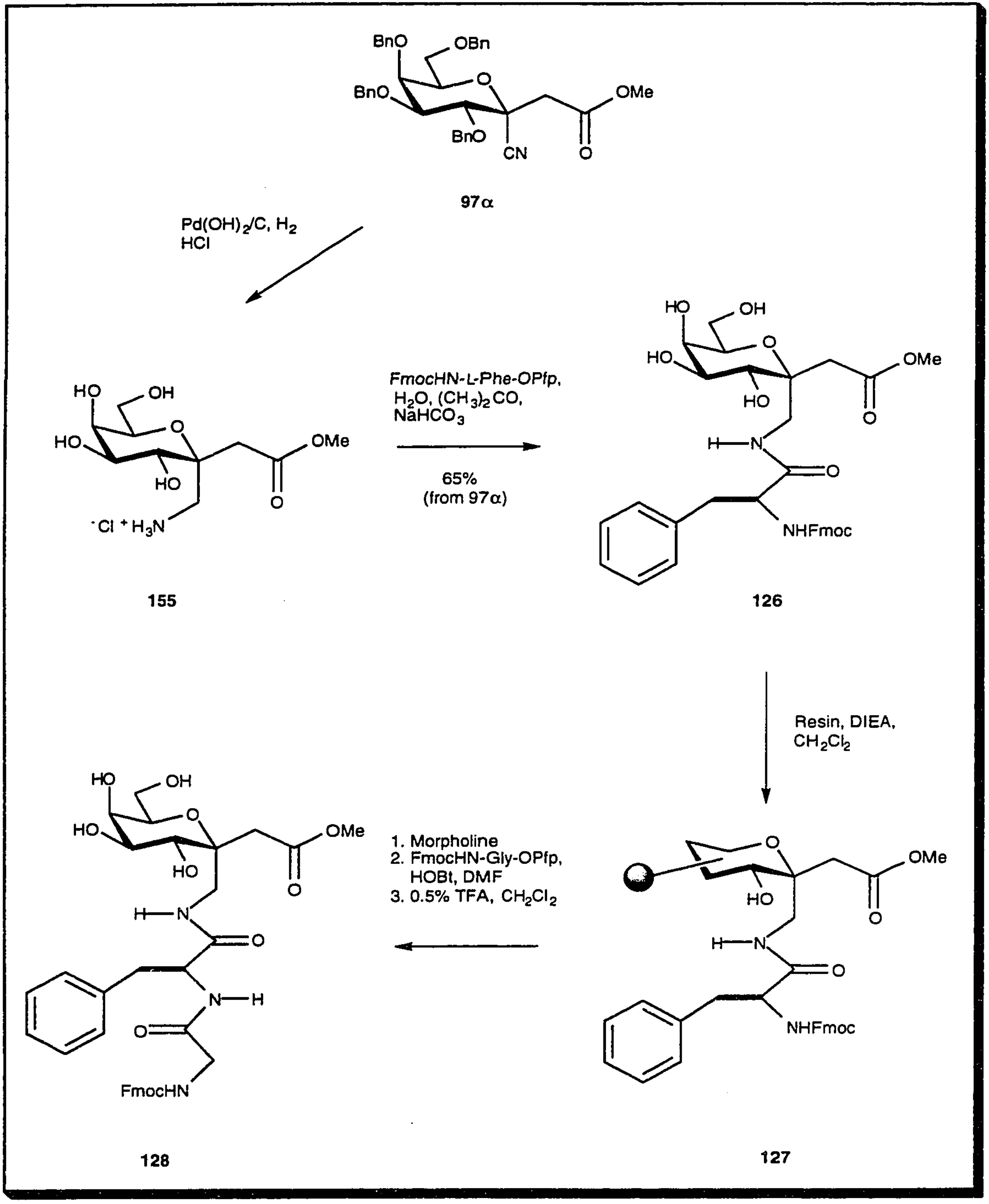
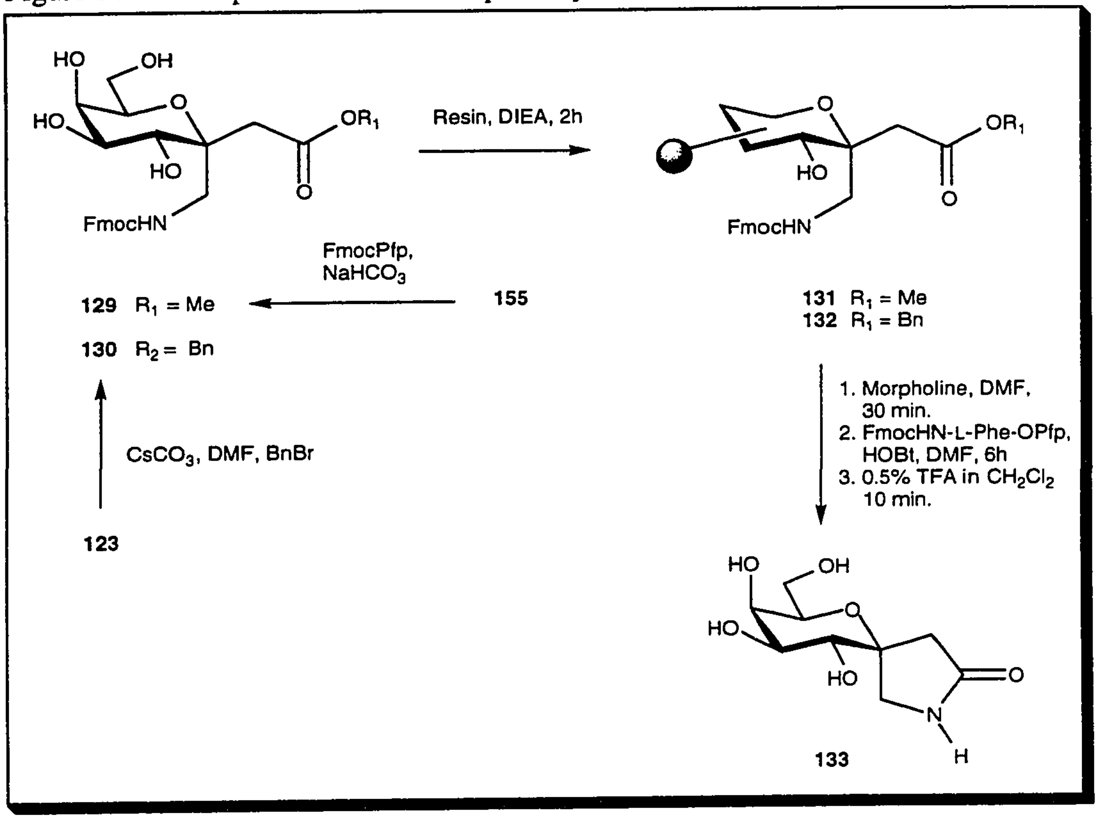


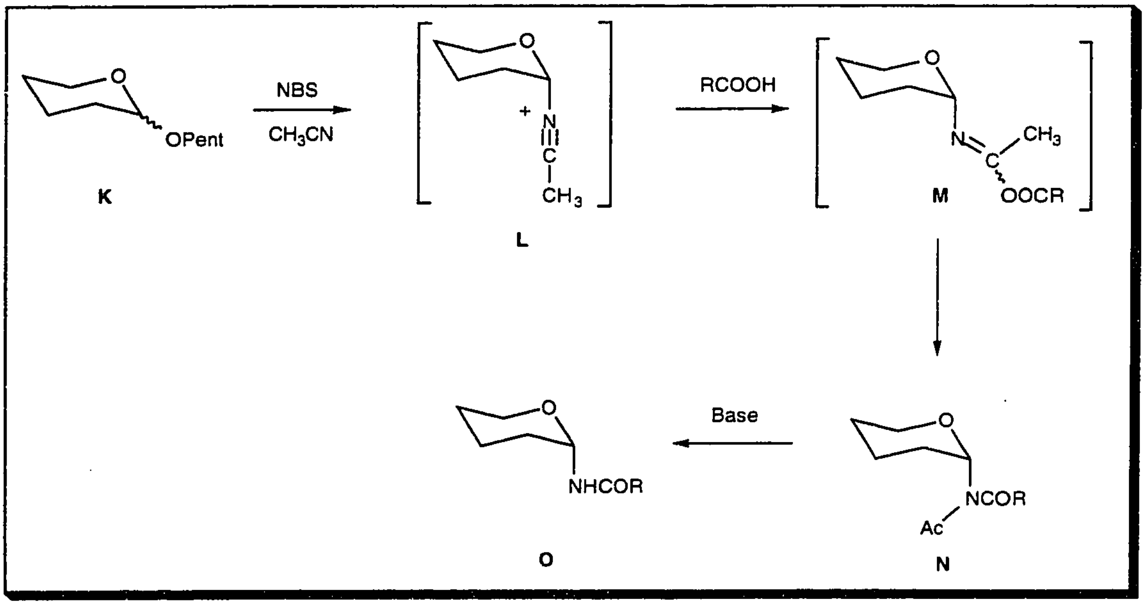
Figure 50: Attempted Solid Phase Peptide Synthesis of SAAs **129** and **130**:



B.10 One Pot Conversion of Ketose **89** into Sugar β-Peptides via a Modified Intramolecular Ritter Reaction:

The difficulties encountered in the acylation of the tertiary glycosylamine **117** (Figure 42) necessitated an alternative approach towards the synthesis of ketose derived glycosylamides. Previously, a Ritter reaction[186] has been used in the synthesis of *N*-linked Glycoproteins[187] (Figure 51).

Figure 51: Ritter Reaction of Pentenyl (Pent) Glycosides in the Presence of a Carboxylic Acid (RCOOH). Activation of the Pentenyl Glycoside (**K**) with NBS in Acetonitrile Leads to the Formation of an α-Acetonitrilium Ion (**L**) which is Trapped by a Carboxylic Acid to Give the Iminoanhydride (**M**). **M** Rearranges to the Stable α-Imide (**N**) which under Basic Conditions Produces the Glycosylamide (**O**):



Treatment of pentenyl glycoside **K** with NBS in acetonitrile gave the iminoanhydride **M** via an initially formed α-acetonitrilium ion **L**, which was trapped by a carboxylic acid to form **M**. The unstable compound **M** rearranged to the stable imide **N** which under basic condition formed the glycosylamide **O**.

Based on that work, we rationalized that this methodology would become even more powerful if the attack of the carboxylic acid would be an intramolecular process, proceeding to a 6-membered ring. The carboxylic acid **157** (Figure 52) appeared to be a suitable starting material. Compound **157** was prepared by cleavage of the *tert*-butyl ester in **89** with TFA in CH_2Cl_2. Treatment of the acid **157** with TMSOTf and benzonitrile (PhCN) in CH_2Cl_2 gave the isolated spiro dihydrooxazinone **134** in 92% yield after 10 minutes. The formation of **134** can be explained by a Ritter reaction assuming irreversible intramolecular trapping of the initially formed nitrilium ion **156** by the free carboxylic acid. The activated cyclic iminoanhydride[188,189] **134** was stable to the work-up conditions (aqueous sodium bicarbonate) and purification (flash-chromatography on silica gel). However, once the activated ester **134** was subjected to excess cycloheptylamine, the diamide **135** containing a sugar β-amino acid core was isolated in 90% yield as the only product.

The stereochemistry of **135** was unambiguously established using NMR spectroscopy. The two amide protons were distinguished easily as a singlet and a doublet (J = 7.4 Hz, Figure 53). The former was examined using a one-dimensional TROESY experiment[190,191], whereby the resonance was excited by a 240 ms selective e-burp1 pulse[192]. Inter-proton enhancements were observed both to H-5 and H-7 (2.5 and 0.5%, respectively of the amide resonance integral, Figure 53). The observation of these ROEs is only compatible with an axial orientation.

Once the stereochemistry of **135** was deduced, a one-pot conversion of the ketose **89** to the diamide **135** was carried out. Removal of the *tert*-butyl group in **89** followed by treatment with TMSOTf and PhCN in CH_2Cl_2 for 2 h at O^0C and quenching with excess of cycloheptylamine gave directly **135** in an overall yield of 71%. In order to demonstrate the

Figure 52: One Pot Conversion of **89** into Sugar β-Peptides via a Modified Intramolecular Ritter Reaction:

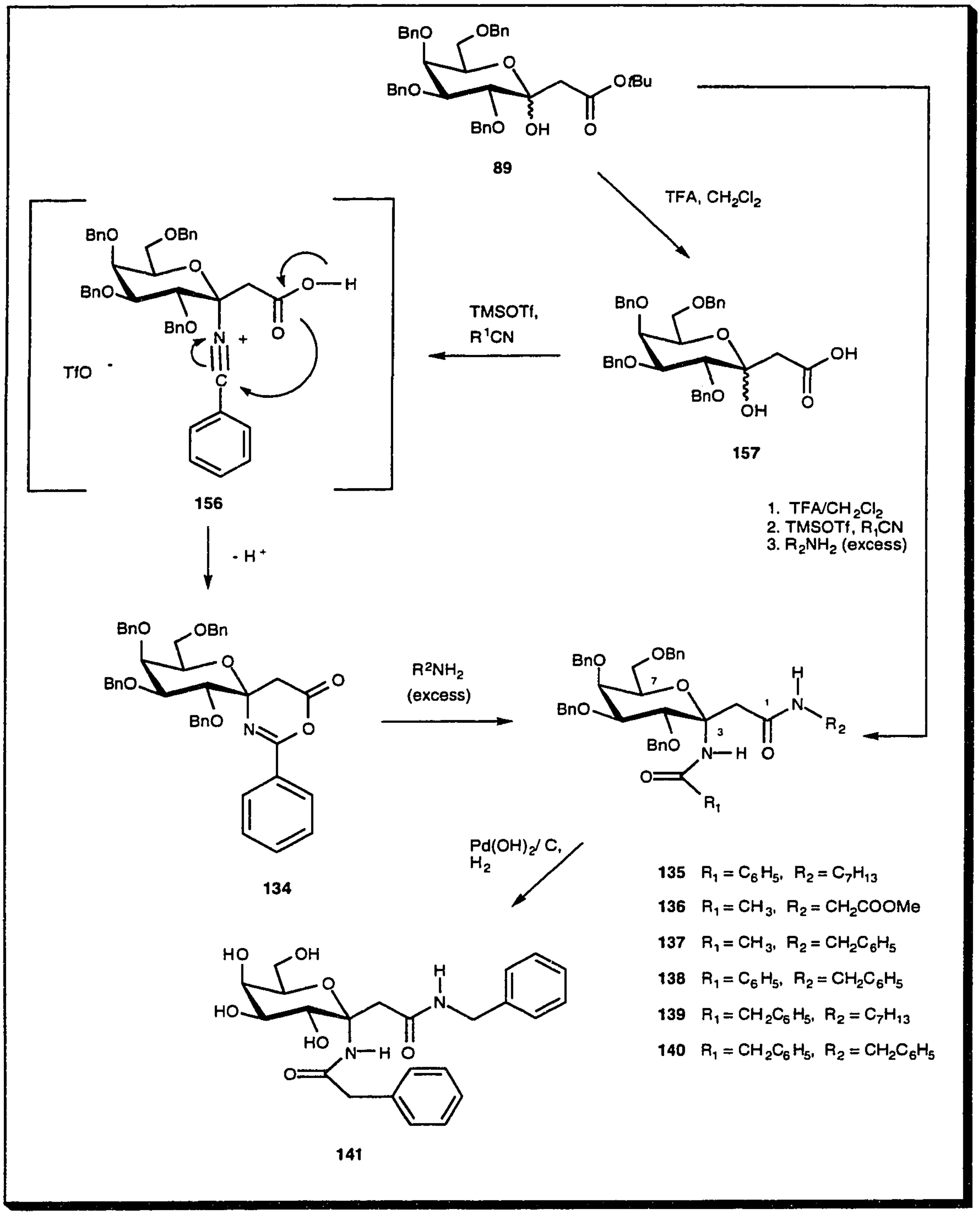
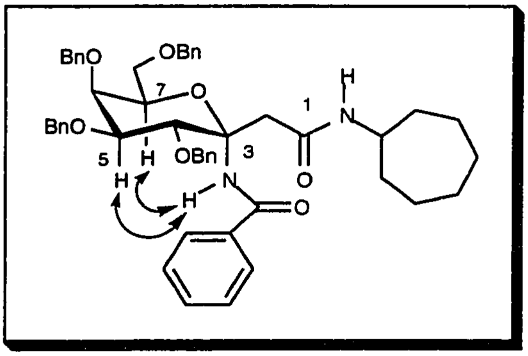


Figure 53: Conformationally Relevant ROE Interactions Observed for **135**:



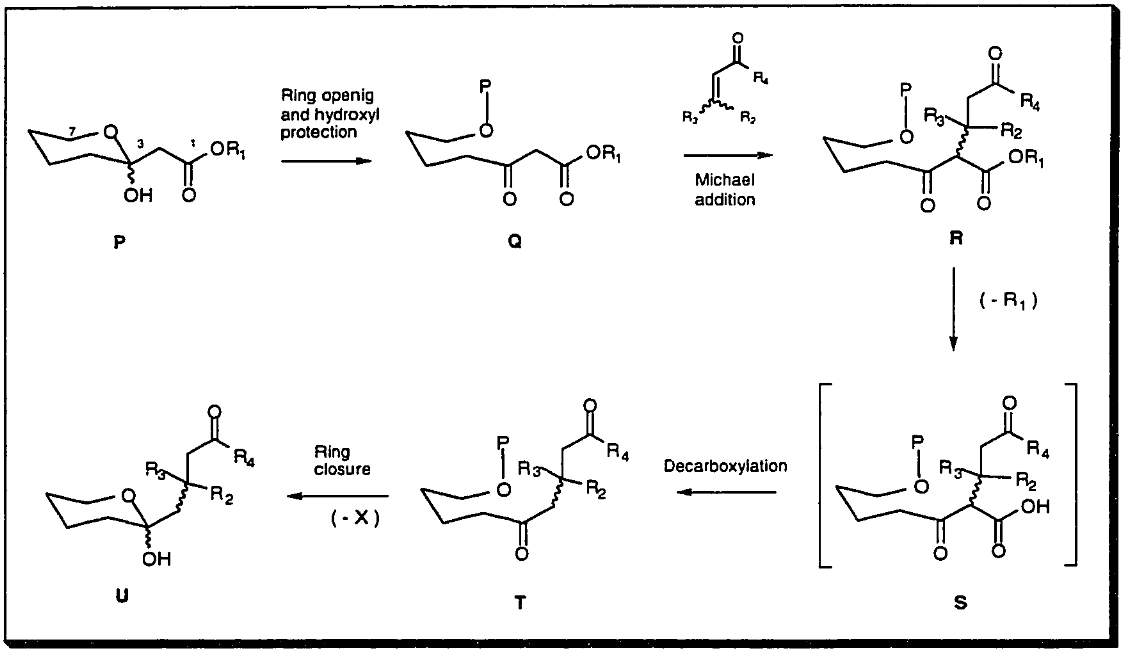
generality of the reaction, we then treated the ketose **157** with two other nitriles, acetonitrile and phenylacetonitrile, and quenched the reaction with three different amines, namely glycine methyl ester, benzylamine, and cycloheptylamine. In all cases, we obtained the desired β-peptidic sugar diamides **136-140** as single stereoisomers in 60-70% overall isolated yield. The stereochemistry in each of **136-140** was verified as described for **135**, using the 1D-TROESY NMR technique. The irreversible axial attack of the nitrile is consistent with the general phenomenon of solvent participation of nitriles in glycoside synthesis generally referred to as "nitrile effect"[174]. Finally deprotection of **140** afforded the unblocked sugar β-peptide **141** in quantitative yield.

The present "Ritter" based method is very effective for generating glycosylamides which are not accessible by acylation of glycosylamines. The commercial availability of large numbers of nitriles and amines suggests that the one-pot reaction presented should be attractive for the preparation of β-peptidic sugar diamide libraries. Furthermore, the highly functionalized α,α-disubstituted pyran backbone in **135-140** with its rigid β-sugar amino acid core might also find use in peptide mimetics[74], and β-peptide synthesis[193].

B.11 Michael Addition with the 1,3 Dicarbonyl Compound **142**:

In part A (Chapter III), the nitro-C-glycoside **69** was studied as a Michael donor in 1,4-conjugate additions. The reaction was generally inefficient which was attributed to the high steric hindrance for the approach of the nucleophile. This was especially true if the Michael acceptor contained substituents at the β-position (R_2,R_3 in Figure 33), since it is well known that conjugate additions are very sensitive to β-substitution[194]. In order to reduce the steric demands in the transition state of the Michael addition, acyclic C-nucleophiles were considered. It was hoped, that acyclic Michael donors would be more reactive and may even allow the use of substituted Michael acceptors. In this respect the masked 1,3 dicarbonyl system **P** may be envisaged as a possible acyclic C-nucleophile **Q** (Figure 54) which may undergo Michael additions. The resulting Michael adduct **R** could be

Figure 54: Michael Addition of the Acyclic β-Keto Ester (**Q**) Generated from the Ketose (**P**) via Protection of the Hydroxyl Group at C-7. Saponification of the Michael Adduct (**R**) Leads to Keto Acid (**S**) which Decarboxylates to the Ketone (**T**). Removal of the Hydroxyl Protecting Group P and Ring Closure Provides the Acetal (**U**):

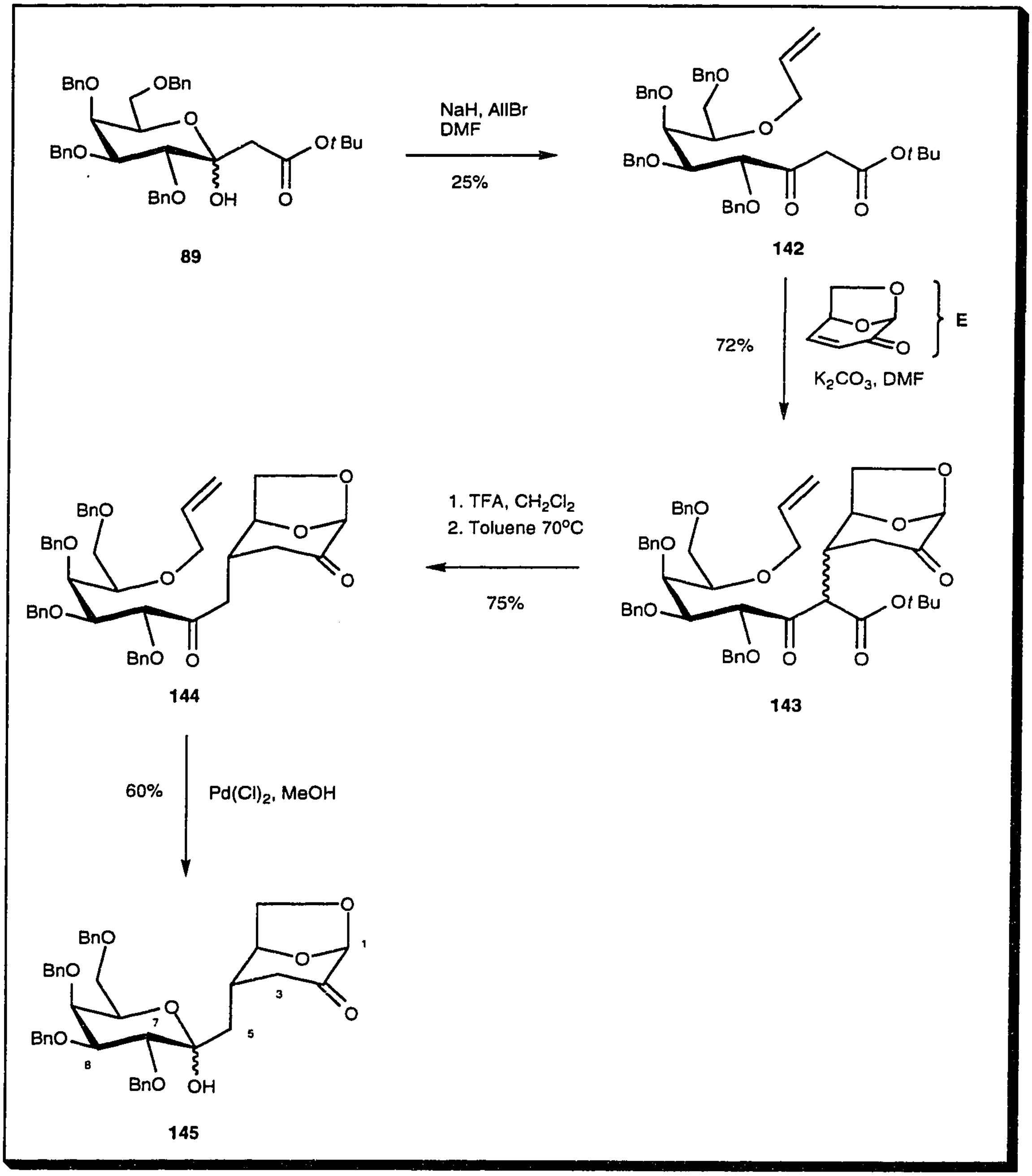


hydrolyzed to the keto acid **S** which affords the ketone **T** after decarboxylation. Removal of the protecting group P with subsequent ring closure produces the ketoside **U** (Figure 54).

This strategy was successfully applied on the acetal **89** as outlined in Figure 55. Treatment of compound **89** with NaH and Allyl bromide (AllBr) for 1 h at 0°C produced the acyclic allyl ether **142** in 25% yield together with 50% unreacted starting material. The yield of **142** could not be improved by prolonged reaction times and led otherwise to complex reaction mixtures of mono- and double- C-alkylated **142**. The β-keto ester **142** was then treated with potassium carbonate (K_2CO_3) and levoglucosenone **E** to furnish the mono Michael adduct **143** as a mixture of two diastereoisomers (ratio 2:1) in 72% yield. Michael diadducts were not observed. Saponification of the *tert*-butyl ester with TFA in CH_2Cl_2, followed by decarboxylation in toluene gave a single ketone **144** in 75% yield. At this point, it was uncertain whether the stereochemistry at the sugar carbon atom adjacent to the carbonyl group was retained or inverted. For instance, the acidic and thermal conditions used for the decarboxylation could in principle have allowed an epimerization at the carbon center adjacent to the carbonyl group. That this configuration was retained at this center was proven via deallylation of **144** with palladium dichloride ($PdCl_2$) and subsequent cyclization to produce the ketoside **145** in 62% yield. The large coupling constant between H-7 and H-8 ($^3J_{7,8}$ = 9.4 Hz) is in agreement with the retained sterochemistry at C-7 during the synthesis. A second, unidentified product obtained in 15% yield showed the same stereochemistry at C-7 but showed unidentified modifications at the C-1 to C-4 part of the molecule **145**.

It is hoped that the successful demonstration of the Michael addition with the β-keto ester **142** to levoglucosenone **E** (Figure 55) may be expanded to other Michael systems. Furthermore, alkylations with reactive alkylhalides may also be feasible and should

Figure 55: Michael Addition with the Acyclic β-Keto Ester (**142**) and Levoglucosenone (E) to Produce the Stereoisomeric Michael Adducts (**143**). This Mixture Is then Converted into the Ketone (**144**) which after Deallylation Affords the Ketoside (**145**):

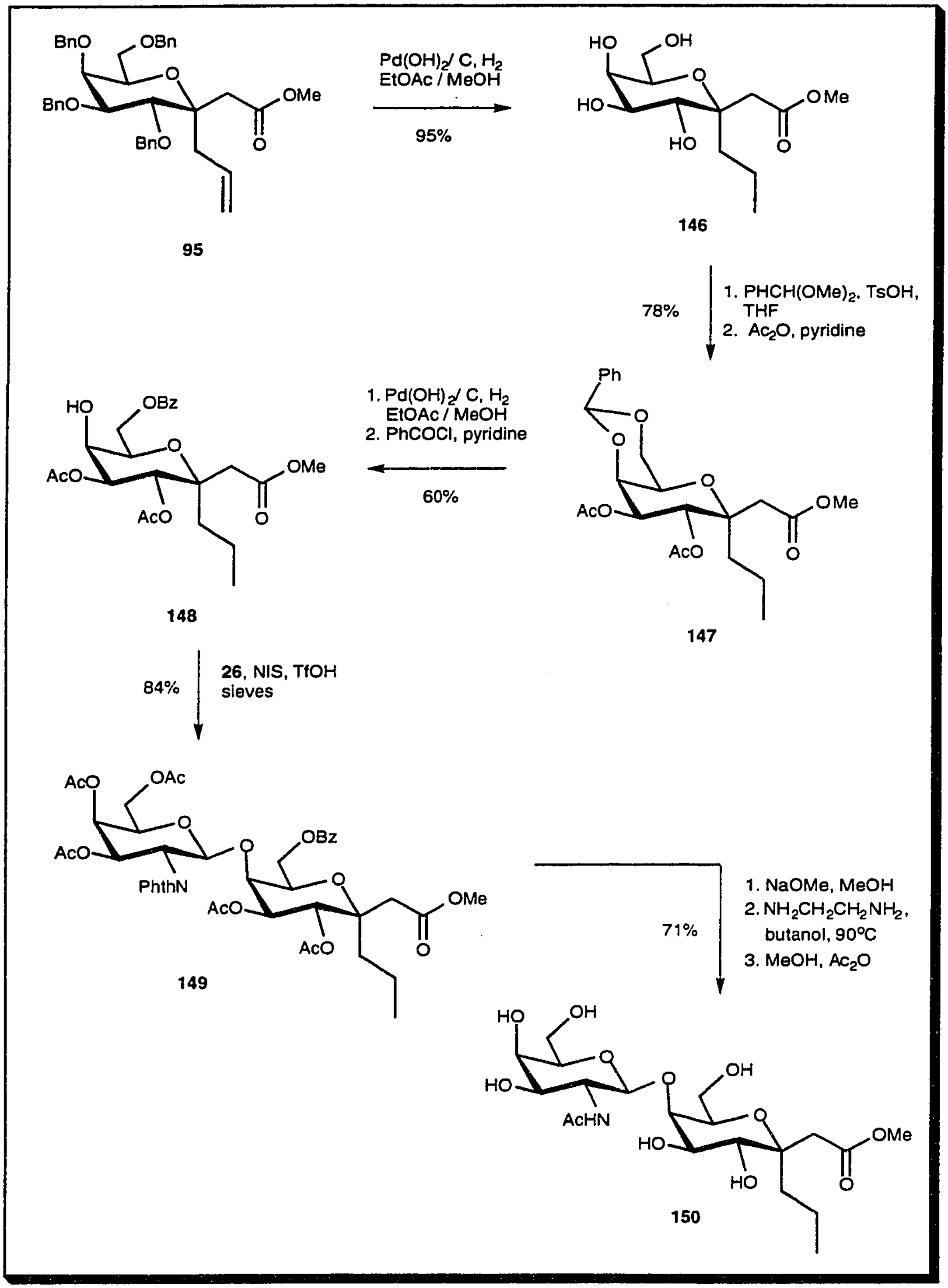


increase the versatility of this strategy. Finally, the acetal **145** might be the starting material for difunctionalization to α,α-disubstituted pyrans (Figure 33) or reduction[195,196] to C-glycosides.

B.12 Incorporation of a α,α-Disubstituted Pyran into the Sequence D-GalNAc-(1→4)-β-D-Gal Recognized by *P. aeruginosa*:

The inhibition results obtained with the modified β-D-GalNAc-(1→4)-β-D-Gal analogs (part G, chapter II) suggested the hydrophobic backbone of the disaccharide receptor as a possible key recognition element. Furthermore, the attachment of a propyl side chain at the 2-position of galactose in the disaccharide substantially enhanced the affinity to the pili, demonstrating the importance of hydrophobic interactions in the stabilization of the pilin-carbohydrate complex. Consequently, it appeared reasonable not to limit the probing of the carbohydrate pilin binding site to only single *O*-alkylated (*O*-methylated or *O*-propylated) disaccharide analogs, but rather to extend it to the hydrophobic backbone (see Figure 32). In this respect, the unblocked backbone modified disaccharide **150** (Figure 56) having a new α-projected propyl side chain at C-1 appeared to be an attractive target compound. The synthesis of compound **150** is outlined in Figure 56. The previously described allyl-C-ketoside **95** was hydrogenated using Pearlman's catalyst[155] to produce the unblocked propyl-C-ketoside **146** in 95% yield. The hydroxyl groups at C-8 and C-6 were selectively protected via acid (TsOH) catalyzed transacetalization with dimethoxytoluene ($PhCH(OMe)_2$) to yield, after acetylation (pyridine, Ac_2O), the blocked acetal **147** in 78% yield. Hydrogenation ($Pd(OH)_2/C$, H_2, EtOAc, MeOH) followed by selective 6-benzoylation gave **148** in 60% yield. Alcohol **148** was glycosylated with the thioglycoside **26** to afford the unnatural C-branched disaccharide **149** in an excellent yield (84%). Deblocking of **149** was done in a three steps sequence. At first the ester protecting groups were removed under basic conditions (NaOMe/MeOH)[153], followed by dephthaloylation ($NH_2CH_2CH_2NH_2$, butanol)[154] and selective N-acylation (Ac_2O, MeOH) to give the unprotected disaccharide **150** in 71% yield. Inhibition data have not yet been obtained with **150**.

Figure 56: Synthesis of a GalNAc-(1→4)-β-D-Gal Analog Incorporating an α,α-Disubstituted Pyran:



C. Conclusions and Suggestions for Future Work:

The synthesis of α,α-disubstituted pyrans was achieved by treatment of α-D-galacto-2-deoxy-oct-3-ulopyranosonate with silyl-based nucleophiles in the presence of Lewis acid. In this way the allyl, azido, cyano, thiophenyl and thioacetyl group could be introduced onto the pyran ring with high α-selectivity. Compound **97***α*, bearing a cyano function at the axial position was reduced and deblocked and the resulting γ-sugar amino acid **122** was used as a building block in solution- and solid phase-synthesis. Sugar β-peptides were accessible via a one pot sequence beginning with α-D-galacto-2-deoxy-octo-3-ulopyranosonic acids. Treatment of this ketoacid with aromatic or aliphatic nitriles under TMSOTf catalyzed conditions resulted in the formation of a glycosyliminoanhydride. Exposure of this intermediate to simple primary amines or amino acids produced 6 novel sugar β-peptides demonstrating the general scope of this new three component reaction. The commercial availability of large numbers of nitriles and amines suggests that the multi component reaction should be attractive for the preparation of β-peptidic sugar diamide libraries. In this respect a future solid-phase approach would be a major improvement and accelerate the synthesis of a large collection of sugar-β-peptides. The library could then be used to screen for inhibitors against a variety of galactose-binding proteins. The results obtained may lead to drug like molecules or at least would address the question whether galactose binding proteins recognize α,α-disubstituted pyrans as glycomimetics. Furthermore, the highly functionalized α,α-disubstituted pyran backbone with its rigid β-sugar amino acid core might find use in peptide mimetic and β-peptide synthesis.

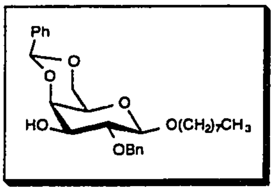
The strategy to use acyclic C-nucleophiles as Michael donors should be extended to a variety of less reactive Michael acceptors, such as 2-cyclohexen-1-one and 2-chlorocyclohexanone (Figure 35) in order to allow a direct comparison with the cyclic C-nucleophile **69**. Finally, the backbone modified α,α-disubstituted pyran incorporated into the sequence β-D-GalNAc-(1→4)-β-D-Gal recognized by *P. aeruginosa* PAK pili has to be tested in a competitive inhibition assay in order to draw conclusions about the properties of the *P. aeruginosa* PAK pili-carbohydrate combining site.

D. Experimental:

General methods:

Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 22° ± 2° C. Analytical thin-layer chromatography (tlc) was performed on silica gel 60-F254 (Merck). Tlc detection was achieved by ultra violet light visualization and by charring with sulfuric acid. All commercial reagents were used as supplied. All chromatography solvents were distilled prior use. Column chromatography was performed using silica gel 60 (Merck 40-60 μM) or beaded silica gel 6RS-8060 (Iatrobeads) manufactured by Iatron Laboratories (Tokyo). ^{1}H-NMR spectra were recorded at 360 MHz (Bruker WM-360) at 500 MHz (Varian Unity-500) and at 600 MHz (Varian INOVA 600) with internal $(CH_3)_4Si$ (δ 0, $CDCl_3$ or CD_3OD). COSY spectra were obtained at 360 MHz (Bruker WM-360), at 500 MHz (Varian Unity-500) and at 600 MHz (Varian INOVA 600). HMBC and TROESY spectra were obtained at 500 MHz (Varian Unity-500) and at 600 MHz (Varian INOVA 600). ^{13}C-NMR spectra were recorded at 75.5 MHz (Bruker AM-300) with internal $(CH_3)_4Si$ (δ 0, $CDCl_3$, CD_3OD or D-5 pyridine). In some cases only partial NMR data are reported. The chemical shifts and coupling constants (as observed splittings) for ^{1}H resonances are reported as though they were first order. The assignments of ^{1}H and ^{13}C resonances were tentative. Organic solutions were concentrated under vacuum at $\leq$ 45°C (bath) / 50 mm Hg. All anhydrous reactions were carried out using starting materials dried over phosphorous pentoxide (P_2O_5) and dry solvents under argon atmosphere. Anhydrous transfers were completed with standard syringe techniques.

Octyl 2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**10**)

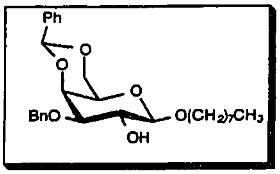


The benzylidene acetal **154** (5.00 g, 13.14 mmol) was dissolved in dichloromethane (300 ml) together with tetrabutylammonium hydrogensulfate (1.29 mg, 4.28 mmol) benzyl bromide (3.15 ml, 26.48 mmol) and sodium hydroxide (5%, 25 ml) solution. The reaction mixture was refluxed for 4 days before more benzyl bromide (1 ml, 8.40 mmol) was added. The refluxing was continued for another two days. The water layer was separated and extracted with dichloromethane (2 x 10 ml) before the combined organic layer was washed with water 1 x 100 ml). The crude material (a mixture of **10**, **11** and **154**) was chromatographed twice (1. dichloromethane /acetone 20 : 1; 2. ethyl acetate / hexane 1 : 4) and gave compound **10** (1.10 g, 18 %) as a white foam: $[\alpha]_D = +15.4^0$ (c 1.0, $CHCl_3$); R_f 0.55 in ethyl acetate/hexane, 6:1; ^1H NMR (360 MHz, $CDCl_3$) δ: 7.55-7.20 (m, 10H, benzyl aromatics), 5.55 (1H, s, PhC$\underline{H}$(O)$_2$), 4.98, 4.73 (2 x d, 2H, J_{gem} = 11.5 Hz, OC$\underline{H}_2$Ph), 4.37 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.33 (dd, 1H, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.22 (dd, 1H, $J_{3,4}$ = 3.5 Hz, $J_{4,5} <$ 1 Hz, H-4), 4.08 (dd, 1H, $J_{5,6b}$ = 1.8 Hz, H-6b), 4.00 (dt, 1H, J_{gem} = 9.9 Hz, J_{vic} = 6.8 Hz, OC$\underline{H}_2$CH$_2$, octyl), 3.74 (dd, br. 1H, $J_{2,3}$ = 9.5 Hz, H-3), 3.62 (dd, 1H, H-2), 3.50 (dt, 1H, J_{gem} = 9.9 Hz, J_{vic} = 6.8 Hz, OC$\underline{H}_2$CH$_2$, octyl), 3.45 (s, br., 1H, H-5), 2.5 (s, br. OH), 1.7-1.2 (m, 12H, 6 x CH$_2$, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH$_3$ octyl); Anal. calcd. for $C_{28}H_{38}O_6$: C 71.46, H 8.14 found: C 71.68, H 8.13.

For further analysis a small amount was acetylated and gave octyl 3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside: ^1H NMR (360 MHz, $CDCl_3$) δ: 7.25-7.55 (m, 10H, benzyl aromatics), 5.50 (s, 1H, PhC$\underline{H}$(O)$_2$), 4.96 (d, 1H, J_{gem} = 11.5 Hz, OC$\underline{H}_2$Ph), 4.93 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.7 Hz, H-3), 4.65 (d, 1H, OC$\underline{H}_2$Ph), 4.47 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.36 (d, br. 1H, $J_{4,5} <$ 1 Hz, H-4), 4.35 (dd, 1H, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$

= 12.5 Hz, H-6a), 4.05 (dd, 1H, $J_{5,6b}$ = 1.7 Hz, H-6b), 4.10 (dt, 1H, J_{gem} = 9.3 Hz, J_{vic} = 6.5 Hz, $OC\underline{H}_2CH_2$, octyl), 3.83 (dd, 1H, H-2), 3.53 (dt, 1H, J_{gem} = 9.9 Hz, J_{vic} = 6.8 Hz, $OC\underline{H}_2CH_2$, octyl), 3.48 (s, br., H-5), 2.05 (s, 3H, OAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl).

Octyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**11**)

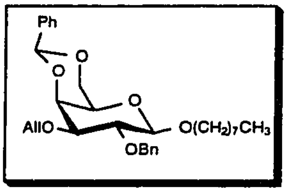


The crude mixture described for compound **10** was chromatographed with ethyl acetate / hexane 1:2. The fastest moving compound was the desired compound **11** (1.65 g, 30 %) which was obtained as a white powder: $[\alpha]_D = +22.4^0$ (c 1.0, $CHCl_3$); R_f 0.64 in ethyl acetate/hexane, 6:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.55-7.25 (m, 10H, benzyl aromatics), 5.45 (s, 1H, $PhC\underline{H}(O)_2$), 4.80 (s, 2H, $OC\underline{H}_2Ph$), 4.31 (dd, 1H, $J_{5,6a}$ = 1.3 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.28 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.13 (d, 1H, $J_{3,4}$ = 3.5 Hz, H-4), 4.03 (dd, 1H, $J_{5,6b}$ = 1.2 Hz, H-6b), 3.98 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 3.94 (dt, 1H, J_{gem} = 9.9 Hz, J_{vic} = 6.8 Hz, $OC\underline{H}_2CH_2$, octyl), 3.53- 3.46 (m, 2H, H-3, $OC\underline{H}_2CH_2$, octyl), 3.34 (s, br., 1H, H-5), 2.54 (s, br., 1H, OH), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3 octyl); Anal. calcd. for $C_{28}H_{38}O_6$: C 71.46, H 8.14 found: C 71.73, H 8.25.

For further analysis a small amount was acetylated and gave octyl 2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside: ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.23-7.55 (m, 10H, benzyl aromatics), 5.48 (1H, s, $PhC\underline{H}(O)_2$), 5.35 (dd, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.9 Hz, H-2), 4.70, 4.60 (2 x d, 2H, J_{gem} = 12.8 Hz), 4.40 (d, 1H, H-1), 4.31 (d, 1H, J_{gem} = 12.1 Hz, H-6a), 4.15 (d, 1H, $J_{3,4}$= 3.4 Hz, H-4), 4.05 (d, 1H, H-6b), 3.87 (dt, 1H, J_{gem} = 9.7 Hz, J_{vic} = 7.3 Hz, $OC\underline{H}_2CH_2$, octyl), 3.83 (dd, 1H, H-2), 3.45 (dt, 1H, J_{gem} = 9.7

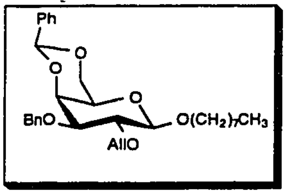
Hz, J_{vic} = 7.3 Hz, $OC\underline{H}_2CH_2$, octyl), 3.35 (s, br., 1H, H-5), 2.05 (s, 3H, OAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl).

Octyl 3-*O*-allyl-2-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**12**)



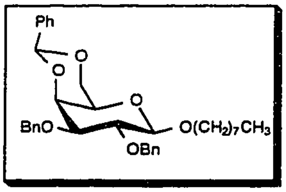
Compound **10** (0.85 g, 1.81 mmol) was dissolved in *N*,*N*-dimethylformamide (50 ml). Sodium hydride (0.45 g, 18.75 mmol) and allyl bromide (0.75 ml, 9.01 mmol) were added. The mixture was stirred for 4 h before methanol (5 ml) was slowly added to destroy the excess of sodium hydride. The mixture was concentrated to dryness, water (50 ml) was added and extracted with dichloromethane (2 x 30 ml). The organic layer was dried (Na_2SO_4) filtered and concentrated. Twenty percent (20%) hexane in ethyl acetate was used as an eluent in the chromatographic purification of the syrup. Product **12** (0.74 g, 80%) was isolated as a clear syrup: $[\alpha]_D = +21.2^0$ (c 1.2, $CHCl_3$); R_f 0.50 in ethyl acetate/hexane, 1:2; ^1H NMR (360 MHz, $CDCl_3$) δ: 7.60-7.25 (m, 10H, benzyl aromatics), 5.95 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.55 (s, 1H, $PhC\underline{H}(O)_2$), 5.30 (m, 1H, $OCH_2CHC\underline{H}_2$), 5.18 (m, 1H, $OCH_2CHC\underline{H}_2$), 4.92, 4.75 (2 x d, 2H, $OC\underline{H}_2Ph$), 4.39 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.33 (dd, 1H, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 12.3 Hz, H-6a), 4.27-4.18 (m, 3H, H-4, 2 x $OC\underline{H}_2CHCH_2$), 4.05 (dd, 1H, $J_{5,6b}$ = 1.7 Hz, H-6b), 3.98 (dt, 1H, J_{gem} = 9.9 Hz, J_{vic} = 6.8 Hz, $OC\underline{H}_2CH_2$, octyl), 3.78 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-2), 3.53-3.42 (m, 2H, H-3, $OC\underline{H}_2CH_2$, octyl), 3.35 (s, br., 1H, H-5), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); Anal. calcd. for $C_{31}H_{42}O_6$: C 72.91, H 8.29 found: C 72.67, H 8.41.

Octyl 2-*O*-allyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**13**)



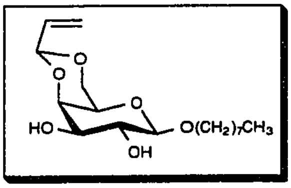
Compound **11** (0.85 g, 1.81 mmol) was dissolved in *N*,*N*-dimethylformamide (50 ml). Sodium hydride (0.45 g, 18.75 mmol) and allyl bromide (0.75 ml, 9.01 mmol) were added. The mixture was stirred for 4 h. Methanol (5 ml) was then slowly added to destroy the excess sodium hydride. The mixture was concentrated to dryness, water (50 ml) was added and extracted with dichloromethane (3 x 30 ml). The organic layer was dried (Na_2SO_4), filtered (celite) and concentrated. Twenty percent (20%) hexane in ethyl acetate was used as the eluent in the chromatographic purification of the syrup. Product **13** (0.74 g, 80%) was isolated as a clear syrup; $[\alpha]_D = +29.7$ (c 1.2, $CHCl_3$); R_f 0.45 in ethyl acetate/hexane, 1:2; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.60-7.26 (m, 10H, benzyl aromatics), 5.99 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.50 (s, 1H, $PhC\underline{H}(O)_2$), 5.30 (m, 1H, $OCH_2CHC\underline{H}_2$), 5.11 (m, 1H, $OCH_2CHC\underline{H}_2$), 4.87, 4.67 (2 x d, 2H, $OC\underline{H}_2Ph$), 4.41 (m, 1H, $OC\underline{H}_2CHCH_2$), 4.33-4.23 (m, 3H, H-1, H-6a, $OC\underline{H}_2CHCH_2$), 4.08 (d, 1H, $J_{3,4} = 3.7$ Hz), 4.00 (dd, 1H, $J_{5,6b} = 1.8$ Hz, $J_{6a,6b} = 12.1$ Hz, H-6b), 3.95 (dt, 1H, $J_{gem} = 9.9$ Hz, $J_{vic} = 6.8$ Hz, $OC\underline{H}_2CH_2$, octyl), 3.69 (dd, 1H, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 9.7$ Hz, H-2), 3.49 (dd, 1H, H-3), 3.46 (dt, 1H, $J_{gem} = 9.9$ Hz, $J_{vic} = 6.8$ Hz, $OC\underline{H}_2CH_2$, octyl), 3.29 (s, br., H-5), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, $J_{vic} = 7.5$ Hz, CH_3, octyl); Anal. calcd. for $C_{31}H_{42}O_6$: C 72.91, H 8.29 found: C 72.67, H 8.41.

Octyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (**14**)



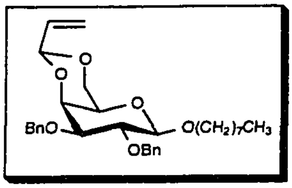
Compound **154** (1.00 g, 2.63 mmol) was dissoved in N,N-dimethylformamide (30 ml). Sodium hydride (0.45 g, 18.75 mmol) and benzyl bromide (1.07 ml, 9.01 mmol) were added. The mixture was stirred for 3 h. Methanol (5 ml) was then slowly added to destroy the excess sodium hydride. The mixture was concentrated to dryness, water (50 ml) was added and extracted with dichloromethane (3 x 30 ml). The organic layer was dried (Na_2SO_4), filtered (celite) and concentrated. Twenty percent (20%) hexane in ethyl acetate was used as an eluent in the chromatographic purification of the syrup. Product **14** (1.40 g, 95%) was isolated as a clear syrup. $[\alpha]_D = +24.5^0$(c 1.2, $CHCl_3$); R_f 0.55 in ethyl acetate/hexane, 1:2; ^1H NMR (360 MHz, $CDCl_3$) δ: 7.60-7.20 (m, 15H, benzyl aromatics), 5.50 (s, 1H, $PhCH(O)_2$), 4.95 (d, 1H, J_{gem} = 12.3 Hz, $OC\underline{H}_2Ph$), 4.82-4.70 (m, 3H, 3 x $OC\underline{H}_2Ph$), 4.38 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.30 (d, 1H, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.10 (d, 1H, $J_{3,4}$ = 3.7 Hz, H-4), 4.05-3.95 (m, 2H, H-6b, $OC\underline{H}_2CH_2$, octyl), 3.84 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-2), 3.56 (dd, 1H, H-3), 3.50 (dt, 1H, J_{gem} = 10.0 Hz, J_{vic} = 7.0 Hz, $OC\underline{H}_2CH_2$, octyl), 3.31 (s, br., 1H, H-5), 1.7-1.2 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); Anal. calcd. for $C_{35}H_{44}O_6$: C 74.97, H 7.91 found: C 74.72, H 8.01.

Octyl 4,6-*O*-allylidene-β-D-galactopyranoside (**15**)



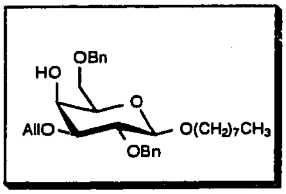
Octyl galactoside (1.70 g, 5.82 mmol) was dissolved in dry acetonitrile (180 ml). P-toluenesulfonic acid (0.17 g, 0.99 mmol) and acroleindimethyl acetal (0.97 ml, 8.73 mmol) was added. The reaction was stirred for 0.5 h. Triethyl amine (0.21 ml, 1.50 mmol) was added and he mixture was concentrated and chromatographed using 14 % hexane in ethyl acetate. Product **15** (1.12 g, 58%) was isolated as a white foam: $[\alpha]_D = -36.9^0$ (c 1.5, $CHCl_3$); R_f 0.40 in ethyl acetate; ^1H NMR (360 MHz, $CDCl_3$) δ: 5.96-5.85 (m, 1H, $(O)_2CHC\underline{H}CH_2$), 5.47 (m, 1H, $(O)_2CHCHC\underline{H}_2$), 5.32 (m, 1H, $(O)_2CHCHC\underline{H}_2$), 5.00 (d, J_{vic} = 5.0 Hz, 1H, $(O)_2C\underline{H}CHCH_2$), 4.28-4.18 (m, 2H, H-1, H-6a), 4.05 (dd, 1H, $J_{3,4}$ = 3.7 Hz, $J_{4,5}$ = 1.1 Hz, H-4), 3.95 (d, 1H, $J_{6a,6b}$ = 10.3 Hz, H-6b), 3.94 (dt, 1H, J_{gem} = 9.9 Hz, J_{vic} = 7.2 Hz, $OC\underline{H}_2CH_2$, octyl), 3.71 (dd, 1H, $J_{1,2}$ = 7.6 Hz, $J_{2,3}$ = 9.6 Hz, H-2), 3.65 (dd, 1H, H-3), 3.46 (dt, 1H, J_{gem} = 9.9 Hz, J_{vic} = 6.8 Hz, $OC\underline{H}_2CH_2$, octyl), 3.38 (s, br., H-5), 2.70-2.40 (s, br, 2H, 2 x OH), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); Anal. calcd. for $C_{17}H_{30}O_6$: C 61.80, H 9.15 found: C 61.71, H 9.17.

Octyl 4,6-*O*-allylidene-2,3-di-*O*-benzyl–β-D-galactopyranoside (**16**)



The acetal **15** (1.05 g 3.07 mmol) was dissolved in N,N-dimethylformamide (30 ml). Sodium hydride (720 mg, 30 mmol) and benzyl bromide (3.75 ml, 31.50 mmol) were added. The reaction mixture was stirred vigorously for 3h. Methanol (5 ml) was then slowly added to destroy the excess sodium hydride. The mixture was stirred for an additional 5 h before it was concentrated under reduced pressure. Water (50 ml) was added and the aqueous phase was extracted with dichloromethane (3 x 30 ml). The solvent was removed under reduced pressure and chromatographed using 33% ethyl acetate in hexane. The desired compound **16** (1.41 g, 90%) was obtained as a transparent syrup; $[\alpha]_D$ = -16.5° (c 2.3, $CHCl_3$); R_f 0.63 in hexane/ethyl acetate, 3:1; ^1H NMR (360 MHz, $CDCl_3$) δ: 7.45-7.20 (m, 10H, benzyl aromatics), 6.00 (ddd, 1H, J_{vic} = 5.0 Hz, J_{vic}(cis) = 10.5 Hz, J_{vic}(trans) = 17.5 Hz, $O_2CHC\underline{H}CH_2$), 5.50 (d, 1H, $OCH_2CHC\underline{H}_2$), 5.30 (d, 1H, $OCH_2CHC\underline{H}_2$), 5.00-4.85 (m, 2H, $O_2C\underline{H}CHCH_2$, 1 x $OC\underline{H}_2Ph$), 4.83-4.67 (m, 3H, 3 x $OC\underline{H}_2Ph$), 4.30 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.20 (dd, 1H, $J_{5,6a}$ = 1.3 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 3.98 (dt, 1H, J_{gem} = 9.9 Hz, J_{vic} = 6.8 Hz, $OC\underline{H}_2CH_2$, octyl), 3.93 (d, 1H, $J_{3,4}$ = 3.7 Hz, H-4), 3.86 (dd, 1H, $J_{5,6b}$ = 1.5 Hz, H-6b), 3.80 (dd, 1H, $J_{2,3}$ = 9.5 Hz, H-2), 3.47 (dd, 1H, H-3), 3.44 (dt, 1H, J_{gem} = 9.9 Hz, J_{vic} = 6.8 Hz, $OC\underline{H}_2CH_2$, octyl), 3.25 (s, br., 1H, H-5), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); Anal. calcd. for $C_{31}H_{42}O_6$: C 72.91, H 8.28 found: C 72.75, H 8.45.

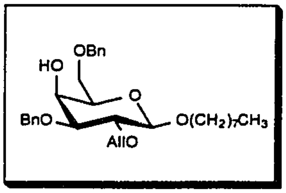
Octyl 3-O-allyl-2,6 di-O-benzyl-β-D-galactopyranoside (**17**)



The acetal **12** (715 mg, 1.40 mmol) was dissolved in tetrahydrofuran (80 ml). Sodium cyanoborohydride (1.05 g, 16.71 mmol) and two crystals of methyl orange were added. To this mixture a cold saturated anhydrous hydrochlorid ether solution was dropwise added under an argon atmosphere. The addition of the acidic ether solution was continued until the development of hydrogen gas ceased and the solution became red. It was advantageous to add more acidic ether (~ 5 ml) after the indicator turned red. The mixture was stirred for another 2 h before Dowex 50 (H^+, 1.9 g) was added. The stirring was continued for an additional 2 h. The mixture was filtered (celite) and concentrated. Water (100 ml) was added and the aqueous phase was extracted with dichloromethane (3 x 60 ml). The combined organic layer was washed with sodium bicarbonate (2 x 20 ml), dried (Na_2SO_4), concentrated and chromatographed using 20% ethyl acetate in hexane as the eluent. The major product **17** (0.54 g, 75%) was obtained as a colourless oil: $[\alpha]_D = -6.3^0$(c 0.8, $CHCl_3$); R_f 0.55 in toluene/ethyl acetate, 3:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.25 (m, 10H, benzyl aromatics), 5.93 (ddt, 1H, J_{vic}trans = 17.2 Hz, J_{vic}(cis) = 10.6 Hz, J_{vic} = 5.7 Hz, $OCH_2C\underline{H}CH_2$), 5.33-5.25 (m, 1H, $OCH_2CHC\underline{H}_2$), 5.19-5.13 (m, 1H, $OCH_2CHC\underline{H}_2$), 4.89, 4.70 (2 x d, 2H, $OC\underline{H}_2Ph$), 4.60 (s, 2H, $OC\underline{H}_2Ph$), 4.35 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.35 (d, 2H, $OC\underline{H}_2CHCH_2$), 4.01 (d, 1H, $J_{3,4}$= 3.1 Hz, H-4), 3.92 (dt, 1H, J_{gem} = 9.4 Hz, J_{vic} = 6.5 Hz, $OC\underline{H}_2CH_2$, octyl), 3.82 (dd, 1H, $J_{5,6a}$ = 6.0 Hz, $J_{6a,6b}$ = 9.9 Hz, H-6a), 3.73 (dd, 1H, $J_{5,6b}$ = 5.9 Hz, H-6b), 3.61-3.47 (m, 3H, H-2, H-5, $OC\underline{H}_2CH_2$, octyl), 3.40 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-3), 2.45 (s, br., 1H, OH), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); Anal. calcd. for $C_{31}H_{44}O_6$: C 72.62, H 8.65 found: C 72.84, H 8.85.

The product was further characterized via acetylation to yield octyl 4-*O*-acetyl 2-*O*-allyl-3,6 di-*O*-benzyl-β-D-galactopyranoside (**18**). ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 10H, benzyl aromatics), 5.95-5.85 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.48 (dd, 1H, $J_{3,4}$ = 3.1 Hz, H-4), 5.33-5.25 (m, 1H, J_{vic}(trans) = 17.3 Hz, $OCH_2CHC\underline{H}_2$), 5.19-5.13 (m, 1H, J_{vic} (cis) = 10.4 Hz, $OCH_2CHC\underline{H}_2$), 4.85, 4.71, 4.55, 4.46 (4 x d, 4H, J_{gem} = 10.8 Hz, J_{gem} = 11.8 Hz, 2 x $OC\underline{H}_2Ph$), 4.37 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1), 4.20 (m, 1H, J_{vic} = 5.4 Hz, J_{gem} = 12.7 Hz, $OC\underline{H}_2CHCH_2$), 4.04 (m, 1H, J_{vic} = 5.9 Hz, $OC\underline{H}_2CHCH_2$), 3.95 (dt, 1H, J_{gem} = 9.4 Hz, J_{vic} = 6.5 Hz, $\underline{OCH_2}CH_2$, octyl), 3.69 (t, br., 1H, H-5), 3.60-3.43 (m, 5H, H-2, H-3, H-6a, H-6b, $OC\underline{H}_2CH_2$, octyl), 2.08 (s, 3H, OAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl).

Octyl 2-*O*-allyl-3,6 di-*O*-benzyl-β-D-galactopyranoside (**19**)

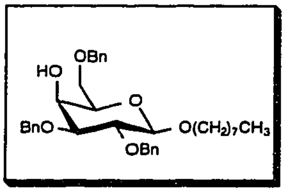


The acetal **13** (0.95 g, 1.86 mmol) was dissolved in tetrahydrofuran (80 ml). Sodium cyanoborohydride (1.14 g, 18.14 mmol) and two crystals of methyl orange were added. To this mixture a cold saturated anhydrous hydrochlorid ether solution was dropwise added under an inert argon atmosphere. The addition of the acidic ether solution was continued until the development of hydrogen gas ceased and the solution became red. It was advantageous to add more acidic ether (~ 5 ml) after the indicator turned red. The mixture was stirred for another 2 h. Dowex 50 (H^+ , 1.9 g) was then added and the mixture was stirred for an additional 2 h. The mixture was filtered (celite) and concentrated. Water (100 ml) was added and the aqueous phase was extracted with dichloromethane (3 x 60 ml). The combined organic layer was washed with sodium bicarbonate (2 x 20 ml), dried (Na_2SO_4), concentrated and chromatographed using 20% ethyl acetate in hexane as the eluent. The major product **19** (0.63 g, 66%) was obtained as a colourless oil; $[\alpha]_D$ = -12.3^0 (c 0.6, $CHCl_3$); R_f 0.62 in toluene/ethyl acetate, 3:1; ^{1}H NMR (360 MHz, $CDCl_3$)

δ: 7.40-7.20 (m, 10H, benzyl aromatics), 5.95 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.33-5.25 (m, 1H, J_{vic} (trans) = 17.3 Hz, $OCH_2CHC\underline{H}_2$), 5.19-5.13 (m, 1H, J_{vic}cis = 10.3 Hz, $OCH_2CHC\underline{H}_2$), 4.58, 4.75 (2 x s, 4H, 2 x $OC\underline{H}_2Ph$), 4.43-4.35 (m, 1H, $OC\underline{H}_2CHCH_2$), 4.28 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.25-4.18 (m, 1H, $OC\underline{H}_2CHCH_2$), 4.00 (d, 1H, $J_{3,4}$ = 3.1 Hz, H-4), 3.92 (dt, 1H, J_{gem} = 9.4 Hz, J_{vic} = 6.5 Hz, $OC\underline{H}_2CH_2$, octyl), 3.78 (dd, 1H, $J_{5,6a}$ = 6.0 Hz, $J_{6a,6b}$ = 10.0 Hz, H-6a), 3.72 (dd, 1H, $J_{5,6b}$ = 5.9 Hz, H-6b), 3.57-3.45 (m, 3H, H-2, H-5, $OC\underline{H}_2CH_2$, octyl), 3.42 (dd, 1H, $J_{2,3}$ = 9.4 Hz, H-3), 2.45 (s, br., 1H, OH), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); Anal. calcd. for $C_{31}H_{44}O_6$: C 72.62, H 8.65 found: C 72.93, H 8.82.

The product was further characterized via acetylation to yield octyl 4-*O*-acetyl-2-*O*-allyl-3,6-di-*O*-benzyl-β-D-galactopyranoside (**20**). ^1H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 10H, benzyl aromatics), 6.05-5.90 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.55 (dd, 1H, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 1.7 Hz, H-4), 5.33-5.25 (m, 1H, J_{vic}trans = 17.3 Hz, $OCH_2CHC\underline{H}_2$), 5.19-5.13 (m, 1H, J_{vic}cis = 10.3 Hz, $OCH_2CHC\underline{H}_2$), 4.75, 4.60, 4.55, 4.45 (4 x d, 4H, 2 x $OC\underline{H}_2Ph$), 4.40-4.32 (m, 1H, $OC\underline{H}_2CHCH_2$), 4.28 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1), 4.25-4.18 (m, 1H, $OC\underline{H}_2CHCH_2$), 3.92 (dt, 1H, J_{gem} = 9.4 Hz, J_{vic} = 6.5 Hz, $OC\underline{H}_2CH_2$, octyl), 3.78 (dd, 1H, $J_{5,6a}$ = 6.0 Hz, $J_{6a,6b}$ = 10.0 Hz, H-6a), 3.72 (dd, 1H, $J_{5,6b}$ = 5.9 Hz, H-6b), 3.70- 3.40 (m, 4H, H-2, H-3, H-5, 1 x $OC\underline{H}_2CH_2$, octyl), 2.10 (s, 3H, OAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl).

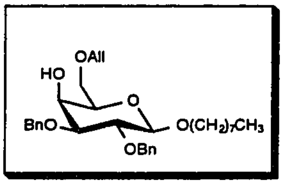
Octyl 2,3,6 tri-*O*-benzyl-β-D-galactopyranoside (**21**)



The acetal **14** (0.67 g, 1.19 mmol) was dissolved in tetrahydrofuran (60 ml). Sodium cyanoborohydride (0.81 g, 12.89 mmol) and two crystals of methyl orange were added. To this mixture a cold saturated anhydrous hydrochlorid ether solution was dropwise added under an inert argon atmosphere. The addition of the acidic ether solution was continued until the development of hydrogen gas ceased and the solution became red. It was advantageous to add more acidic ether (~ 5 ml) after the indicator turned red. The mixture was stirred for another 2 h before Dowex 50 (H^+, 1.9 g) was added. The stirring was continued for an additional 2 h. The mixture was filtered (celite) and concentrated. Water (100 ml) was added and the aqueous phase was extracted with dichloromethane (3 x 60 ml). The combined organic layer was washed with sodium bicarbonate (2 x 25 ml), dried (Na_2SO_4), concentrated and chromatographed using 20% ethyl acetate in hexane as the eluent. The product **21** (0.52 g, 77%) was obtained as a colourless oil; $[\alpha]_D = +12.0^0$ (c 0.8, $CHCl_3$); R_f 0.15 in dichloromethane; ^1H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 15H, benzyl aromatics), 4.93, 4.73 (2 x d, 2H, J_{gem} = 11.0 Hz, $OC\underline{H}_2Ph$) 4.73, 4.59 (2 x s , 4H, $OC\underline{H}_2Ph$), 4.35 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.03 (d, 1H, $J_{3,4}$ = 3.1 Hz, H-4), 3.95 (dt, 1H, J_{gem} = 9.4 Hz, J_{vic} = 6.4 Hz, $OC\underline{H}_2CH_2$, octyl), 3.81 (dd, 1H, $J_{5,6a}$ = 6.0 Hz, $J_{6a,6b}$ = 9.9 Hz, H-6a), 3.73 (dd, 1H, $J_{5,6a}$ = 5.9 Hz, H-6b), 3.63 (dd, 1H, $J_{2,3}$ = 9.0 Hz, H-2), 3.55 (t, 1H, H-5), 3.53-3.47 (m, 2H, H-3, $OC\underline{H}_2CH_2$, octyl), 2.45 (s, br., 1H, OH), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); Anal. calcd. for $C_{35}H_{46}O_6$: C 74.70, H 8.24 found: C 74.91, H 8.33.

The product was further characterized via acetylation to yield octyl 4-*O*-acetyl-2,3,6 tri-*O*-benzyl-β-D-galactopyranoside (**22**). ^1H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 15H, benzyl aromatics), 5.57 (d, 1H, $J_{3,4}$ = 2.0 Hz, H-4), 4.88, 4.76, 4.71, 4.57, 4.52, 4.48 (6 x d , 6H, J_{gem} = 10.9 Hz, 11.5 Hz, 11.9 Hz, $OC\underline{H}_2Ph$), 4.38 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 3.95 (dt, 1H, J_{gem} = 9.6 Hz, J_{vic} = 6.7 Hz, $OC\underline{H}_2CH_2$, octyl), 3.62-3.48 (m, 6H, H-2, H-3, H-5, H-6a, H-6b, $O\underline{CH}_2CH_2$, octyl), 2.10 (s, 3H, OAc), 1.7-1.2 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl).

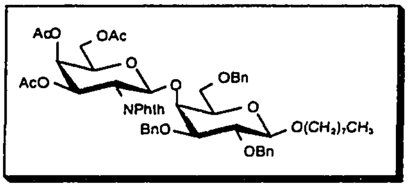
Octyl 4-*O*-acetyl-6-*O*-allyl-2,3 di-*O*-benzyl-β-D-galactopyranoside (**23**)



The acetal **12** (1.26 g, 2.46 mmol) was dissolved in tetrahydrofuran (100 ml). Sodium cyanoborohydride (1.55 g, 24.67 mmol) and two crystals of methyl orange were added. To this mixture a cold saturated anhydrous hydrochlorid ether solution was dropwise added under an argon atmosphere. The addition of the acidic ether solution was continued until the development of hydrogen gas ceased and the solution became red. It was advantageous to add more acidic ether (~ 10 ml) after the indicator turned red. The mixture was stirred for another 2 h before Dowex 50 (H^+, 1.9 g) was added. The stirring was continued for an additional 2 h. The mixture was filtered over celite and concentrated. Water (150ml) was added and the aqueous phase was extracted with dichloromethane (3 x 70 ml). The combined organic layer was washed with sodium bicarbonate (2 x 30 ml), dried (Na_2SO_4), concentrated and chromatographed using 20% ethyl acetate in hexane as the eluent. The product **23** (0.95 g, 75%) which was impured (~10%) with unreacted **14** was obtained as a white foam. Further chromatographic purification did not enhance the purity. This crude mixture was used for further reactions.

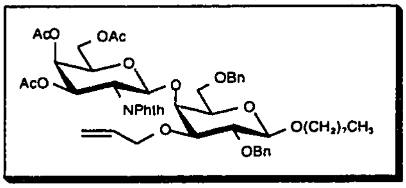
A small amount of this crude mixture was acetylated and purified using 20% ethyl acetate in hexane as the eluent to yield octyl 4-*O*-acetyl-6-*O*-allyl 2,3 di-*O*-benzyl-β-D-galactopyranoside (**24**) as a white foam; $[\alpha]_D = -5.7^0$(c 0.7, $CHCl_3$); R_f 0.4 in toluene/ethyl acetate 3 : 1; ^1H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 10H, benzyl aromatics), 5.95-5.85 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.57 (d, 1H, $J_{3,4}$ = 2.1 Hz, H-4), 5.33-5.25 (m, 1H, J_{vic}trans = 17.2 Hz, $OCH_2CHC\underline{H}_2$), 5.19-5.13 (m, 1H, J_{vic}cis = 10.3 Hz, $OCH_2CHC\underline{H}_2$), 4.90, 4.77, 4.73, 4.53 (4 x d, 4H, J_{gem} = 10.8 Hz, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.39 (d, 1H, $J_{1,2}$= 7.3 Hz, H-1), 4.07-3.93 (m, 3H, 2 x $OC\underline{H}_2CHCH_2$, $O\underline{CH}_2CH_2$, octyl), 3.68 (t, 1H, $J_{5,6}$ = 5.8 Hz, H-5), 3.62-3.48 (m, 5H, H-2, H-3, H-6a, H-6b, O$\underline{CH}_2$CH$_2$, octyl), 2.15 (s, 3H, OAc), 1.70-1.20 (m, 12H, 6 x CH$_2$, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH$_3$, octyl); Anal. calcd. for $C_{33}H_{46}O_7$: C 71.45, H 8.36 found: C 71.70, H 8.46.

Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**27**)



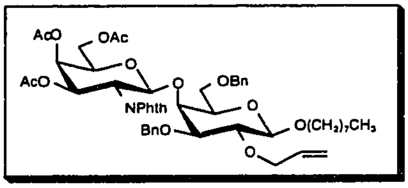
A mixture of the alcohol **21** (204.90 mg, 0.36 mmol), the thioglycoside **26** (225.00 mg, 0.48 mmol) and molecular sieves (0.6 g, 4 Å) in dry dichloromethane (20 ml) was stirred under argon for 30 min at 0°C. *N*-iodosuccinimide (140.4 mg, 62.4 mmol) was added directly followed by a addition of trifluoromethanesulfonic acid (0.08 mmol). The reaction mixture was stirred for an additional 1 h at 0°C. Saturated sodium bicarbonate solution (10 ml) was then added. The organic layer was washed with saturated sodium thiosulfate (5 ml) and water (5 ml), dried (Na_2SO_4) and concentrated to give a viscous syrup. Twelve percent ethyl acetate in toluene was used in chromatographic purification of the syrup. Product **27** (225.00 mg, 64 %) was isolated as a clear syrup; $[\alpha]_D$ = -23.8^0(c 0.8, CHCl$_3$); R_f 0.37 in toluene/ethyl acetate, 3:1; ^{1}H NMR (360 MHz, CDCl$_3$) δ: 7.90-7.55 (m, 4H, phthalimido), 7.40-6.90 (m, 15H, benzyl aromatics), 6.09 (dd, 1H, $J_{2',3'}$ = 11.6 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 5.49 (d, 1H, H-4'), 5.33 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 4.66 (dd, 1H, H-2'), 4.65 (s, 2H, OC$\underline{H}_2$Ph), 4.43 (d, 1H, $J_{6'a,6'b}$ = 10.8 Hz, H-6'a), 4.37, 4.18 (2 x d, 2H, J_{gem} = 12.6 Hz, OC$\underline{H}_2$Ph), 4.15 (d, 1H, $J_{1,2}$ = 7.3 Hz, H-1), 4.13 (d, 1H, OC$\underline{H}_2$Ph), 4.08-4.00 (m, 2H, H-4, OC$\underline{H}_2$Ph), 3.88 (dt, 1H, J_{gem} = 9.5 Hz, J_{vic} = 6.6 Hz, OC$\underline{H}_2$CH$_2$, octyl), 3.75 (dd, 1H, $J_{5,6a}$ = 5.5 Hz, $J_{6a,6b}$ = 10.1 Hz, H-6a), 3.65 (dd, 1H, $J_{5,6b}$ = 6.0 Hz, H-6b), 3.62-3.55 (m, 2H, H-5', H-6'b), 3.40 (dt, 1H, J_{gem} = 9.5 Hz, J_{vic} = 6.6 Hz, OC$\underline{H}_2$CH$_2$, octyl), 3.33(t, 1H, H-5), 3.18 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 2.7 Hz, H-3), 3.09 (dd, 1H, H-2), 2.20, 2.05, 1.90 (3 x s, 9H, 3 x OAc), 1.70-1.20 (m, 12H, 6 x CH$_2$, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH$_3$, octyl); ^{13}C NMR (75.5 MHz, CDCl$_3$) δ: 170.42 x 2, 169.94, 168.50, 167.67 (5 x CO), 138.42-123.23 (aromatics), 103.37, 99.45 (C-1, C-1'), 79.82 (C-H), 79.73 (C-H), 76.30 (C-H), 75.06 (C-H$_2$), 73.50 (C-H$_2$), 73.23 (C-H), 72.95 (C-H$_2$), 70.23 (C-H), 69.98 (C-H$_2$), 69.69 (C-H$_2$), 67.60 (C-H), 66.50 (C-H), 61.34 (C-H$_2$), 51.36 (C-2'), 31.89, 29.73, 29.49, 29.30, 26.20, 22.72 (6x C-H$_2$, octyl), 20.78, 20.66 (2 x OAc), 14.14 (C-H$_3$, octyl); Anal. calcd. for $C_{55}H_{65}O_{15}N_1$: C 67.40 H 6.68, N 1.43, found: C 67.34, H 6.74, N 1.45

Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3-*O*-allyl-2,6-di-*O*-benzyl-β-D-galactopyranoside (**28**)



A mixture of the alcohol **17** (364.0 mg, 0.71 mmol), the donor **25**^{198} (1.42 g, 2.85 mmol), molecular sieves (4.2 g, 4 Å), tetramethylurea (0.43 ml, 3.61 mmol) and silvertriflate (845.00 mg, 3.28 mmol) in dry dichloromethane (50 ml) was stirred under argon at 0°C for 2 h. The temperature was raised to room temperature and the stirring continued for an additional 24 h. The mixture was filtered (celite), water (50 ml) was added and the water phase was extracted with dichloromethane (3 x 40 ml). The organic layer was dried (Na$_2$SO$_4$), concentrated, and chromatographed using 15% ethyl acetate in toluene as the eluent. The product **28** was isolated as a colourless oil (0.25 g, 45%) together with unreacted alcohol **17** (60 mg); $[\alpha]_D = -3.25^0$ (c 2.0, CHCl$_3$); R_f 0.37 in toluene/ethyl acetate, 3:1; ^{1}H NMR (360 MHz, CDCl$_3$) δ: 7.90-7.55 (m, 4H, phthalimido), 7.40-6.95 (m, 10H, benzyl aromatics), 6.05 (dd, 1H, $J_{2',3'}$ = 11.6 Hz, $J_{3',4'}$ = 3.4 Hz, H-3'), 5.77-5.68 (m, 1H, OCH$_2$C$\underline{H}$CH$_2$), 5.49 (d, 1H, H-4'), 5.32 (d, 1H, $J_{1',2'}$ = 8.3 Hz, H-1'), 5.13-5.04 (m, 2H, OCH$_2$CHC$\underline{H}_2$), 4,64 (dd, 1H, H-2') 4.57 (s, 2H, OC$\underline{H}_2$Ph), 4.36 (d, 1H, J_{gem} = 10.4 Hz, OC$\underline{H}_2$Ph), 4.20-4.03 (m, 4H), 3.92-3.78 (m, 4H), 3.68 (t, 1H, H-5), 3.65 (m, 1H, OC$\underline{H}_2$CHCH$_2$), 3.55 (d, 1H), 3.45-3.37 (m, 2H, H-5, $OC\underline{H}_2CH_2$, octyl), 3.13 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 2,9 Hz, H-3), 3.03 (dd, 1H, $J_{1,2}$ = 7.6 Hz, H-2), 2.15, 2.05, 1.89 (3 x s, 9H, 3 x OAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 170.40 x 2, 169.90, 168.40, 167.60 (5 x CO), 138.42-117.98 (aromatic, olefinic), 103.32, 99.96 (C-1, C-1'), 79.82 (C-H), 79.74 (C-H), 77.22 (C-H), 75.08 (C-H_2), 73.56 (C-H_2), 73.23 (C-H), 72.46 (C-H_2), 70.17 (C-H), 69.92 (C-H_2), 69.64 (C-H_2), 67.59 (C-H), 66.49 (C-H), 61.36 (C-H_2) 51.33 (C-2'), 31.88, 29.71, 29.48, 29.29, 26.18, 22.71 (6 x C-H_2,octyl), 20.77, 20.69, 20.64 (3 x OAc), 14.13 (C-H_3, octyl); Anal. calcd. for $C_{51}H_{63}O_{15}N_1$: C 65.86, H 6.82, N 1.50 found: C 65.59, H 6.77, N 1.53.

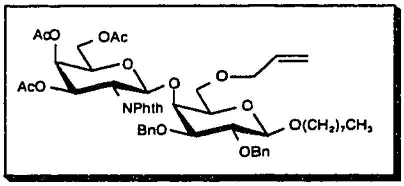
Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-2-*O*-allyl-3,6-di-*O*-benzyl-β-D-galactopyranoside (**29**)



A mixture of the alcohol **19** (0.62 g, 1.21 mmol), the donor **25** (1.80 g, 3.61 mmol), molecular sieves (3,70 g, 4 Å), tetramethylurea (0.55 ml, 4.91 mmol) and silver trifluoromethanesulfonate (1.08g, 4.21 mmol) in dry dichloromethane (50 ml) was stirred under argon at 0°C for 2 h. The temperature was raised to room temperature and the stirring continued for an additional 24 h. The mixture was filtered (celite), water (50 ml) was added and the water phase was extracted with dichloromethane (3 x 40 ml). The organic layer was dried (Na_2SO_4), concentrated, and chromatographed using 15% ethyl acetate in toluene as the eluent. The product **29** was isolated as a colourless oil (567.00 mg, 74%) together with unreacted alcohol **19** (0.20 g); $[\alpha]_D = -19.3^0$(c 1.0, $CHCl_3$); R_f 0.35 in toluene/ethyl acetate, 3:1; ^1H NMR (360 MHz, $CDCl_3$) δ: 7.90-7.55 (m, 4H, phthalimido), 7.40-7.20 (m, 10H, benzyl aromatics), 6.05 (dd, 1H, $J_{2',3'}$ = 11.5 Hz, $J_{3',4'}$ = 3.4 Hz, H-3'), 5.63-5.51 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.48 (d, 1H, H-4'), 5.32 (d, 1H,

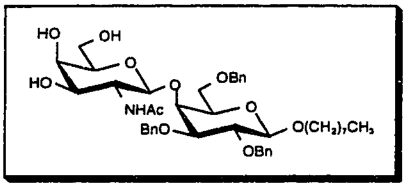
$J_{1',2'}$ = 8.4 Hz, H-1'), 5.05-4.94 (m, 2H, $OCH_2CHC\underline{H}_2$), 4,61 (dd, 1H, H-2') 4.52 (s, 2H, $OC\underline{H}_2Ph$), 4.35 (d, 1H, J_{gem} = 12.6 Hz, $OC\underline{H}_2Ph$), 4.18-4.00 (m, 5H, H-1, H-5', H-6'a, H-6'b, $OC\underline{H}_2Ph$), 3.91-3.81 (m, 2H, $OC\underline{H}_2CHCH_2$, $OC\underline{H}_2CH_2$, octyl), 3.73 (dd, 1H, $J_{5,6a}$ = 5.5 Hz, $J_{6a,6b}$ = 10.2 Hz, H-6a), 3.63 (dd, 1H, $J_{5,6b}$ = 6.0 Hz, H-6b), 3.59 (d, 1H, $J_{3,4}$ = 2.7 Hz, H-4), 3.36 (dt, 1H, J_{gem} = 9.4 Hz, J_{vic} = 6.9 Hz, $O\underline{CH}_2CH_2$, octyl), 3.32 (t, 1H, H-5), 3.23-3.15 (m, 1H, $OC\underline{H}_2CHCH_2$), 3.10 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-3), 2.94 (dd, 1H, $J_{1,2}$ = 7.7 Hz, H-2), 2.20, 2.05, 1.90 (3 x s, 9H, 3 x OAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 170.41 x 2, 169.93, 168.45, 167.58 (5 x CO), 138.35-116.31 (aromatics, olefinic), 103.32, 99.95 (C-1, C-1'), 79.60 (C-H), 79.46 (C-H), 77.05 (C-H), 73.86 (C-H_2), 73.50 (C-H_2), 73.22 (C-H), 72.84 (C-H_2), 70.20 (C-H), 69.93 (C-H_2), 69.71(C-H_2), 67.62 (C-H), 66.49 (C-H), 61.33 (C-H_2) 51.32 (C-2'), 31.87, 29.61, 29.42, 29.29, 26.06, 22.71 (6 x C-H_2, octyl, C-H_2), 20.77, 20.70, 20.65 (3 x OAc), 14.14 (C-H_3, octyl); Anal. calcd. for $C_{51}H_{63}O_{15}N_1$: C 65.86, H 6.82, N 1.50 found: C 65.76, H 6.72, N 1.53.

Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-6-*O*-allyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (**30**)



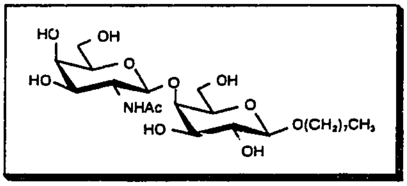
A mixture of the alcohol **23** (335.0 mg, 0.65 mmol), the donor **25** (870.0 mg, 1.74 mmol), molecular sieves (1.34 g, 4Å), tetramethylurea (0.28 ml, 2.35 mmol) and silver trifluoromethanesulfonate (449.50 mg, 1.75 mmol) in dry dichloromethane (50 ml) was stirred under argon at 0°C for 2 h. The temperature was raised to room temperature and the stirring continued for an additional 24 h. The mixture was filtered (celite), water (50 ml) was added and the water phase was extracted with dichloromethane (3 x 40 ml). The organic layer was dried (Na_2SO_4), concentrated, and chromatographed using 15% ethyl acetate in toluene as the eluent. The product **29** was isolated as a colourless oil (241 mg, 50%) together with unreacted alcohol **23** (72.0 mg); $[\alpha]_D = -24.4^0$ (c 0.9, CHCl$_3$); R$_f$ 0.41 in toluene/ethyl acetate, 3:1; ^{1}H NMR (360 MHz, CDCl$_3$) δ: 7.90-7.55 (m, 4H, phthalimido), 7.40-6.95 (m, 10H, benzyl aromatics), 6.08 (dd, 1H, $J_{2',3'}$ = 11.6 Hz, $J_{3',4'}$ = 3.4 Hz, H-3'), 5.88 (ddt, 1H, J_{vic} (trans) = 17.2 Hz, J_{vic} (cis) = 10.4 Hz, J_{vic} = 5.5 Hz, OCH$_2$C$\underline{H}$CH$_2$), 5.55 (d, 1H, H-4'), 5.34 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 5.28-5.21 (m, 1H, OCH$_2$CHC$\underline{H}_2$), 5.19-5.14 (m, 1H, OCH$_2$CHC$\underline{H}_2$), 4,63 (dd, 1H, H-2'), 4.43 (d, 1H, J_{gem} = 10.4 Hz, OC$\underline{H}_2$Ph), 4.38 (d, 1H, J_{gem} = 12.6 Hz, OC$\underline{H}_2$Ph), 4.21-4.04 (m, 5H), 4.00-3.96 (m, 2H), 3.88 (dt, 1H, J_{gem} = 9.5 Hz, J_{vic} = 6.6 Hz, OC$\underline{H}_2$CH$_2$, octyl), 3.70 (dd, 1H, $J_{5,6a}$ = 5.9 Hz, $J_{6a,6b}$ = 10.0 Hz, H-6a), 3.62-3.56 (m, 3H), 3.41(dt, 1H, J_{gem} = 9.5 Hz, J_{vic} = 6.6 Hz, OC$\underline{H}_2$CH$_2$, octyl), 3.31 (t, 1H, $J_{5,6}$ = 6.8 Hz, H-5), 3.19 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 2.7 Hz, H-3), 3.09 (dd, 1H, $J_{1,2}$ = 7.6 Hz, H-2), 2.20, 2.08, 1.90 (3 x s, 9H, 3 x OAc), 1.70-1.20 (m, 12H, 6 x CH$_2$, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH$_3$, octyl); ^{13}C NMR (75.5 MHz, CDCl$_3$) δ: 170.46, 170.41, 169.94, 168.49, 167.65 (5 x CO), 138.35-116.96 (aromatics, olefinic), 103.37, 99.93 (C-1, C-1'), 79.81 (C-H), 79.71 (C-H), 77.05 (C-H), 75.05 (C-H$_2$), 73.17 (C-H), 72.91 (C-H$_2$), 72.32 (C-H$_2$), 70.21 (C-H) 69.96 (C-H$_2$), 69.44 (C-H$_2$), 67.62 (C-H), 66.51 (C-H), 61.37 (C-H$_2$) 51.36 (C-2'), 31.89, 29.72, 29.49, 29.29, 26.20, 22.71 (octyl, C-H$_2$), 20.77 x 2, 20.66 (3 x OAc), 14.14 (C-H$_3$, octyl); Anal. calcd. for $C_{51}H_{63}O_{15}N_1$: C 65.86, H 6.82, N 1.50, found: C 65.52, H 6.74, N 1.52.

Octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**31**)



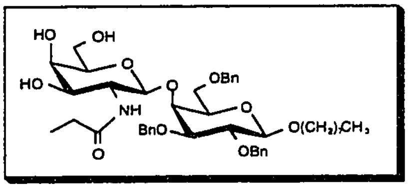
The disaccharide **27** (80.0 mg, 81.6 µmol) was dissolved in dry methanol (9 ml). The pH was adjusted to 9 using a sodium methoxide solution (1M). The mixture was stirred for 1 h. Amberlite (IRC 50) was added to neutralize the reaction solution. The solution was filtered (celite) and concentrated. The solid residue was then dissolved in a mixture of butanol (14 ml) and ethylenediamine (3.5 ml) and heated at 90°C for 17 h under an inert argon atmosphere. The reaction mixture was then cooled, concentrated, and codistilled to dryness with toluene (3 x 10 ml). The yellow crude was dissolved in dry methanol and acetic anhydride (20.8 µl, 220 µmol) was added. The residue was concentrated in vacuuo and chromatographed using 6% methanol in dichloromethane. The product **31** (45.0 mg, 68% was obtained as a white foam; R_f 0.24 in dichloromethane/methanol, 15:1; ^{1}H NMR (360 MHz, $CDCl_3$ + 5% D_2O) δ: 7.40-7.20 (m, 15H, benzyl aromatics), 4.83 (ABq, 2H, J_{gem} = 11.0 Hz, $OC\underline{H}_2Ph$), 4.73 (AB_q, 2H, J_{gem} = 10.2 Hz, $OC\underline{H}_2Ph$), 4.59 (ABq, 2H, J_{gem} = 12.2 Hz, $OC\underline{H}_2Ph$), 4.37 (d, 1H, $J_{1',2'}$ = 8.6 Hz, H-1'), 4.34 (d, 1H, $J_{1,1}$ = 7.5 Hz, H-1), 4.09 (dd, 1H, J = 8.1 Hz, J = 10.5 Hz), 4.04 - 3.86 (m, 3H), 3.78 (d, 1H, J = 3.5 Hz), 3.75 - 3.67 (m, 2H), 3.60-3.40 (m. 7H), 1.7-1.2 (m, 12H, 6 x CH_2, octyl), 1.50 (s, 3H, NHAc), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 174.07 (C=O), 138.39, 138.03, 136.68 (3 x C, benzyl), 128.92-127.95 C-H, aromatics), 103.76, 102.88 (C-1, C-1'), 81.66 (C-H), 80.16 (C-H), 76.63 (C-H), 75.33 (C-H), 75.29 (C-H), 75.10 (C-H_2), 73.59 (C-H_2), 71.85 (C-H), 70.22 (C-H_2), 67.98 (C-H_2), 67.85 (C-H), 65.89 (C-H_2), 62.52 (C-H_2), 51.36 (C-2'), 31.86, 29.73, 29.43, 29.27, 26.19, 22.69 (C-H_2, octyl), 22.10 (NHAc), 14.14 (C-H_3, octyl); MS (ES) $[M+Na]^+$ 788.3.

Octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**1**)



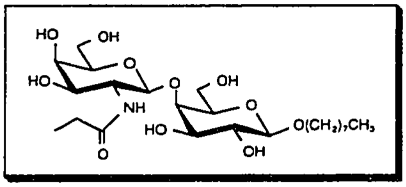
The disaccharide **31** (39.6 mg, 50.7 µmol) was dissolved in methanol (3 ml). The mixture was hydrogenated (1 atm) over Pd-C (45 mg) for 10 h. The mixxture was then filtered through a millipore filter and concentrated giving product **1** (23.0 mg, 96%) as a white powder. For use in the biological assay **1** was dissolved in water (10 ml) and adsorbed on a sepak column. The column was washed with distilled water (3 x 10 ml) before **1** was eluted using methanol (HPLC grade). The fractions containing **1** were collected and concentrated to dryness. The residue was dissolved in distilled water (50 ml) and lyophilized; $[\alpha]_D$= -11.9 (c 0.60, MeOH); ^{1}H NMR (360 MHz, D_2O) δ: 4.64 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.37 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 3.38 (dd, 1H, $J_{2',3'}$ = 9.8 Hz, H-2'), 2.05 (s, 3H, NHAc), 1.65-1.20 (m, 12H, octyl), 0.85 (t, 3H, CH_3, octyl); ^{13}C NMR (75.5 MHz, D_2O) δ: 175.85 (C=O), 103.62, 103.44 (C-1, C-1'), 76.74, 75.67, 74.85, 73.65, 71.84, 71.73 (6x C-H), 71.34 (C-H_2), 68.69 (C-H), 61.91, 61.12 (2x C-H_2), 53.57 (C-2'), 32.00, 29.64, 29.37, 29.29, 25.92 (5 x C-H_2, octyl), 23.26 (C-H_3, NHAc), 22.90 (C-H_2, octyl), 14.28 (C-H_3, octyl); MS (ES): $[M+Na]^+$ 518.2.

Octyl 2-deoxy-2-propionamido-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**32**)



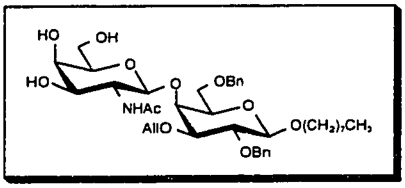
The disaccharide **27** (71.50 mg, 72.90 μmol) was dissolved in dry methanol (9 ml). The pH was adjusted to 9 using a sodiummethoxide solution (1M). The mixture was stirred for 1 h. Amberlite (IRC 50) was added to neutralize the reaction solution. The solution was filtered (celite) and concentrated. The solid residue was then dissolved in a mixture of butanol (14 ml) and ethylenediamine (3.5 ml) and heated at 90°C for 17 h under an inert argon atmosphere. The reaction mixture was cooled, concentrated, and codistilled to dryness with toluene (3 x 5 ml). The yellow crude was dissolved in dry methanol and propionic anhydride (28.2 μl, 220 μmol) was added. The residue was concentrated in vacuuo and chromatographed using 6% methanol in dichloromethane. The product **32** (43.0 mg, 76% was obtained as a white foam; R_f 0.27 in dichloromethane/methanol, 15:1; ^{1}H NMR (360 MHz, $CDCl_3/D_2O$ 30 :1) δ: 7.40-7.20 (m, 15H, benzyl aromatics), 4.83 (ABq, 2H, J_{gem} = 11.1 Hz, $OC\underline{H}_2Ph$), 4.73 (ABq, 2H, J_{gem} = 10.2 Hz, $OC\underline{H}_2Ph$), 4.60 (ABq, 2H, J_{gem} = 12.4 Hz, $OC\underline{H}_2Ph$), 4.38 (d, 1H, $J_{1',2'}$ = 8.6 Hz, H-1'), 4.34 (d, 1H, $J_{1,1}$ = 7.5 Hz, H-1), 4.10 (dd, 1H, J = 8.1 Hz, J = 10.5 Hz), 4.04-3.86 (m, 3H), 3.78 (d, 1H, J = 3.1 Hz), 3.75-3.68 (m, 2H), 3.60-3.40 (m, 7H), 2.45 (q, 2H, $COC\underline{H}_2CH_3$), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.90, 0.85 (2 x t, 6H, J_{vic} = 7.6 Hz, J_{vic} = 7.0 Hz, CH_3, octyl and propionamide); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 177.74 (C=O), 138.49, 138.03, 136.76 (3 x C, benzyl), 128.88-127.83 (C-H, aromatics), 103.79, 102.87 (C-1, C-1'), 81.61 (C-H), 80.32 (C-H), 76.42 (C-H), 75.37 x 2 (C-H), 75.32 ($C-H_2$), 75.08 ($C-H_2$), 73.58 ($C-H_2$), 71.88 (C-H), 70.22 ($C-H_2$), 68.00 (C-H), 67.86 ($C-H_2$), 62.51 ($C-H_2$), 55.99 (C-2'), 31.86, 29.72, 29.43, 29.27, 28.83, 26.19, 22.68 (6 x $C-H_2$, octyl, propionamide), 14.12, 9.72 (2 x $C-H_3$, octyl, propionamide); MS (MALDI) $[M+Na]^+$ 802.0.

Octyl 2-deoxy-2-propionamido-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**8**)



The disaccharide **32** (31.4 mg, 40.3 μmol) was dissolved in methanol (3 ml). The mixture was hydrogenated (1 atm) over Pd-C (40 mg) for 10 h before it was filtered through a millipore filter and concentrated giving product **8** (17.5 mg, 87%) as a white powder. For use in the biological assay **8** was dissolved in water (10 ml) and adsorbed on a sepak column. The column was washed with distilled water (3 x 10 ml) before **8** was eluted using methanol (HPLC grade). The fractions containing **8** were collected and concentrated to dryness. The residue was redissolved in distilled water (50 ml) and lyophilized; $[\alpha]_D$= -16.5 (c 0.60, MeOH); ^{1}H NMR (360 MHz, D_2O) δ: 4.64 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.37 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 3.38 (dd, 1H, $J_{2',3'}$ = 9.8 Hz, H-2'), 2.23 (q, 2H, $COC\underline{H}_2CH_3$), 1.65-1.20 (m, 12H, octyl), 1.10, 0.85 (2 x t, 6H, CH_3, propionamide, octyl,); ^{13}C NMR (75.5 MHz, D_2O) δ: 179.60 (C=O), 103.60, 103.29 (C-1, C-1'), 76.45, 75.66, 74.87, 73.68, 71.90, 71.77 (6 x C-H), 71.32 ($C-H_2$), 68.74 (C-H), 61.92, 61.14 (2 x $C-H_2$), 53.57 (C-2'), 31.93, 30.33, 29.60, 29.29, 29.20 25.86, 22.84 (7 x $C-H_2$, octyl, propionamide), 14.23, 10.32 (2 x $C-H_3$, octyl, propionamide); MS (FAB): M + Na]$^+$ = 532.2.

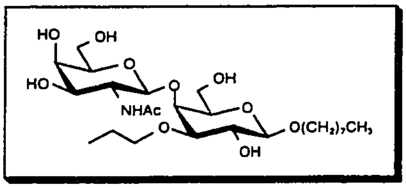
Octyl 2-acetamido-2-deoxy-2-β-D-galactopyranosyl-(1→4)-3-allyl-2,6-di-*O*-benzyl-β-D-galactopyranoside (**33**)



The disaccharide **28** (93 mg, 100 μmol) was dissolved in dry methanol (9 ml). The pH was adjusted to 9 using a sodiummethoxide solution (1M). The mixture was stirred for 1.5 h. Amberlite (IRC 50) was then added to neutralize the reaction solution. The solution was filtered (celite) and concentrated. The solid residue was then dissolved in a mixture of butanol (14 ml) and ethylenediamine (3.5 ml) and heated at 90°C for 17 h under an inert argon atmosphere. The reaction mixture was cooled, concentrated, and codistilled to dryness with toluene (3 x 5 ml). The yellow crude was dissolved in dry methanol and acetic anhydride (24.50 μl, 260 μmol) was added. The residue was concentrated in vacuuo and chromatographed using 6% methanol in dichloromethane. The product **33** (50 mg, 70%) was obtained as a white foam; R_f 0.28 in dichloromethane/methanol, 15:1; ^1H NMR (360 MHz, CD_3OD) δ: 7.40-7.20 (m, 10H, benzyl aromatics), 6.05-5.94 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.36-5.28 (m, 1H, J_{vic}(trans) = 17.2 Hz), 5.23-5.17 (m, 1H, J_{vic}(cis) = 10.3 Hz, $OCH_2CHC\underline{H}_2$), 4.78 (ABq, 2H, J_{gem} = 11.7 Hz, $OC\underline{H}_2Ph$), 4.64 (d, 1H, $J_{1',2'}$ = 8.1 Hz, H-1'), 4.57 (s, 2H, $OC\underline{H}_2Ph$), 4.50-4.43 (m, 1H, $OC\underline{H}_2CHCH_2$), 4.37 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1), 4.29-4.15 (m, 2H), 4.12 (d, 1H, J = 2.3 Hz), 3.92-3.42 (m, 12H), 2,00 (s, 3H, NHAc), 1.70-1.20 (m, 12H, 6 x

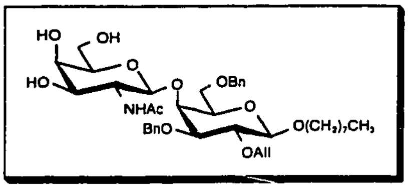
CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 174.48 (C=O), 140.32, 139.82 (2 x C, benzyl), 136.41 (C-H, olefinic), 129.37-128.54 C-H, aromatics), 118.37 (C-H_2, olefinic), 104.92, 103.44 (C-1, C-1'), 82.37 (C-H), 81.07 (C-H), 76.51 (C-H), 76.16 (C-H_2), 76.08 (C-H), 74.57 (C-H), 74.33 (C-H_2), 73.83 (C-H), 73.41 (C-H_2), 71.36 (C-H_2), 70.89 (C-H_2), 69.41 (C-H), 62.39 (C-H_2), 55.73 (C-2'), 32.98, 30.92, 30.51, 30.42, 28.44 , 23.72, (6 x C-H_2, octyl), 23.43 (C-H_3, NHAc), 14.42 (C-H_3, octyl). MS (MALDI) $[M+Na]^+$ 738.5.

Octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-3-*O*-propyl-β-D-galactopyranoside (**6**)



The disaccharide **33** (18 mg, 25.1 μmol) was dissolved in methanol (2 ml). The mixture was hydrogenated (1atm) over Pd-C (22 mg) for 6 h before it was filtered through a millipore filter and concentrated giving product **6** (12.5 mg, 93 %) as a white powder. For use in the biological assay **6** was dissolved in water (10 ml) and adsorbed on a sepak column. The column was washed with distilled water (3 x 10 ml) before **6** was eluted using methanol (HPLC grade). The fractions containing **6** were collected and concentrated to dryness. The residue was redissolved in distilled water (50 ml) and lyophilized; $[\alpha]_D$ = -14.5° (c 0.5, MeOH); ^{1}H NMR (500 MHz, D_2O) δ: 4.53 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 4.37 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1), 3.45 (dd, 1H, $J_{2',3'}$ = 9.8 Hz, H-2'), 2.08 (s, 3H, NHAc), 1.70-1.20 (m, 14H, octyl, propyl), 0.92, 0.85 (2 x t, 6H, CH_3, octyl, propyl); ^{13}C NMR (75.5 MHz, D_2O) δ: 175.74 (C=O), 103.67, 102.78 (C-1, C-1'), 81.77 (C-H), 75.68 (C-H), 74.98 (C-H), 72.84 (C-H_2), 72.66 (C-H), 71.71 (C-H), 71.27 (C-H_2), 70.96 (C-H), 68.68 (C-H), 61.91 (C-H_2), 61.20 (C-H_2), 53.67 (C-2'), 32.04, 29.65 x 2, 29.39, 25.35 (5 x C-H_2), 23.79 (C-H_3, NHAc), 23.25 (C-H_2), 22.93 (C-H_2), 14.32, 10.60 (2 x C-H_3, octyl, propyl); MS (FAB) 538.3 $[M+H]^+$.

Octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-*O*-allyl-3,6-di-*O*-benzyl-β-D-galactopyranoside (**34**)

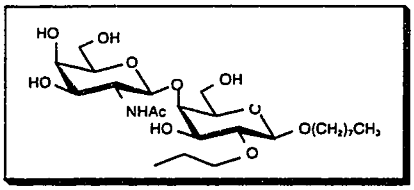


The disaccharide **29** (100 mg, 101.7 μmol) was dissolved in dry methanol (9 ml). The pH was adjusted to 9 using a sodiummethoxide solution (1M). The mixture was stirred for 1 h. Amberlite (IRC 50) was then added to neutralize the reaction solution. The solution was filtered (celite) and concentrated. The solid residue was then dissolved in a mixture of butanol (14 ml) and ethylenediamine (3.5 ml) and heated at 90°C for 17 h under an inert argon atmosphere. The reaction mixture was cooled, concentrated, and codistilled to dryness with toluene (3 x 5 ml). The yellow crude was dissolved in dry methanol and acetic anhydride (20.8 μl, 220 μmol) was added. The residue was concentrated in vacuuo and chromatographed using 6% methanol in dichloromethane as the eluent. The product **34** (52.4 mg, 68%) was obtained as a white foam; R_f 0.24 in dichloromethane/methanol, 15:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.10 (m, 10H, benzyl aromatics), 7.10 (d, br., 1H, $J_{NH.2'}$ = 2.8 Hz, N$\underline{H}$Ac), 6.03-5.92 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.36-5.28 (m, 1H, J_{vic}trans = 17.3 Hz, J_{vic}cis = 10.4Hz, $OCH_2CHC\underline{H}_2$), 5.23-5.17 (m, 1H, $OCH_2CHC\underline{H}_2$), 4.76 (ABq, 2H, J_{gem} = 10.1 Hz, $OC\underline{H}_2Ph$), 4.58 (ABq, 2H, J_{gem} = 12.2 Hz, $OC\underline{H}_2Ph$), 4.50-4.43 (m, 1H, $OC\underline{H}_2CHCH_2$), 4.37 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 4.27 (d, 1H, $J_{1,1}$ = 7.4 Hz, H-1), 4.16-4.10 (m, 1H, $OC\underline{H}_2CHCH_2$), 4.07 (dd, 1H, J = 8.0 Hz, J = 10.4 Hz), 3.98 (d, 1H, J = 2.8 Hz), 3.96 (dd, 1H, J = 7.8 Hz), J = 11.7 Hz, 3.92 - 3.84 (m, 2H), 3.77 (dd, 1H, J = 3.4, J ~ 0.9 Hz), 3.75-3.65 (m, 2H), 3.58-3.40 (m, 6H), 3.20-2.60 (s, br. 3 x OH), 1.7-1.2 (m, 12H, 6 x CH_2, octyl), 1.60 (s, 3H, NHAc), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl);

A small amount of **34** was acetylated to give octyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-2-*O*-allyl-3,6-di-*O*-benzyl-β-D-galactopyranoside **151**; $[\alpha]_D$

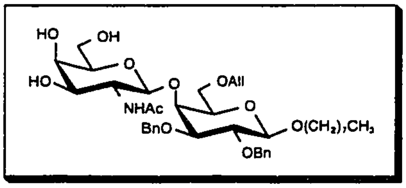
= -37.2°(c 1.0, $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 10H, benzyl aromatics), 6.03-5.91 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.64 (d, 1H, $J_{2',NH}$ = 7.8 Hz, N-H), 5.35-5.27 (m, 1H, J_{vic} (trans) = 17.3 Hz, $OCH_2CHC\underline{H}_2$), 5.27 (dd, 1H, $J_{4',5'}$ ~ 1Hz, $J_{3',4'}$ = 3.4 Hz, H-4'), 5.22-5.16 (m, 1H, J_{vic}cis = 10.3 Hz, $OCH_2CHC\underline{H}_2$), 4.83 (m, 2H, H-3', $OC\underline{H}_2Ph$), 4.65 (m, 2H, H-1', $OC\underline{H}_2Ph$), 4.55 (AB_q, 2H, J_{gem} = 12.0 Hz, $OC\underline{H}_2Ph$), 4.50-4.40 (m, 1H, $OC\underline{H}_2CHCH_2$), 4.27 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.15-4.05 (m, 3H), 4.01 (dd, 1H, J = 6.0 Hz, J = 11.0 Hz), 3.94 (d, 1H, J = 2.5), 3.90 (dt, 1H, J_{gem} = 9.4 Hz, J_{vic} = 6.9 Hz, $OC\underline{H}_2CH_2$, octyl), 3.83-3.74 (m, 2H), 3.65 (dd, 1H, J = 5.5 Hz, J = 10.0 Hz), 3.55-3.37 (m, 4H), 2.20, 2.05, 1.95, 1.68 (4 x s, 12H, 3 x OAc, NHAc), 1.65-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 170.53, 170.40 x 2, 169.97 (4 x CO), 138.30, 137.55 (2 x C, benzyl), 135.16 (C-H, olefinic), 129.03-127.69 (C-H, benzyl), 116.62 (C-H_2, olefinic), 103.32, 99.95 (C-1, C-1'), 81.19 (C-H), 79.89 (C-H), 76.50 (C-H), 74.64 (C-H_2), 74.01 (C-H_2), 73.62 (C-H_2), 73.34 (C-H), 71.94 (C-H), 70.87 (C-H), 70.04 (C-H_2), 69.47 (C-H_2), 69.64(C-H_2), 61.33 (C-H_2) 50.95 (C-2'), 31.88, 29.69, 29.42, 29.30, 26.14, (5 x C-H_2, octyl), 23.18 (C-H_3), 22.71 (C-H_2), 20.81, 20.76, 20.70 (3 x C-H_3), 14.14 (C-H_3, octyl); Anal. calcd. for $C_{45}H_{63}O_{14}N_1$: C 64.19, H 7.54, N 1.66 found: C 63.85, H 7.25, N 1.62.

Octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-*O*-propyl-β-D-galactopyranoside (**7**)



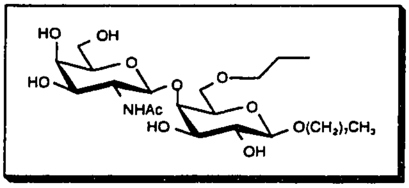
The disaccharide **34** (18 mg, 25.1 μmol) was dissolved in methanol (2 ml). The mixture was hydrogenated (1 atm) over Pd-C (22 mg) for 6 h. The mixture was filtered through a millipore filter and concentrated giving product **7** (11.7 mg, 89%) as a white powder. For use in the biological assay **7** was dissolved in water (10 ml) and adsorbed on a sepak column. The column was washed with distilled water (3 x 10 ml) before **7** was eluted using methanol (HPLC grade). The fractions containing **7** were collected and concentrated to dryness. The residue was redissolved in distilled water (50 ml) and lyophilized; $[\alpha]_D$= -7.3° (c 0.5, MeOH); ^{1}H NMR (500 MHz, D_2O) δ: 4.65 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 4.30 (d, 1H, $J_{1',2'}$ = 7.5 Hz, H-1'), 3.15 (dd, 1H, $J_{2',3'}$ = 9.8 Hz, H-2'), 2.08 (s, 3H, NHAc), 1.65-1.20 (m, 14H, octyl, propyl), 0.92, 0.85 (2x t, 6H, CH_3, octyl, propyl); ^{13}C NMR (75.5 MHz, D_2O) δ: 175.09 (C=O), 104.17, 103.48 (C-1, C-1'), 80.24 (C-H), 76.81 (C-H), 75.68 (C-H), 75.56 (C-H_2), 74.63, 73.34, 72.15 (3 x C-H), 70.84 (C-H_2), 61.91 (C-H_2), 60.88 (C-H_2), 53.69 (C-2'), 32.51, 30.14, 30.05, 29.69, 26.74, 23.81 (6 x C-H_2), 23.46 (C-H_3, NHAc), 23.27 (C-H_2), 14.48, 10.94 (2 x C-H_3, octyl, propyl); MS (FAB) 538.3 $[M+H]^+$.

Octyl 2-acetamido-2-deoxy-2-β-D-galactopyranosyl-(1→4)-6-*O*-allyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (**35**)



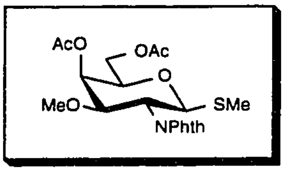
The disaccharide **30** (100 mg, 107.5 μmol) was dissolved in dry methanol (9 ml). The pH was adjusted to 9 using a sodium methoxide solution (1M). The mixture was stirred for 2 h. Amberlite (IRC 50) was then added to neutralize the reaction solution. The solution was filtered (celite) and concentrated. The solid residue was then dissolved in a mixture of butanol (14 ml) and ethylenediamine (3.5 ml) and heated at 90°C for 17 h under an inert argon atmosphere. The reaction mixture was then cooled, concentrated, and codistilled to dryness with toluene (3 x 8 ml). The yellow crude was dissolved in dry methanol and acetic anhydride (31 μl, 0.33 mmol) was added. The residue was concentrated in vacuuo and chromatographed using 2% methanol in ethyl acetate. The product **35** (55 mg, 65%) was obtained as a white foam; R_f 0.32 in dichloromethane/methanol, 15:1; ^{1}H NMR (360 MHz, CD_3OD) δ: 7.40-7.20 (m, 10H, benzyl aromatics), 5.98-5.84 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.31-5.22 (m, 1H, J_{vic}trans = 17.3 Hz), 5.18-5.11 (m, 1H, J_{vic}(cis) = 10.3 Hz, $OCH_2CHC\underline{H}_2$), 4.80 (AB_q, 2H, J_{gem} = 11.2 Hz, $OC\underline{H}_2Ph$), 4.74 (AB_q, 2H, J_{gem} = 11.0 Hz, $OC\underline{H}_2Ph$), 4.63 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.55 (s, 1H), 4.37 (d, 1H, $J_{1',2'}$ = 7.6 Hz, H-1), 4.14 (d, 1H, J = 2.6 Hz), 4.05-4.00 (m, 2H), 3.93-3.40 (m, 12H), 1.75 (s, 3H, NHAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, C-H_3, octyl; ^{13}C NMR (75.5 MHz, CD_3OD) δ: 174.59 (C=O), 140.30, 139.29, (2 x C, benzyl), 136.18 (C-H, olefinic), 129.93-128.60 (C-H, aromatics), 117.21 (C-H_2, olefinic), 104.91, 103.50 (C-1, C-1'), 82.72 (C-H), 81.16 (C-H), 76.62 (C-H), 76.29 (C-H), 76.19 (C-H_2), 74.66 (C-H_2), 74.59, (C-H), 74.36 (C-H), 73.27 (C-H_2), 71.00 (C-H_2), 70.90 (C-H_2), 69.33 (C-H), 62.38 (C-H_2), 55.69 (C-2'), 32.98, 30.90, 30.50, 30.41, 27.34 , 23.72, (6 x C-H_2), 23.07 (C-H_3, NHAc), 14.42 (C-H_3, octyl). MS (FAB) $[M+Na]^+$ 738.6.

Octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-6-*O*-allyl-2,3-di-*O*-benzyl-β-D-galactopyranoside (**5**)



The disaccharide **35** (18.0 mg, 25.1 μmol) was dissolved in methanol (2 ml). The mixture was hydrogenated (1 atm) over Pd-C (22 mg) for 6 h before it was filtered through a millipore filter and concentrated giving product **5** (12.5 mg, 93%) as a white powder. For use in the biological assay **5** was dissolved in water (10 ml) and adsorbed on a sepak column. The column was washed with distilled water (3 x 10 ml) before **5** was eluted using methanol (HPLC grade). The fractions containing **5** were collected and concentrated to dryness. The residue was dissolved in distilled water (50 ml) and lyophilized; $[\alpha]_D$ = -16.5° (c 0.5, MeOH); ^{1}H NMR (360 MHz, D_2O) δ: 4.68 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 4.37 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 3.42 (dd, 1H, $J_{2',3'}$ = 9.2 Hz, H-2'), 2.10 (s, 3H, NHAc), 1.70-1.20 (m, 14H, octyl, propyl), 0.92, 0.85 (2 x t, 6H, CH_3, octyl, propyl); ^{13}C NMR (75.5 MHz, D_2O) δ: 175.84 (C=O), 103.90, 103.30 (C-1, C-1'), 77.64 (C-H), 75.53 (C-H), 74.30 (C-H), 73.85 (C-H), 73.75 (C-H_2), 72.15 (C-H), 71.70 (C-H), 71.33 (C-H_2), 70.97 (C-H_2), 68.55 (C-H), 61.76 (C-H_2), 53.79 (C-2'), 32.40, 29.91 x 3, 26.30 (5x C-H_2), 23.36 (C-H_3, NHAc), 23.27 (C-H_2), 23.21 (C-H_2), 14.45, 10.91 (2 x C-H_3, octyl, propyl); MS (FAB) 538.3 $[M + H]^+$.

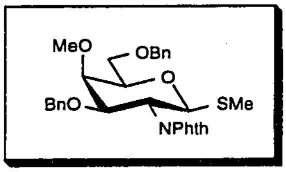
Methyl 4,6-di-*O*-acetyl-2-deoxy-3-*O*-methyl-2-phthalimido-1-thio-β-D-galactopyranoside (**40**)



The methyl ether **36**^{199} (0.20 g, 394.0 mmol) was dissolved in a mixture of dry dichloromethane (14 ml) and trifluoroacetic acid (4.8 ml) and was stirred for 2 h. Toluene (20 ml) was added and the mixture was concentrated to dryness. The residue was dissolved in toluene (15 ml) and codistilled (2 x) to dryness to remove traces of trifluoroacetic acid. A mixture of 30% pyridine in acetic anhydride (5 ml) was added to the solid residue and stirring continued for an additional 12 h. The solvent was removed under reduced pressure and chromatographed using 33% ethyl acetate in hexane. The product **39** (131 mg, 79%, α/β mixture 1:5) was obtained as a white foam; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.85-7.65 (m, 4H, pthalimido), 6.28 (d, $J_{1\alpha.2\alpha}$ = 3.6 Hz, H-1α), 6.26 (d, $J_{1\beta.2\beta}$ = 8.8 Hz, H-1β), 5.67 (dd, $J_{3\alpha.4\alpha}$ = 3.2 Hz, $J_{4\alpha.5\alpha}$ ~ 1Hz, H-4α), 5.58 (d, $J_{3\beta.4\beta}$ = 3.1 Hz, H-4β), 4.97 (dd, $J_{5\alpha.6a\alpha}$ = 3.3 Hz, $J_{6a\alpha.6b\alpha}$ = 11.7 Hz, H-6aα), 4.70 (dd, $J_{5\beta.6b\beta}$ = 3.3 Hz, $J_{6a\beta.6b\beta}$ = 11.7 Hz, H-6bα), 4.50 (dd, $J_{2b.3b}$ = 11.2 Hz, H-2β), 4.38 (dd, H-3β), 4.40-4.02 (m, H-5β, H-6aβ, H-6bβ, H-2α, H-3α, H-5α), 3.35 (s, OCH_3(α)), 3.25 (s, OCH_3 (β)), 2.20, 2.08, 1.99 (3 x OAc (β), 2.17, 2.05, 2.03 (3 x OAc (α)).

The α/β mixture **39** (131 mg, 291.0 μmol) was dissolved in dichloromethane (8 ml) and methylthiotrimethylsilane (124 μl, 873 μmol) and TMSOTf (56.2 μl, 291 μmole) were added at 0^0C. After 0.5 h the temperature was raised to room temperature and the mixture continued to stirr for 12 h. The temperature was lowered to 0^0C and an additional amount of TMSOTf (56.2 µl, 291.0 µmol) was added. After the addition, the temperature was slowly warmed up to room temperature. After 36 h the organic layer was diluted with dichloromethane (20 ml), saturated sodium bicarbonate solution (5 ml) was added to quench the reaction. The organic layer was dried (Na_2SO_4), concentrated and chromatographed using 25% ethyl acetate in hexane as the eluent. The desired product **40** (106.70 mg, 90%) was obtained as a white powder; R_f 0.34 in hexane/ethyl acetate, 2:1; ^1H NMR (360 MHz, $CDCl_3$) δ: 7.85-7.65 (m, 4H, pthalimido), 5.57 (d, 1H, $J_{3,4} = 2.7$ Hz, H-4), 5.16 (d, 1H, $J_{1,2} = 10.4$ Hz), 4.48 (t, 1H, $J_{2,3} = 10.6$ Hz, H-2), 4.29 (dd, 1H, H-3), 4.18 (m, 2H, H-6a, H-6b), 4,03 (t, 1H, $J_{5,6} = 6.2$ Hz), 3.25 (s, 3H, OCH_3), 2.18, 2.17, 2.05 (3 x s, 9H). MS (FAB) $[M+H]^+$ 438.1.

Methyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-methyl-2-phthalimido-1-thio-β-D-galactopyranoside (**42**)

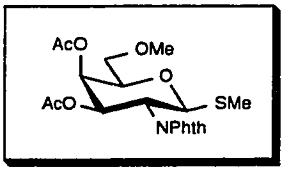


The methyl ether **37**[199] (275.0 mg, 455.50 µmol) was dissolved in a mixture of dry dichloromethane (14 ml) and trifluoroacetic acid (4.8 ml) and was stirred for 2 h. Toluene (20 ml) was added and the mixture was concentrated to dryness. The residue was dissolved in toluene (15 ml) and codistilled with toluene (2 x 10 ml) to dryness to remove traces of trifluoroacetic acid. The mixture was redissolved in 30% pyridine in acetic anhydride (5 ml) and was stirred for 12 h. The solvent was removed under reduced pressure and the residue was chromatographed using 33% ethyl acetate in hexane as the eluent. The product **41** (180 mg, 74%, α/β mixture 1:3) was obtained as a white foam; ^1H NMR (360 MHz, $CDCl_3$) δ: 7.85-7.65 (m, 4H, pthalimido), 7.40-7.10 (m, 10H, benzyl), 6.34 (d, $J_{1\alpha,2\alpha} = 3.4$ Hz, H-1α), 6.32 (d, 1H, $J_{1\beta,2\beta} = 8.9$ Hz, H-1β), 5.18 (dd, $J_{2\alpha,3\alpha} = 11.7$ Hz, $J_{3\alpha,4\alpha} = 2.6$ Hz, H-

3α), 5.04 (dd, H-2α), 4.82-4.68 (m, 4H, $OC\underline{H}_2Ph$ (α)), 4.64 (ABq, 2H, $OC\underline{H}_2Ph$ (β)), 4.62 (dd, $J_{2\beta,3\beta}$ = 11.2 Hz, H-2β), 4.58 (ABq, 2H, $OC\underline{H}_2Ph$ (β)), 4.49 (dd, 1H, $J_{3\beta,4\beta}$ = 2.7 Hz, H-3β), 4.28 (dd, $J_{5\alpha,6a\alpha}$ = 8.0 Hz, $J_{5\alpha,6b\alpha}$ = 5.6 Hz, H-5α), 4.02 (d, $J_{3\alpha,4\alpha}$ = 2.5 Hz, H-4α), 3.95 (dd, $J_{5\beta,6a\beta}$ = 8.2 Hz, $J_{5\beta,6b\beta}$ = 5.3 Hz, H-5β), 3.92 (d, H-4β), 3.83 (t, H-6aβ), 3.79 (dd, $J_{6a\alpha,6b\alpha}$ = 11.7 Hz, H-6aα), 3.73 (dd, $J_{6a\beta,6b\beta}$ = 9.0 Hz, H-6bα), 3.68 (dd, H-6bβ), 3.66 (s, OCH_3 (β)), 3.62 (s, OCH_3 (α)), 2.10 (OAc (α), 2.00 (OAc (α)).

The α/β mixture **41** (180.0 mg, 330 μmol) was dissolved in dichloroethane (12 ml) and methylthiotrimethylsilane (0.16 ml, 1.16 mmol) and TMSOTf (64 μl, 0.33 mmol) were added at 0^0C. After 0.5 h the temperature was raised to room temperature and the mixture continued to stirr for 12 h. The temperature was lowered to 0^0C and more TMSOTf (64 μl, 0.33 mmol) was added before the temperature was slowly warmed up to room temperature. After 36 h the organic layer was diluted with dichlormethane (20 ml) and saturated sodium bicarbonate solution (5 ml) was added to quench the reaction. The organic layer was dried (Na_2SO_4), concentrated and chromatographed using 25% ethyl acetate in hexane as the eluent. The desired product **42** (149.6 mg, 85%) was obtained as a white powder; R_f 0.36 in hexane/ethyl acetate, 2:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.85-7.65 (m, 4H, pthalimido), 7.40-7.00 (m, 10H, benzyl), 5.09 (d, 1H, $J_{1,2}$ = 10.3 Hz, H-1), 4.66 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 4.64 (d, 1H, J_{gem} = 12.2 Hz, $OC\underline{H}_2Ph$), 4.56 (ABq, 2H, J_{gem} = 11.8 Hz, $OC\underline{H}_2Ph$), 4.33 (m, 2H, H-3, $OC\underline{H}_2Ph$), 3.82 (d, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 3.77 (ddd, 1H, $J_{4,5}$ ~ 1Hz, $J_{5,6}$ = 5.3 Hz, $J_{5,6}$ = 6.1 Hz, H-5), 3.74-3.64 (m, 2H, H-6a, H-6b), 3.45 (s, 3H, OCH_3), 2.0, (s, 3H, SCH_3). MS (FAB) $[M+H]^+$ 438.1.

Methyl 3,4-di-*O*-acetyl-2-deoxy-6-*O*-methyl-2-phthalimido-1-thio-β-D-galactopyranoside (**44**)

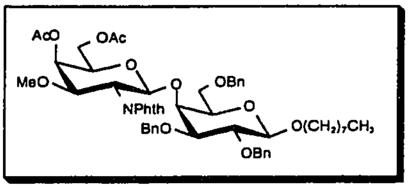


The methyl ether **38**[199] (0.25 g, 0.54 mmol) was dissolved in a mixture of dry dichloromethane (12 ml) and trifluoroacetic acid (4.8 ml) and was stirred for 3.5 h. Toluene (20 ml) was added and the mixture was concentrated to dryness. The residue was redissolved in toluene (15 ml) and codistilled with toluene (2 x 10 ml) to dryness to remove traces of trifluoroacetic acid. The solid residue was then dissolved in a mixture of 30% pyridine in acetic anhydride (5 ml) and stirred for 12 h. The solvent was removed under reduced pressure and chromatographed using 33% ethyl acetate in hexane. The product **43** (186 mg, 78%, α/β mixture 1:20) was obtained as a white foam; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.85-7.65 (m, 4H, pthalimido), 6.46 (dd, $J_{2\alpha,3\alpha}$ = 11.5 Hz, $J_{3\alpha,4\alpha}$ = 3.0 Hz, H-3α), 6.42 (d, 1H, $J_{1\beta,2\beta}$ = 8.8 Hz, H-1β), 6.33 (d, $J_{1\alpha,2\alpha}$ = 3.0 Hz, H-1aα), 5.90 (dd, $J_{2\beta,3\beta}$ = 10.4 Hz, $J_{3\beta,4\beta}$ = 3.4 Hz, H-3β), 5.66 (d, H-4α), 5.53 (d, H-4β), 5.04 (dd, H-2α), 4.64 (dd, H-2β), 4.41 (t, 1H, H-5α), 4.12 (t, 1H, H-5β), 3.56-3.37 (H-6aβ, H-6bβ, H-6aα, H-6bα), 3.35 (s, OCH_3(α)), 3.32 (s, OCH_3 (β)), 2.20, 2.05, 1.85 (3 x OAc(β)), 2.18, 2.03, 1.88 (3x OAc (α)).

The α/β mixture **43** (170 mg, 378 μmol) was dissolved in dichloroethane (10 ml) and methylthiotrimethylsilane (187 μl, 1.32 mmol) and TMSOTf (73 μl, 378 μmol) were added at 0°C. After 0.5 h the temperature was raised to room temperature and the mixture continued to stirr for 12 h. The temperature was lowered to 0°C and more TMSOTf (73 μl, 0.33 mmol) was added. The temperature was slowly (1 h) raised to room temperature . After 36 h the organic layer was diluted with dichloromethane (20 ml) and saturated sodium bicarbonate solution (5 ml) was added to quench the reaction. The organic layer was dried (Na_2SO_4), concentrated and chromatographed using 25% ethyl acetate in hexane as eluent.

The desired product **44** (132.3 mg, 80%) was obtained as a oil; R_f 0.33 in hexane/ethyl acetate, 1:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.85-7.65 (m, 4H, pthalimido), 5.85 (dd, 1H, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 2.5 Hz, H-3), 5.53 (dd, 1H, $J_{4.5}$ ~ 1Hz, H-4), 5.33 (d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), 4.60 (t, 1H, H-2), 4.03 (t, 1H, $J_{5.6}$ = 6.2 Hz, H-5), 3.55 (dd, 1H, J_{gem} = 10.1 Hz, H-6a), 3.43 (dd, 1H, H-6b), 3.35 (s, 3H, OCH_3), 2.20, 2.18, 1.85 (3 x s, 9H, 2 x OAc, SMe). MS (FAB) $[M+H]^+$ 438.1.

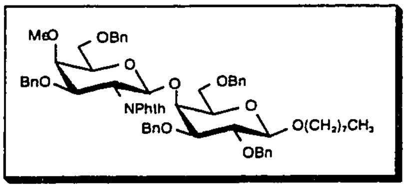
Octyl 4,6-di-*O*-acetyl-2-deoxy-3-*O*-methyl-2-phthalimido-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**45**)



The thioglycoside **40** (180 mg, 411.5 µmol), the alcohol **21** (224.9 mg, 0.40 mmol) and molecular sieves (4 Å, 2.0 g) were suspended in dichloromethane (30 ml) and stirred for 0.5 h. The mixture was cooled to 0°C and *N*-iodosuccinimide (110 mg, 494 µmol) and trifluoromethanesulfonic acid (100 µmol) was added subsequently, while the mixture kept stirring at 0°C. After 0.5 h saturated sodium bicarbonate solution (10 ml) was added and the organic layer was separated, washed with saturated sodium thiosulfate (10 ml) and water (6 ml) and dried (Na_2SO_4). The organic layer was concentrated and chromatographed using 12% ethyl acetate in toluene as the eluent. The product **45** (266 mg, 70%) was isolated as a colourless oil; R_f 0.50 in toluene/ethyl acetate, 3:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.90-7.55 (m, 4H, phthalimido), 7.40-6.95 (m, 15H, benzyl aromatics), 5.56 (d, 1H, $J_{3',4'}$ = 1.2 Hz, H-4'), 5.22 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 4.55-4.25 (m, 6H, H-2', H-3', 4 x $OC\underline{H}_2Ph$), 4.22-4.13 (m, 3H, H-1, H-6'a), 4.03 (dd, 1H, $J_{5',6'b}$ = 6.1 Hz, $J_{6'a,6'b}$ = 11.0 Hz, H-6'b), 3.95 (t, 1H, H-5'), 3.88 (dt, 1H, J_{gem} = 9.5 Hz, J_{vic} = 6.2 Hz, $OC\underline{H}_2CH_2$, octyl), 3.76 (dd, 1H, $J_{5,6a}$ = 5.6 Hz, $J_{6a,6b}$ = 10.1 Hz, H-6a), 3.65 (dd, 1H, $J_{5,6b}$ = 6.0 Hz, H-6b), 3.64-3.57 (m, 2H, H-4, OCH_2Ph), 3.38 (dt, 1H, J_{gem} = 9.5 Hz, J_{vic} = 6.6 Hz, $OC\underline{H}_2CH_2$, octyl), 3.33 (t, 1H, H-

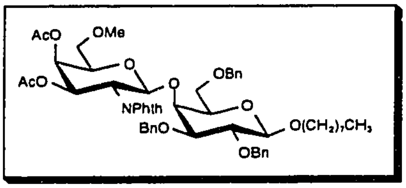
5), 3.29 (s, 3H, OCH_3), 3.17 (dd, 1H, $J_{2,3}$ = 12.2 Hz, H-3), 3.13 (dd, 1H, $J_{1,2}$ = 7.2 Hz, H-2), 2.20, 2.05, (2 x s, 6H, 2 x OAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 170.56, 170.51, 169.17, 167.87 (4 x C=O), 138.52-122.87 (C, aromatics), 103.38, 100.29 (C-1, C-1'), 80.03 (C-H), 79.73 (C-H), 77.30 (C-H), 75.31 (C-H), 75.07 (C-H_2), 73.51 (C-H_2), 73.33 (C-H), 72.92 (C-H_2), 70.58 (C-H), 69.86 (C-H_2), 69.81(C-H_2), 64.83 (C-H), 62.04 (C-H_2), 57.63 (OCH_3), 52.89 (C-2'), 31.91, 29.75, 29.51, 29.31, 26.23, 22.73 (6 x C-H, octyl), 20.94, 20.78 (2 x OAc), 14.16 (CH_3, octyl); MS (FAB) $[M+Na]^+$ 974.4.

Octyl 3,6-di-*O*-benzyl-2-deoxy-4-*O*-methyl-2-phthalimido-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**46**)



The thioglycoside **42** (168 mg, 314.8 μmol), the alcohol **21** (154.10 mg, 273.80 μmol) and molecular sieves (4 Å, 2.0 g) were suspended in dichloromethane (30 ml) and stirred for 0.5 h. The mixture was cooled to 0°C and *N*-iodosuccinimide (99.2 mg, 441 μmol) and trifluoromethanesulfonic acid (68 μmol) were added subsequently, while the mixture kept stirring at 0°C. After 0.5 h saturated sodium bicarbonate solution (10 ml) was added and the organic layer was separated, washed with sodium thiosulfate (10 ml) and water (6 ml) and dried (Na_2SO_4). The organic layer was concentrated and chromatographed using 20% ethyl acetate in hexane as the eluent. The product **46** (252 mg, 88%) was isolated as a colourless oil; R_f 0.58 in ethyl acetate/hexane, 1:2; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.85-6.90 (m, 29H, aromatics), 5.16 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1'), 4.55 (dd, 1H, $J_{3',4'}$ = 2.9 Hz, $J_{2',3'}$ = 11.2 Hz, H-3'), 4.70-4.14 (m, 10H, 5 x $OC\underline{H}_2Ph$), 4.10 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1), 3.84-3.45 (m, 9H), 3.60 (s, 3H, OCH_3), 3.36 (dt, 1H, J_{gem} = 9.5 Hz, J_{vic} = 6.2 Hz, $OC\underline{H}_2CH_2$, octyl), 3.28 (t, 1H, $J_{5,6}$ = 5.9 Hz, H-5), 3.13-3.06 (m, 2H), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 168.97, 167.80 (2 x C=O), 138.70-122.68 (C, aromatics), 103.22, 100.15 (C-1, C-1'), 79.96 (C-H), 79.50 (C-H), 76.30 (C-H), 76.22 (C-H), 74.96 (C-H_2), 74.26 (C-H), 73.56 (C-H_2), 73.44 (C-H), 73.37 (C-H_2), 72.80 (C-H_2), 72.48 (C-H), 71.56 (C-H_2), 69.80(C-H_2), 69.49 (C-H_2), 68.37 (C-H_2), 61.26 (C-2'), 53.13 (OCH_3), 31.90, 29.76, 29.49, 29.30, 26.20, 22.73 (6 x C-H_2, octyl), 14.16 (CH_3, octyl); MS (FAB) $[M+H]^+$ 1048.8.

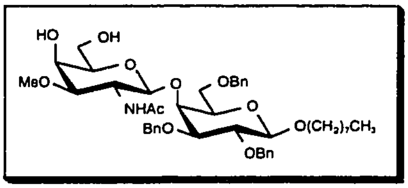
Octyl 3,4-di-*O*-acetyl-2-deoxy-6-*O*-methyl-2-phthalimido-β-D-galactopyranosyl-(1→4) - 2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**47**)



The thioglycoside **44** (107 mg, 245 μmol), the alcohol **21** (124 mg, 220.4 μmol) and molecular sieves (4 Å, 1.0 g) were suspended in dichloromethane (30 ml) and stirred for 0.5 h. The mixture was cooled to 0°C and *N*-iodosuccinimide (71.5 mg, 318 μmol) and trifluoromethanesulfonic acid (31 μmol) were added subsequently, while the mixture kept stirring at 0°C. After 0.5 h saturated sodium bicarbonate solution (10 ml) was added and the organic layer was separated, washed with saturated sodium thiosulfate (10 ml) and water (6 ml) and dried (Na_2SO_4). The organic layer was concentrated and chromatographed using 20% ethyl acetate in hexane as the eluent. The product **47** (160 mg, 71%) was isolated as a colourless oil; R_f 0.50 in toluene/ethyl acetate, 1:2; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.85-6.90 (m, 19H, aromatics), 6.05 (dd, 1H, $J_{2',3'}$ = 11.5 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 5.47 (dd, 1H, $J_{4',5'}$ ~ 1.0 Hz, H-4'), 5.30 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 4.61 (dd, 1H, H-2'), 4.52 (s, 2H, $OC\underline{H}_2Ph$), 4.25 (ABq, 2H, J_{gem} = 12.5 Hz, $OC\underline{H}_2Ph$), 4.13 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1), 4.01 (ABq, 2H, J_{gem} = 10.4 Hz, $OC\underline{H}_2Ph$), 3.94 (t, 1H, $J_{5',6'}$ = 6.0 Hz, H-5'), 3.86 (dt, 1H, J_{gem} = 9.5 Hz, J_{vic} = 6.2 Hz, $OC\underline{H}_2CH_2$, octyl), 3.78 (dd, 1H, $J_{5,6a}$ = 5.8 Hz, $J_{6a,6b}$ = 10.0 Hz, H-6a), 3.60-3.69 (m, 2H, H-6b, H-4), 3.42-3.30 (m, 4H, H-5, H-6'a, H-6'b, $OC\underline{H}_2CH_2$), 3.23 (s, 3H, OCH_3), 3.16 (dd, 1H, $J_{3,4}$ =

2.6 Hz, $J_{2,3}$ = 9.9 Hz, H-3), 3.08 (dd, 1H, H-2), 2.20, 1.85 (2 x s, 6H, OAc), 1.7-1.2 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 170.39, 169.88, 168.97, 167.50 (4 x C=O), 138.45-123.20 (C, aromatics), 103.39, 99.96 (C-1, C-1'), 79.90 (C-H), 79.70 (C-H), 76.80 (C-H), 75.05 (C-H_2), 73.38 (C-H_2), 73.25 (C-H), 72.84 (C-H_2), 71.41 (C-H), 70.75 (C-H_2), 69.96 (C-H_2), 69.71 (C-H_2), 67.82 (C-H), 67.27 (C-H), 59.29 (C-2'), 51.56 (OCH_3), 31.88, 29.72, 29.49, 29.29, 26.20, 22.71 (6 x C-H_2, octyl), 20.80, 20.68 (2 x OAc), 14.16 (CH_3, octyl); MS (FAB) $[M+Na]^+$ 974.4.

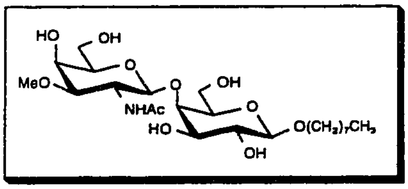
Octyl 2-deoxy-3-*O*-methyl-2-phthalimido-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**48**)



The disaccharide **45** (200 mg, 0.21 mmol) was dissolved in dry methanol (9 ml). The pH was adjusted to 9 using a sodium methoxide solution (1M). The mixture was stirred for 1 h. Amberlite (IRC 50) was added to neutralize the reaction solution. The solution was filtered (celite) and concentrated. The solid residue was then dissolved in a mixture of butanol (14 ml) and ethylenediamine (3.5 ml) and heated at 90°C for 17 h under an inert argon atmosphere. The reaction mixture was cooled, concentrated, and codistilled to dryness (3 x 5 ml) with toluene. The yellow crude was dissolved in dry methanol (3.5 ml) and acetic anhydride (59.4 µl, 630 µmol) was added. The residue was concentrated in vacuuo and chromatographed using 6% methanol in dichloromethane as the eluent. The product **48** (109.1 mg, 66%) was obtained as a white foam; R_f 0.53 in ethyl acetate/methanol, 1:4; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 15H, aromatics), 5.63 (d, br., 1H, $J_{2',NH}$ = 7.1 Hz, NH), 4.85 (ABq, 2H, J_{gem} = 11.0 Hz, $OC\underline{H}_2Ph$), 4.70 (ABq, 2H, J_{gem} = 11.2 Hz, $OC\underline{H}_2Ph$), 4.57 (ABq, 2H, J_{gem} = 12.2 Hz, $OC\underline{H}_2Ph$), 4.28 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'), 4.34 (d, 1H, $J_{1,1}$ = 7.5 Hz, H-1), 4.05-3.85 (m, 6H), 3.73-

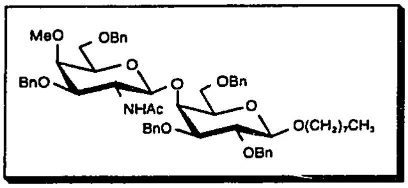
3.38 (m, 9H), 3.37 (s, 3H, OCH_3), 2.85, 2.55 (2 x s, br., 2H, OH), 1.75 (s, 3H, CH_3, NHAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, CH_3, octyl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 170.74 (C=O), 138.69, 138.15, 137.98 (3 x C, benzyl), 128.67-127.73 (C-H, aromatics), 103.97, 101.80 (C-1, C-1'), 81.08 (C-H), 80.94 (C-H), 79.79 (C-H), 75.22 (C-H_2), 74.74 x 2 (C-H), 73.68 (C-H_2), 73.54 (C-H_2), 72.19 (C-H), 70.13 (C-H_2), 68.41 (C-H_2), 65.51 (C-H), 62.41 (C-H_2), 57.62 (C-2'), 53.16 (OCH_3), 31.87, 29.76, 29.46, 29.28, 26.20, (5 x C-H_2, octyl), 23.64 (CH_3, NHAc), 22.70 (C-H_2, octyl), 14.12, (CH_3, octyl); MS (FAB) $[M+H]^+$ 780.4. Addition of D_2O (10 μl) led to the disappearance of the signals at 5.63, 2.85 and 2.55.

Octyl 2-acetamido-3-*O*-methyl-2-deoxy-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**4**)



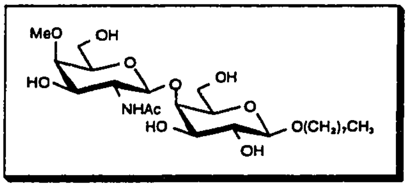
The disaccharide **48** (16 mg, 21 μmol) was dissolved in methanol (2 ml). The mixture was hydrogenated (1 atm) over Pd-C (22 mg) for 6 h before it was filtered through a millipore filter and concentrated giving product **4** (9.50 mg, 91 %) as a white powder. For use in the biological assay **4** was dissolved in water (10 ml) and adsorbed on a sepak column. The column was washed with distilled water (3 x 10 ml) before **4** was eluted using methanol (HPLC grade). The fractions containing **4** were collected and concentrated to dryness. The residue was redissolved in distilled water (50 ml) and lyophilized; $[\alpha]_D = -6.5°$ (c 0.5, MeOH); ^1H NMR (500 MHz, D_2O) δ: 4.66 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 4.37 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.21 (d, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 4.10 (d, 1H, $J_{3',4'}$ = 2.6 Hz, H-4'), 3.95 (dd, 1H, $J_{2,3}$ = 11.0 Hz, H-2), 3.49 (dd, 1H, H-3), 2.10 (s, 3H, NHAc), 1.70-1.20 (m, 12H, CH_2, octyl), 0.85 (t, 3H, CH_3, octyl); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 174.01 (C=O), 104.96, 104.35 (C-1, C-1'), 82.39, 77.56, 76.70, 75.59. 74.99, 72.58, 70.80, 65.63, 62.70, 61.32, 57.31, 53.31, 32.99, 30.84, 30.56, 30.38, 27.08, 23.69, 23.28, 14.39; MS (FAB) $[M + H]^+$ 510.3.

Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**49**)



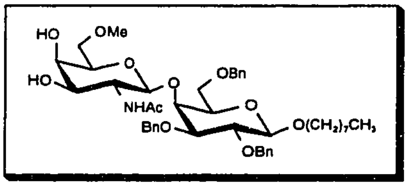
The disaccharide **46** (152.5 mg, 145 μmol) was dissolved in a mixture of butanol (14 ml) and ethylenediamine (3.5 ml) and heated at 90°C for 17 h under an inert argon atmosphere. The reaction mixture was then cooled, concentrated, and codistilled to dryness with toluene (3 x 10 ml). The yellow crude was dissolved in dry methanol (3.5 ml) and acetic anhydride (55 μl, 0.58 mmol) was added. The residue was concentrated in vacuuo and chromatographed using 6% methanol in dichloromethane as the eluent. The product **49** (93.5 mg, 67% was obtained as a white foam; R_f 0.36 in ethyl acetate/toluene, 1:3; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 25H, aromatics), 5.63 (d, br., 1H, $J_{2',NH}$ = 6.7 Hz, NH), 4.95 (d, 1H, J_{gem} = 11.0 Hz, $OC\underline{H}_2Ph$), 4.77 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.73-4.45 (m, 9H, $OC\underline{H}_2Ph$), 3.95-3.87 (m, 2H), 3.80-3.55 (m, 7H), 3.55 (s, 3H, OCH_3), 3.50-3.40 (m, 6H), 1.75 (s, 3H, CH_3, NHAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, CH_3, octyl); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 170.26 (C=O), 138.79, 138.64, 138.51, 138.04, 138.00 (5 x C, benzyl), 128.58-127.56 (C-H, aromatics), 103.79, 101.55 (C-1, C-1'), 81.30 (C-H), 79.92 (C-H), 79.74 (C-H), 75.19 (C-H_2), 74.92 (C-H), 74.35 (C-H), 73.78 (C-H), 73.57 x 2 (C-H_2), 73.50 (C-H_2), 73.35 (C-H), 72.17 (C-H_2), 70.02 (C-H_2), 69.87 (C-H_2), 68.45 (C-H_2), 61.33 (C-2'), 53.88 (OCH_3), 31.89, 29.79, 29.48, 29.30, 26.23, (5 x C-H_2, octyl), 23.73 (CH_3, NHAc), 22.71 (C-H_2, octyl), 14.14, (CH_3, octyl). MS (FAB) $[M+H]^+$ 960.5. Addition of D_2O to the ^{1}H NMR sample led to the disapearance of the signal at 5.63.

Octyl 2-acetamido-4-*O*-methyl-2-deoxy-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**3**)



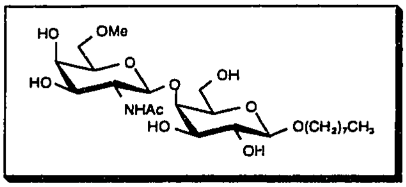
The disaccharide **49** (57 mg, 59 μmol) was dissolved in methanol (3 ml). The mixture was hydrogenated (1 atm) over Pd-C (57 mg) for 6 h before it was filtered through a millipore filter and concentrated giving product **3** (26 mg, 86%) as a white powder. For use in the biological assay **3** was dissolved in water (10 ml) and adsorbed on a reverse phase sepak column. The column was washed with distilled water (3 x 10 ml) before **3** was eluted using methanol (HPLC grade). The fractions containing **3** were collected and concentrated to dryness. The residue was redissolved in distilled water (50 ml) and lyophilized; $[\alpha]_D$ = -18.7° (c 0.5, MeOH); ^{1}H NMR (360 MHz, D_2O) δ: 4.66 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 4.40 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 3.60 (s, 3H, OCH_3), 3.43 (dd, 1H, $J_{2',3'}$ = 9.8 Hz, H-2'), 2.10 (s, 3H, NHAc), 1.70-1.20 (m, 12H, CH_2, octyl), 0.85 (t, 3H, CH_3, octyl); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 175.77 (C=O), 103.84, 103.56 (C-1, C-1'), 79.21, 76.93, 75.98, 75.02, 73.87, 72.44, 71.91 (7 x C-H), 71.44 (C-H_2), 62.37 (C-2'), 61.60, 61.21 (2 x C-H_2), 54.12 (OCH_3), 32.17, 29.84, 29.55, 29.45, 26.10, (5 x C-H_2, octyl), 23.36 (C-H_3, NHAc), 23.05 (C-H_2, octyl), 14.39 (C-H_3, octyl); MS (FAB) $[M + Na]^+$ 532.2.

Octyl 2-deoxy-6-*O*-methyl-2-phthalimido-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-β-D-galactopyranoside (**50**)



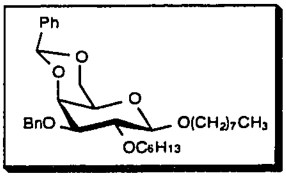
The disaccharide **47** (130 mg, 137 μmol) was dissolved in dry methanol (9 ml). The pH was adjusted to 9 using a sodium methoxide solution (1M). The mixture was stirred for 1 h. Amberlite (IRC 50) was then added to neutralize the reaction. The solution was filtered and concentrated. The solid residue was then dissolved in a mixture of butanol (14 ml) and ethylenediamine (3.5 ml) and heated at 90°C for 17 h under an inert argon atmosphere. The reaction mixture was then cooled, concentrated, and codistilled to dryness with toluene (3 x 8 ml). The yellow crude was dissolved in dry methanol (4 ml) and acetic anhydride (52 µl, 0.55 mmol) was added. The residue was concentrated in vacuuo and chromatographed using 17% ethyl acetate in toluene as the eluent. The product **50** (78 mg, 73% was obtained as a white foam; R_f 0.68 in ethyl acetate/methanol, 1:4; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 15H, aromatics), 6.63 (d, br., 1H, $J_{2',NH}$ = 7.0 Hz, NH), 4.82 (ABq, 2H, J_{gem} = 11.0 Hz, $OC\underline{H}_2Ph$), 4.75 (ABq, 2H, J_{gem} = 10.1 Hz, $OC\underline{H}_2Ph$), 4.58 (ABq, 2H, J_{gem} = 11.9 Hz, $OC\underline{H}_2Ph$), 4.42 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 4.37 (d, 1H, $J_{1,1}$ = 7.2 Hz, H-1), 4.07 (d, 1H, J = 2.2 Hz), 3.99-3.85 (m, 2H), 3.77 (d, 1H, J = 3.4 Hz), 3.74-3.47 (m, 9H), 3.38 (dd, 1H, J = 3.4 Hz, J = 9.3 Hz), 3.29 (s, 3H, OCH_3), 2.95, 2.85 (2x s, br., 2H, OH), 1.75 (s, 3H, CH_3, NHAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, CH_3, octyl); Addition of D_2O (30 µl) led to the disappearance of signals at 6.63, 2.95 and 2.85; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 173.86 (C=O), 138.49, 138.39, 136.54 (3 x C, benzyl), 128.67-127.73 (C-H, aromatics), 103.65, 102.60 (C-1, C-1'), 81.74, 80.18, 76.72, 75.30, 75.25, 75.12, 74.01, 73.49, 73.43, 71.92, 70.11, 69.33, 67.47, 59.18, 55.95, 31.79, 29.67, 29.38, 29.20, 26.15, 22.62, 22.21, 14.20. MS (FAB) $[M+Na]^+$ 802.4.

Octyl 2-acetamido-6-*O*-methyl-2-deoxy-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (**2**)



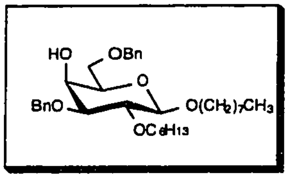
The disaccharide **50** (50 mg, 64 µmol) was dissolved in methanol (3 ml). The mixture was hydrogenated (1 atm) over Pd-C (52 mg) for 6 h. The mixture was then filtered through a millipore filter and concentrated giving product **2** (30 mg, 92%) as a white powder. For use in the biological assay **2** was dissolved in water (10 ml) and adsorbed on a reverse phase sepak column. The column was washed with distilled water (3 x 10 ml) before **2** was eluted using methanol (HPLC grade). The fractions containing **2** were collected and concentrated to dryness. The residue was redissolved in distilled water (50 ml) and lyophilized; $[\alpha]_D = -13.3°$ (c 0.5, MeOH); ^{1}H NMR (360 MHz, D_2O) δ: 4.64 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.35 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.07 (d, 1H, $J_{3',4'}$ = 2.8 Hz, H-4'), 3.43 (s, 3H, OCH_3), 3.39 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, H-2'), 2.10 (s, 3H, NHAc), 1.70-1.20 (m, 12H, CH_2, octyl), 0.85 (t, 3H, CH_3, octyl); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 176.20 (C=O), 104.17, 103.96 (C-1, C-1'), 77.17, 74.98, 74.30, 73.99, 72.97, 72.17 x 2, 71.74, 69.30, 61.13, 59.71, 53.92, 32.59, 30.30, 30.19, 29.97, 23.49, 23.74, 23.46, 14.80; MS (FAB)MS $[M + Na]^+$ 532.2.

Octyl 4,6-*O*-benzylidene-3-*O*-benzyl-2-O-hexyl-β-D-galactopyranoside (**51**)



Compound **11** (300 mg, 638 μmmol) was dissolved in *N*,*N*-dimethylformamide (10 ml). Sodium hydride (160 mg, 6.67 mmol) and 1-bromohexane (269 μl, 9.0 mmol) were added. The mixture was stirred for 4 h. Methanol (5 ml) was then slowly added to destroy excess sodium hydride. The mixture was concentrated to dryness. Water (50 ml) was added and the product was extracted with dichloromethane (2 x 30 ml). The organic layer was dried (Na_2SO_4), filtered and concentrated. Chromatographic purification using 12% ethyl acetate in pentane as the eluent gave product **51** (287 mg, 81%) as a clear syrup; $[\alpha]_D = -10.5°$ (c 0.5, $CHCl_3$); R_f 0.50 in ethyl acetate/pentane, 1:4; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.50-7.15 (m, 10H, benzyl aromatics), 5.38 (s, 1H, PhC<u>H</u>$(O)_2$), 4.67 (ABq, 2H, J_{gem} = 12.5 Hz, OC<u>H</u>$_2$Ph), 4.18 (d, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.17 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 3.97 (d, 1H, $J_{3,4}$ = 3.6 Hz, H-4), 3.89 (dd, 1H, $J_{5,6b}$ = 1.5 Hz, H-6b), 3.87-3.73 (m, 2H, 2x(OC<u>H</u>$_2$CH$_2$-), 3.64-3.57 (m, 1H, OC<u>H</u>$_2$CH$_2$-), 3.52 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-3), 3.40-3.32 (m, 2H, H-3, OC$\underline{H}_2$CH$_2$-), 3.18 (s, br., H-5), 1.70-1.20 (m, 20H, 10 x CH$_2$, octyl, hexyl), 0.85 (t, 6H, J_{vic} = 6.8 Hz, CH$_3$, octyl, hexyl); Anal. calcd. for $C_{32}O_6H_{50}$: C 73.61, H 9.08 found: C 73.38, H 8.87.

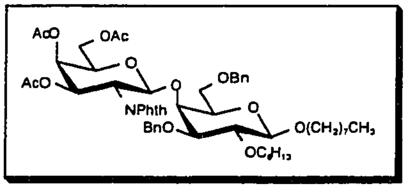
Octyl 3,6 di-*O*-benzyl-4-*O*-hexyl-β-D-galactopyranoside (**52**)



The acetal **51** (0.26 g, 469 μmol) was dissolved in tetrahydrofuran (20 ml). Sodium cyanoborohydride (295 mg, 4.68 mmol) and two crystals of methyl orange were added. To this was added dropwise a cold, saturated anhydrous hydrochlorid ether solution under an inert argon atmosphere. The addition of the acidic ether solution was continued until the development of hydrogen gas ceased and the solutuon became red. It was advantageous to add more acidic ether (~ 5 ml) after the indicator turned red. The mixture was stirred for another 2 h and then Dowex 50 (H$^+$, 0.8 g) was added. The stirring was continued for an additional 2 h. The mixture was filtered (celite) and concentrated. Water (30 ml) was added and the aqueous phase was extracted with dichloromethane (3 x 20 ml). The combined organic layers were washed with sodium bicarbonate (2 x 20 ml), dried (Na_2SO_4), concentrated and chromatographed using 10% ethyl acetate in hexane as the eluent. The major product **52** (186 mg, 71%) was obtained as a colourless oil; $[\alpha]_D$ = -13.2^0(c 0.5, CHCl$_3$); R_f 0.55 in toluene/ethyl acetate, 5:1; ^{1}H NMR (360 MHz, CDCl$_3$) δ: 7.40-7.20 (m, 10H, benzyl aromatics), 4.63 (ABq, 2H, J_{gem} = 11.9 Hz, OC$\underline{H}_2$Ph), 4.48 (s, 2H, OC$\underline{H}_2$Ph), 4.15 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 3.88 (m, 1H, $J_{3,4}$ = 2.4 Hz, H-4), 3.84-3.71 (m, 2H, OCH$_2$-, octyl, hexyl), 3.68 (dd, 1H, $J_{5,6a}$ = 6.0 Hz, $J_{6a,6b}$ = 9.9 Hz, H-6a), 3.60 (dd, 1H, $J_{5,6b}$ = 6.6 Hz, H-6b), 3.57 (dt, 1H, J_{gem} = 9.2 Hz, J_{vic} = 6.7 Hz, OC$\underline{H}_2$-), 3.45-3.30 (m, 4H, H-2, H-3, H-5, OC$\underline{H}_2$- octyl, hexyl), 2.35 (s, br., 1H, OH), 1.7-1.2 (m, 20H, 10 x CH$_2$, octyl, hexyl), 0.85 (t, 6H, J_{vic} = 7.5 Hz, CH$_3$, octyl, hexyl); Anal. calcd. for $C_{34}H_{52}O_6$: C 73.34, H 9.41 found: C 73.59, H 9.65.

The product was further characterized via acetylation to yield octyl 4-*O*-acetyl-3,6 di-*O*-benzyl-2-*O*-hexyl-β-D-galactopyranoside (**53**); ^{1}H NMR (360 MHz, CDCl$_3$) δ: 7.37-7.25 (m, 10H, benzyl aromatics), 5.53 (d, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 4.80 (ABq, 2H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.50 (ABq, 2H, J_{gem} = 12.3 Hz, OC$\underline{H}_2$Ph), 4.27 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 3.92 (dt, 1H, J_{gem} = 9.5 Hz, J_{vic} = 6.5 Hz, OC$\underline{H}_2$), 3.80 (dt, 1H, J_{gem} = 9.8 Hz, J_{vic} = 6.8 Hz, OC$\underline{H}_2$), 3.70-3.62 (2H, H-5, OC$\underline{H}_2$), 3.58-3.48 (m, 3H, H-6a, H-6b, OC$\underline{H}_2$), 3.45 (dd, 1H, $J_{2,3}$ = 9.6 Hz, H-3), 3.35 (dd, 1H, H-2), 2.08 (s, 3H, OAc), 1.70-1.20 (m, 20H, 10 x CH$_2$, octyl, hexyl), 0.85 (t, 6H, J_{vic} = 7.5 Hz, CH$_3$, octyl, hexyl).

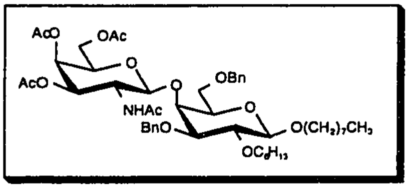
Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-hexyl-β-D-galactopyranoside (**54**)



A mixture of the alcohol **52** (145 mg, 260 μmol), the thioglycoside **26** (146 mg, 312 μmole) and molecular sieves (0.6 g, 4 Å) in dry dichloromethane (15 ml) was stirred under argon for 30 min at 0°C. *N*-Iodosuccinimide (77.3 mg, 344 μmol) was added directly followed by a addition of trifluoromethanesulfonic acid (37 μmol). The reaction mixture was stirred for an additional 1 h at 0 °C. Saturated sodium bicarbonate solution (10 ml) was then added. The organic layer was washed with saturated sodium thiosulfate (5 ml) and water (5 ml) and (Na$_2$SO$_4$) and concentrated to give a viscous syrup which was subjected to chromatographic purification with 7% ethyl acetate in toluene as the eluent to give product **54** (151.00 mg, 60%) as a clear syrup; $[\alpha]_D$ = -14.8^0 (c 0.8, CHCl$_3$); R$_f$ 0.44 in toluene/ethyl acetate, 3:1; ^{1}H NMR (360 MHz, CDCl$_3$) δ: 7.90-7.55 (m, 4H, phthalimido), 7.40-6.90 (m, 10H, benzyl aromatics), 6.04 (dd, 1H, $J_{2',3'}$ = 11.6 Hz, $J_{3',4'}$ = 3.5 Hz, H-3'), 5.49 (d, 1H, H-4'), 5.32 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 4.60 (dd, 1H, H-2'), 4.52 (s, 2H, OC$\underline{H}_2$Ph), 4.23 (ABq, 2H, J_{gem} = 12.7 Hz, OC$\underline{H}_2$Ph), 4.18-4.10 (m, 2H, H6'a, OC$\underline{H}_2$CH$_2$-), 4.07-4.00 (m, 2H, H1, H6'b), 3.88-3.80 (dt, 1H, J_{gem} = 9.5 Hz,

J_{vic} = 6.5 Hz, OC$\underline{H}_2$CH$_2$-), 3.70 (dd, 1H, $J_{5,6a}$ = 5.6 Hz, $J_{6a,6b}$ = 10.1 Hz, H-6a), 3.64 (dd, 1H, $J_{5,6b}$ = 6.0 Hz, H-6b), 3.58 (d 1H, $J_{3,4}$ = 2.7 Hz, H-4), 3.40-3.30 (m, 3H, H-5, H-5', OC$\underline{H}_2$CH$_2$-), 3.07 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-3), 2.85 (dd, 1H, $J_{1,2}$ = 7.7 Hz, H-2), 2.63 (dt, 1H, J_{gem} = 8.8 Hz, J_{vic} = 6.8 Hz, OC$\underline{H}_2$CH$_2$-), 2.10, 2.03, 1.95 (3 x s, 9H, 3 x OAc), 1.70-1.20 (m, 20H, 10 x CH$_2$, octyl, hexyl), 0.90 (t, 3H, J_{vic} = 7.5 Hz, CH$_3$, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH$_3$, octyl); Anal. calcd. for $C_{54}H_{71}O_{15}N_1$: C 66.58 H 7.34, N 1.43, found: C 66.93, H 7.49, N 1.44.

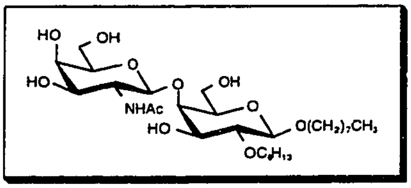
Octyl 2-acetamido-3,4,6 tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-hexyl-β-D-galactopyranoside (**55**)



The disaccharide **54** (100 mg, 103 μmol) was dissolved in dry methanol (9 ml). The pH was adjusted to 9 using a sodium methoxide solution (1M). The mixture was stirred for 1 h. Amberlite (IRC 50) was then added to neutralize the solution. The solution was filtered (celite) and concentrated. The solid residue was then dissolved in a mixture of butanol (14 ml) and ethylenediamine (3.5 ml) and heated at 90°C for 17 h under an inert argon atmosphere. The reaction mixture was cooled, concentrated, and codistilled to dryness with toluene (3 x 6 ml). The yellow crude was dissolved in a mixture of 33% pyridine in acetic anhydride (10 ml) and was stirred for 12 h. The mixture was concentrated under vacuum and chromatographed using 20% ethyl acetate in in toluene as the eluent. The product **55** (63 mg, 69%) was obtained as a colourless oil; R_f 0.25 in toluene/ethyl acetate, 3:1; $[\alpha]_D = -9.3^0$ (c 1.0, CHCl$_3$); ^{1}H NMR (360 MHz, CDCl$_3$) δ: 7.40-7.20 (m, 10H, benzyl aromatics), 5.70 (d, br., 1H, $J_{2',NH}$ = 7.6 Hz, NH), 5.25 (d, 1H, $J_{3',4'}$ = 3.1 Hz, H-4'), 4.80 (dd, 1H, $J_{2',3'}$ = 10.9 Hz, H-3'), 4.72 (ABq, 2H, J_{gem} = 10.7 Hz, OC$\underline{H}_2$Ph), 4.59 (d, 1H, $J_{1',2}$ = 8.7 Hz, H-1'), 4.52 (ABq, 2H, J_{gem} = 12.0 Hz, OC$\underline{H}_2$Ph), 4.19 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.12-4.04 (m, 2H), 3.98 (dd, 1H, $J_{5',6'a}$ =

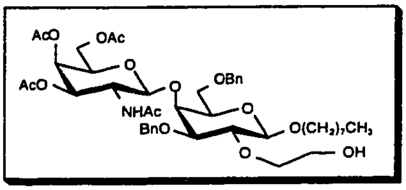
5.9 Hz, $J_{6'a,6'b}$ = 11.1 Hz, H-6'b), 3.90-3.80 (m, 3H), 3.77-3.70 (m, 2H), 3.63-3.58 (m, 1H), 3.56-3.38 (m, 4H), 3.30 (dd, 1H, H-2'), 2.15, 1,98, 1.95, 1.72 (4 x s, 12H, 3x OAc, NHAc), 1.7-1.2 (m, 20H, 10 x CH_2, octyl, hexyl), 0.90 (t, 3H, J_{vic} = 7.5 Hz, CH_3), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3); Addition of D_2O (20 µl) led to the disapperance of the signal at 5.70.

Octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-*O*-hexyl-β-D-galactopyranoside (**56**)



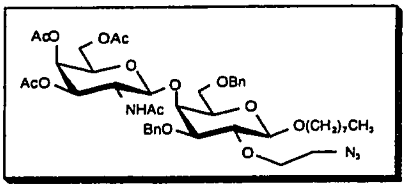
The disaccharide **55** (55 mg, 62 µmol) was dissolved in methanol (3 ml) and sodium methoxide solution was added (pH = 9). After 2 h, Amberlite (IRC 50, 50 mg) was added to neutralize the solution. The mixture was filtered, concentrated and dissolved in methanol (3 ml) and $Pd(OH)_2/C$ (45 mg) was added. Hydrogenation (6 h, 1 atm) of the crude material gave **56** (31 mg, 85%) as a colourless oil. For use in the biological assay **56** (6 mg was dissolved in water (50 ml) and adsorbed on a reverse phase sepak column. The coloumn was washed with distilled water (3 x 30 ml) and **56** was then eluted using methanol (HPLC grade). The fractions containing **56** were collected and concentrated to dryness. The residue was redissolved in distilled water (50 ml) and lyophilized; $[\alpha]_D$ = -5.0° (c 0.5, MeOH); ^{1}H NMR (360 MHz, CD_3OD) δ: 4.62 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1'), 4.23 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1), 3.18 (dd, 1H, $J_{2',3'}$ = 9.6 Hz, H-2'), 2.03 (s, 3H, NHAc), 1.70-1.82 (m, 20H, CH_2, octyl, hexyl), 0.90 (t, 3H, CH_3), 0.85 (t, 3H, CH_3); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 174.20 (C=O), 105.01, 104.41 (C-1, C-1'), 81.34. 78.65, 76.89, 75.32, 74.62, 74.45 (6 x C-H), 74.40 (C-H_2), 70.77 (C-H_2), 69.56 (C-H), 62.66 (C-H_2), 61.23 (C-H_2), 55.56 (C-H), 33.00, 32.97, 31.32, 30.89 x 2, 30.54, 30.45, 27.32, 26.95, 23.73 (10 x C-H_2), 23.15 (C-H_3), 14.41 (2 x C-H_3); MS (MALDI) $[M + Na]^+$ 602.4.

Octyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-(2-hydroxyethyl)-β-D-galactopyranoside (**57**)



The allyl ether **34** (1.90 g, 2.25 mmol) was dissolved in a (1:1) mixture of dichloromethane and methanol (100 ml). The temperature was lowered to -79°C and a gentle flow of ozon was bubbled through the solution for 12 min while stirring. During this time the solution became blue. The ozon flow was stopped and dimethylsulfide (~ 3.5 ml) was added at -79 °C which decolourized the solution instantly. The temperature was slowly raised to room temperature over a period of 2 h. The solution was acidified with trifluoroacetic acid (2 ml) and sodium cyanoborohydride (1.5 g, 23.8 mmol) was added in small portions over a period of 1 h. After stirring the mixture for 12 h, acidic resin (Dowex 50, 3.5 g) was added. The stirring continued for another 12 h. Water (200 ml) was added and the organic layer separated, dried (Na_2SO_4) and concentrated. Chromatographic purification using 25% ethyl acetate in toluene as the eluent gave **57** (1.20 g, 63%) as a colourless oil; $[\alpha]_D$ = 16.5° (c 0.5, $CHCl_3$); R_f 0.25 in ethyl acetate/toluene, 3:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 10H, benzyl aromatics), 5.55 (d, 1H, $J_{2',NH}$ = 8.0 Hz, NH), 4.69 (d, 1H, $J_{1',2'}$ = 8.6 Hz, H-1'), 4.77 (ABq, 2H, J_{gem} = 10.9 Hz, $OC\underline{H}_2Ph$), 4.53 (s, 2H, $OC\underline{H}_2Ph$), 4.25 (d, 1H, $J_{1,2}$ = 7.4 Hz, H-1), 4.00-3.40 (m, 19H), 2,08, 2.00, 1.95, 1.75 (4 x s, 12H, 3 x OAc, NHAc), 1.70-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); Addition of D_2O (10 μl) led to the disappearance of the signal at 5.55; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 170.42, 170.36, 170.32, 170.20 (4 x C=O), 138.27, 137.13, (2 x C, benzyl), 129.08-127.68 (C-H, aromatics), 103.08, 102.03, (C-1, C-1'), 81.82 (C-H), 79.60 (C-H), 74.85 (C-H), 74.52 ($C-H_2$), 73.89 ($C-H_2$), 73.62 ($C-H_2$), 74.57 (C-H), 73.49 (C-H), 71.12 (C-H), 70.72 (C-H), 70.11 ($C-H_2$), 69.53 ($C-H_2$), 62.41 ($C-H_2$), 61.33 ($C-H_2$), 51.25 (C-2'), 31.85, 29.54, 29.38, 29.25, 26.00 (5 x $C-H_2$), 23.31 ($C-H_3$), 22.68 ($C-H_2$), 20.76, 20.74, 20.72 (3 x C-H_3), 14.13 (C-H_3, octyl); Anal. calcd. for $C_{44}H_{63}O_{15}N_1$: C 62.47 H 7.50, N 1.65, found: C 62.73, H 7.57, N 1.63.

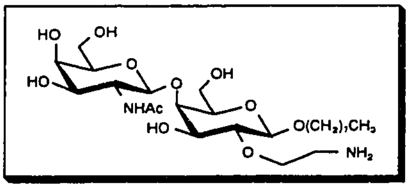
Octyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-*O*-(2-azidoethyl)-β-D-galactopyranoside (**58**)



The alcohol **57** (1,10 g, 1.30 mmol) was dissolved in dry pyridine (30 ml) and methanesulfonyl chloride (201 μl, 2.60 mmol) was added at 0°C. The mixture was stirred for 2 h and then concentrated. Filtration over a short path silica coloumn using 33% toluene in ethyl acetate as the eluent gave the mesylate (1.05) g which was of sufficient purity to introduce the azido function directly. The crude material (1.05 g) was dissolved in *N,N*- dimethylformamide (20 ml) and sodium azide (376 mg, 5.78 mmol) was added. The reaction solution was heated to 80°C and stirred at this temperature for 5 h. Then the mixture was cooled to room temperature and the solvent was removed under reduced pressure. Water (30 ml) and dichloromethane (30 ml) was added. The organic layer was separated, dried (Na_2SO_4) and concentrated. Thirtythree percent ethyl acetate in toluene was used as eluent in chromatographic purification. The product **58** (777 mg, 68%) was isolated as an oil; $[\alpha]_D = -20.4^0$ (c 0.9, $CHCl_3$); R_f 0.36 in toluene/ethyl acetate, 1:2; ^1H NMR (500 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 10H, benzyl aromatics), 5.65 (d, br., 1H, $J_{2',NH}$ = 7.9 Hz, NH), 5.24 (dd, 1H, $J_{3',4'}$ = 2.6 Hz, $J_{4',5'}$ ~ 1Hz, H-4'), 4.76 (dd, 1H, $J_{2',3'}$ = 11.0 Hz, H-3'), 4.75 (d, 1H, $J_{1',2}$ = 8.6 Hz, H-1'), 4.72 (ABq, 2H, J_{gem} = 10.5 Hz, OC$\underline{H}_2$Ph), 4.53 (ABq, 2H, J_{gem} = 12.0 Hz, OC$\underline{H}_2$Ph), 4.24 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1), 4.14-4.04 (m, 3H), 3.99 (dd, 1H, $J_{5',6'b}$ = 5.9 Hz, $J_{6'a,6'b}$ = 11.0 Hz, H-6'b), 3.92 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4), 3.86 (dt, 1H, J_{gem} = 9.2 Hz, J_{vic} = 6.6 Hz, OC$\underline{H}_2$), 3.77-3.70 (m, 3H), 3.63 (dd, 1H, J = 5.8 Hz, J = 10.0 Hz), 3.52-3.30 (m, 6H), 2.11, 1.99, 1.93, 1.64 (4 x s, 12H, 3 x OAc, NHAc), 1.60-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} =

7.5 Hz, CH_3, octyl); Addition of D_2O (30 µl) led to the disapperance of the signal at 5.65; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 170.38, 170.28, 170.24, 169.69 (4 x C=O), 138.22, 137.43, (2 x C, benzyl), 129.08-127.68 C-H, aromatics), 103.29, 102.82, (C-1, C-1'), 81.08 (C-H), 80.24 (C-H), 76.32 (C-H), 74.46 ($C-H_2$), 73.47 ($C-H_2$), 73.22 (C-H), 71.81 (C-H), 71.52 ($C-H_2$), 70.77 (C-H), 69.88 ($C-H_2$), 69.32 ($C-H_2$), 66.52 (C-H), 61.21 ($C-H_2$), 51.39 ($C-H_2$), 50.74 (C-2'), 31.78, 29.58, 29.31, 29.19, 26.03 (5 x $C-H_2$), 23.14 ($C-H_3$), 22.60 ($C-H_2$), 20.69, 20.65, 20.58 (3 x $C-H_3$), 14.05 ($C-H_3$, octyl); Anal. calcd. for $C_{44}H_{62}O_{14}N_4$: C 60.67, H 7.17, N 6.43, found: C 60.71, H 7.27, N 6.31: IR (KBr) 2103 cm^{-1} (s), 1752(s) cm^{-1}.

Octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-*O*-(2-aminoethyl)-β-D-galactopyranoside (**59**)

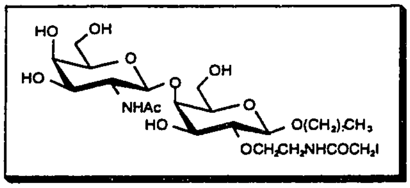


The azido compound **58** (0.79 g, 0.90 mmol) was dissolved in dry methanol (30 ml) and sodium methoxide solution was added until the solution reached basic pH (pH ~ 9). After 4 h, Amberlite (IRC 50, H^+, 250 mg) was added to neutralize the solution. The solution was filtered (celite), concentrated and redissolved in a (1:1) mixture of acetic acid and methanol. Palladiumhydroxid ($Pd(OH)_2/C$, 220 mg) was added and the solution was hydrogenated (1 atm) for 3 d. The solution was filtered (celite) and evaporated to dryness. The product **59** (300.56 mg, 62%) was isolated as a white foam and was of sufficient purity for further reactions. For use in the biological assay, **59** (10 mg) was dissolved in water (10 ml) and adsorbed on a reverse phase sepak column. The column was first washed with distilled water (3 x 10 ml) and then **59** was eluted using methanol (HPLC grade). The fractions containing **59** were collected and concentrated to dryness. The residue was dissolved in distilled water (50 ml) and lyophilized; $[\alpha]_D$ = +6.5° (c 0.5, CH_3OH); R_f 0.17 chloroform/methanol/water, 65:35:8; ^1H NMR (360

MHz, CD_3OD) δ: 4.58 (d, 1H, J = 8.3 Hz), 4.41 (d, 1H, J = 7.8 Hz), 4.05 (d, 1H, J = 2.8 Hz), 3.04 (t, 2H, $OCH_2\underline{CH_2}NH_2$), 2.05 (s, 3H, NHAc), 1.65-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 170.23 (C=O), 107.10, 106.99, (C-1, C-1'), 84.12, 81.07, 79.38, 78.00, 76.78, 76.38 (6 x C-H), 73.32 x 2 (C-H_2), 72.15 (C-H), 65.26 (C-H_2), 63.69 (C-H_2), 57.95 (C-H), 43.86 (C-H_2), 35.57, 33.43, 33.07, 32.97, 29.77, 26.26 (6 x C-H_2), 25.95 (C-H_3), 16.98 (C-H_3, octyl); Anal. calcd. for $C_{44}H_{62}O_{14}N_4$: C 60.67 H 7.17, N 6.43, found: C 60.71, H 7.27, N 6.31: IR (KBr) 2103 cm^{-1} (s), 1752(s) cm^{-1}; MS (FAB) $[M + H]^+$ 539.3.

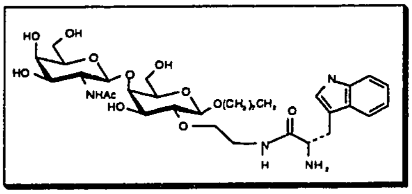
For further characterization, a small amount of **59** (5 mg) was dissolved in methanol (2 ml) and acetic anhydride (10.5 µl) was added. After stirring for 1.5 h, the mixture was concentrated to give octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-*O*-(2-ethyl-acetamido)-β-D-galactopyranoside (**60**); ^1H NMR (360 MHz, DMSO) δ: 7.85 (t, 1H, NHAc), 7.65 (d, 1H, NHAc), 1.80, 1.70 (2 x s, 6H, CH_3, NHAc); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 174.71, 173.28 (C=O), 104.81, 104.48 (C-1, C-1'), 81.86, 78.04, 76,84, 75.41, 74.25, 74.17 (6 x C-H), 72.70 (C-H_2), 70.83 (C-H_2), 69.59 (C-H), 62.68 (C-H_2), 61.19 (C-H_2), 55.41 (C-H), 40.90 (C-H_2), 33.00, 30.87, 30.49, 30.39, 27.23, 23.71 (6 x C-H_2), 23.27 (C-H_3), 22.68 (C-H_3), 14.43 (C-H_3, octyl); MS (FAB) $[M+Na]^+$ 603.1.

Octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-*O*-(2-[iodoacetamido]-ethyl)-β-D-galactopyranoside (**61**)



The amine **59** (10 mg, 18.56 µmol), was dissolved in dry methanol (1ml) and iodoacetic anhydride (9.8 mg, 27.8 μmol) was added. The flask was wrapped with aluminium foil. After 0.5 h more iodoacetic anhydride (2.3 mg, 6.5 μmol) was added. The mixture was stirred for 0.5 h and was then concentrated and purified by a reverse phase (C-18) column. The product **61** (11.5 mg, 87%) was obtained as a white powder after lyophilization; $[\alpha]_D = -2.0^0$ (c 0.5, CH_3OH); R_f 0.73 chloroform/methanol/water, 65:35:8; ^1H NMR (360 MHz, CD_3OD) δ: 4.63 (d, 1H, J = 8.4 Hz), 4.27 (d, 1H, J = 7.7 Hz), 4.03 (d, 1H, J = 2.9 Hz), 3.70 (s, 2H, $COC\underline{H}_2I$), 3.23 (dd, 1H, $J_{2',3'}$ = 9.5 Hz, H-2'), 2.05 (s, 3H, NHAc), 1.65-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 174.23, 171.33 (C=O), 104.76, 104.72, (C-1, C-1'), 81.79, 78.36, 76.85, 75.40, 74.28 x 2, (6x C-H), 72.18 ($C-H_2$), 70.81 ($C-H_2$), 69.58 (C-H), 62.68 ($C-H_2$), 61.17 ($C-H_2$), 55.54 (C-H), 41.40 ($C-H_2$), 33.01, 30.89, 30.50, 30.40, 27.23, 23.71 (6 x $C-H_2$, octyl), 23.39 ($C-H_3$, NHAc), 14.44 ($C-H_3$, octyl), 2.04 ($C-H_2$, $\underline{C}H_2I$); FAB $[M + Na]^+$ = 729.2.

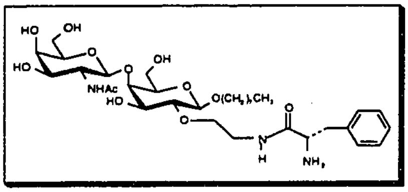
Octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-*O*-(2-N-(L-tryptophanylamine) ethylamido)-β-D-galactopyranoside (**63**)



The amino compound **59** (13 mg, 24.1 μmol) was dissolved in *N,N*-dimethylformamide (2 ml) and PfpOOC-L-Trp-NH-Fmoc (46.2 mg, 78.0 μmol) was added. The reaction mixture was stirred for 5 h and was then concentrated under reduced pressure. Chromatographic purification using 11% methanol in ethyl acetate as the eluent gave the Fmoc-protected amide **62** (19 mg, 84%) as a slightly yellow coloured powder. This material was then dissolved in piperidine (2 ml) and stirred for 2 h. Removal of the solvent under reduced pressure and chromatographic purification using a ternary mixture (ethyl acetate/methanol/triethylamine, 6:4:1) gave the desired product **63** (10.1 mg, 58%).

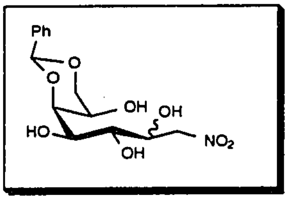
For use in the biological assay **63** was further purified by reverse phase (C-18) chromatography using methanol as eluent. The lyophilized product **63** was obtained as a white powder; $[\alpha]_D = -1.4^0$ (c 0.4, CH_3OH); ^1H NMR (360 MHz, CD_3OD) δ: 7.60 (d, 1H, J = 8.0 Hz, aromatic), 7.35 (d, 1H, J = 8.0 Hz, aromatic), 7.11 (s, 1H, aromatic), 6.97-7.10 (m, 2H, aromatic), 4.59 (d, 1H, J = 8.5 Hz, H-1), 4.15 (d, 1H, J = 7.8 Hz, H-1'), 3.97 (d, 1H, J = 2.7 Hz), 3.19 (dd, 1H, $J_{2',3'}$ = 9.8 Hz, H-2'), 3.05 (dd, 1H, J_{vic} = 7.0 Hz, J_{gem} = 12.3 Hz), 2.00 (s, 3H, NHAc), 1.65-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 174.76, 173.77 (2 x C=O), 137.19 (C, aromatic), 127.73 (C, aromatic), 123.93, 121.93, 118.93, 118.37, 111.45 (5 x C-H, aromatic), 109.59 (C, aromatic), 103.53 x 2 (C-1, C-1'), 80.72, 77.47, 75.81, 74.37, 73.27, 73.19 (6 x C-H), 71.37 ($C-H_2$), 69.80 ($C-H_2$), 68.54 (C-H), 61.68 ($C-H_2$), 60.12 ($C-H_2$), 55.65 (C-H), 54.38 (C-H), 39.93 ($C-H_2$), 31.98, 30.51, 29.80, 29.46, 29.39, 26.12, 22.69 (7 x $C-H_2$), 22.28 ($C-H_3$, NHAc), 13.42 ($C-H_3$, octyl); MS (MALDI) $[M+Na]^+$ 745.4.

Octyl 2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→4)-2-*O*-(2-N-(L-phenylalanineamine) ethylamido)-β-D-galactopyranoside (**65**)



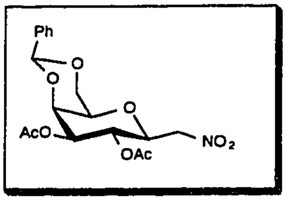
The amino compound **59** (13 mg, 24.1 μmol) was dissolved in *N,N*-dimethylformamide (2 ml) and PfpOOC-L-Phe-NH-Fmoc (40 mg, 72 μmol) was added. The reaction mixture was stirred for 5 h and was then concentrated under reduced pressure. Chromatographic purification using 11% methanol in ethyl acetate as the eluent gave the Fmoc-protected amide **64** (19.00 mg, 81%) as a white powder. This material was then dissolved in piperidine (2 ml) and stirred for 2 h. Removal of the solvent under reduced pressure and chromatographic purification using a ternary mixture (ethyl acetate/methanol/triethylamine, 6:4:1) as the eluent gave the desired product **65** (12.5 mg, 95%). For use in the biological assay **65** was further purified by reverse phase (C-18) chromatography using methanol as eluent. The lyophilized product **65** was obtained as a white powder; $[\alpha]_D$ = +4.2^0 (c 0.5, CH_3OH); ^{1}H NMR (360 MHz, CD_3OD) δ: 7.40-7.20 (m, 5H, aromatic), 4.61 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.24 (d, 1H, $J_{1',2'}$ = 7.7 Hz, H-1'), 4.00 (d, 1H, J = 3.1 Hz), 3.21 (dd, 1H, $J_{2',3'}$ = 9.4 Hz, H-2'), 3.04 (dd, 1H, J_{vic} = 6.2 Hz, J_{gem} = 13.4 Hz), 2.80 (dd, 1H, J_{vic} = 7.4 Hz, J_{gem} = 13.4 Hz), 2.00 (s, 3H, NHAc), 1.65-1.20 (m, 12H, 6 x CH_2, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH_3, octyl); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 176.29, 174.76 (2 x C=O), 138.64 (C, aromatic), 130.45 x 2, 129.61 x 2, 127.87 (5 x C-H, aromatic), 104.71, 104.46 (C-1, C-1'), 81.78, 78.45, 76.85, 75.41, 74.32, 74.26 (6 x C-H), 72.40 (C-H_2), 70.80 (C-H_2), 69.56 (C-H), 62.68 (C-H_2), 61.15 (C-H_2), 57.72 (C-H), 55.52 (C-H), 42.20 (C-H_2), 40.71 (C-H_2), 33.01, 30.88, 30.51, 30.42, 27.20, 23.70 (6 x C-H_2, octyl), 23.32 (C-H_3, NHAc), 14.42 (C-H_3, octyl); MS (FAB) $[M+Na]^+$ 708.0.

5,7-*O*-Benzylidene-1-deoxy-1-nitro-D-*glycero*-L-*manno* (L-*gluco*)-heptitol (**67**)



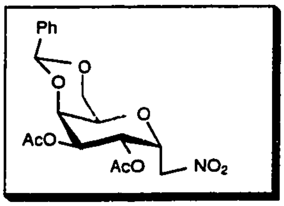
The acetal **66** (2.70 g, 10 mmol) was dissolved in a mixture containing nitromethane (7 ml) and tetrahydrofuran (40 ml). Molecular sieves (AW 300, 6 g), 1,8 diazabicyclo[5.4.0]undec-7-ene (1.06 ml, 7 mmol) and 2-hydroxypyridine (0.53 g, 5 mmol) were added. The resulting mixture stirred for 24 h. Stirring the mixture for a longer periode of time were avoided since it gave a significant amount of dinitro compound **68**. The reaction mixture was neutralized with Amberlite resin (IRC 50 H^+, 6 g), filtered and concentrated. A small amount of the product was chromatogaphically purified using 5% methanol in chloroform as the eluent to give a syrup; R_f 0.70 ethyl acetate/methanol/water, 12:3:1; ^{1}H NMR (360 MHz, CD_3OD) δ: 7.25-7.05 (m, 5H, aromatics), 5.35 (s, 1H, PhC<u>H</u>$(O)_2$), 4.60 (dd, 1H, J = 2.5 Hz, J = 12.4 Hz), 4.28-4.11 (m, 2H), 4.02 (dd, 1H, J = 1.8 Hz, J = 12.0 Hz), 3.90-3.83 (m, 2H), 3.72 (dd, 1H, J = 1.3 Hz, J = 9.4 Hz), 3.58 (m, 1H), 3.45 (dd, 1H, J = 0.9 Hz, J = 8.5 Hz), IR (cm $^{-1}$); 1549 (s), 1386 (s); MS (FAB) $[M + H]^+$ 330.1.

3,4-Di-*O*-acetyl-2,6-anhydro-5,7-*O*-benzylidene-7-deoxy-7-nitro-D-glycero-L-*manno*-heptitol (**69**)



The crude mixture **67** (2.80 g) was dissolved in tetrahydrofuran (40 ml), 1,8 diazabicyclo[5.4.0]undec-7-ene (1.06 ml, 7.0 mmol), 2-hydroxypyridine (0.53 g, 5 mmol) and molecular sieves (AW 300, 6g) were added and stirred for 5 days. The reaction mixture was neutralized with Amberlite resin (IRC 50 H^+, 6.00 g), filtered and concentrated. The product (20.1 mg) was dissolved in dichloromethane (2 ml) and acetic anhydride (120 μl) and imidazole (18 mg) were added. The resulting mixture was stirred for 3 d. Chromatographic purification using 9% ethyl acetate in toluene as the eluent gave the β-C-glycoside **69** (17.0 mg, 68 %) as a white powder. A lower moving spot was also isolated and characterized as α-C-glycoside **70**. The product **69** was characterized as follows; R_f 0.76 ethyl acetate/toluene, 1:2; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.50-7.30 (m, 5H, aromatics), 5.48 (s, 1H, PhC<u>H</u>$(O)_2$), 5.28 (t, 1H, J = 10.0 Hz, H-3), 5.02 (dd, 1H, $J_{3,4}$ = 10.0 Hz, $J_{4,5}$ = 3.5 Hz, H-4), 4.65 (dd, 1H, $J_{1a,1b}$ = 13.6 Hz, $J_{1a,2}$ = 10.1Hz, H-1a), 4.42 (dd, 1H, $J_{5,6}$ ~ 0.9 Hz, H-5), 4.36 (dd, 1H, $J_{1b,2}$ = 2.6 Hz, H-1b), 4.31-4.22 (m, 2H, H-2, H-7a), 3.98 (dd, 1H, $J_{7a,7b}$ = 13.1Hz, $J_{6,7b}$ = 1.8 Hz, H-7b), 3.55 (m, 1H, H-6), 2.05, 2.07 (2 x s, 6H, 2 x OAc); MS (FAB) $[M+H]^+$ 396.1.

3,4-Di-*O*-acetyl-2,6-anhydro-5,7-*O*-benzylidene-1-deoxy-1-nitro-D-*glycero*-L-*gluco*-heptitol (**70**)

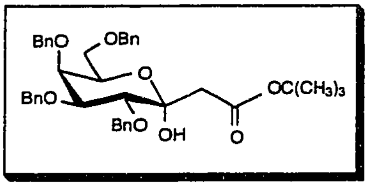


The lower moving spot **70** (4.20 mg, 17%) of the above described mixture was isolated and showed the folowing data: R_f 0.45 ethyl acetate/toluene, 1:2; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.50-7.30 (m, 5H, aromatics), 5.67 (dd, 1H, $J_{3,4}$ = 10.1 Hz, $J_{2,3}$ = 6.1 Hz, H-3), 5.48 (m, 1H, PhC<u>H</u>$(O)_2$), 5.25-5.17 (m, 1H, H-2), 5.02 (dd, 1H, $J_{4,5}$ = 3.3 Hz, H-4), 4.78 (dd, 1H, $J_{1a,1b}$ = 12.5 Hz, $J_{1a,2}$ = 10.5 Hz, H-1a), 4.55 (dd, 1H, $J_{1b,2}$ = 4.1 Hz, H-1b), 4.46 (dd, 1H, $J_{5,6}$ = 1.4 Hz, H-5), 4.24 (dd, 1H, $J_{6,7a}$ = 1.6 Hz, $J_{7a,7b}$ = 12.7 Hz, H-7a), 4.03 (dd, 1H, $J_{6,7b}$ = 1.9 Hz, H-7b), 3.77-3.73 (m, 1H, H-6), 2.05, 2.07 (2 x s, 6H, 2 x OAc); FAB $[M+H]^+$ = 396.1.

General procedure for attempted conjugate addition and alkylation of 3,4-di-*O*-acetyl-2,6-anhydro-5,7-*O*-benzylidene-7-deoxy-7-nitro-D-glycero-L-*manno*-heptitol (**69**)

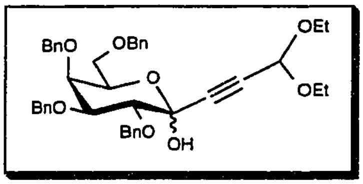
The nitro compound **69** (20.0 mg, 64 μmol) was dissolved in dry acetonitrile or THF (2 ml), cesium fluoride or 1,8 diazabicyclo[5.4.0]undec-7-ene (64 μmol) and the Michael acceptor or α-halo ketone (1.28 mmol) were added. After stirring the reaction for 3 h, formic acid (128 μmol) was added and the reaction was concentrated under reduced pressure and chromatographically purified.

tert -Butyl (4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-3-octulopyranosid)onate (**89**)



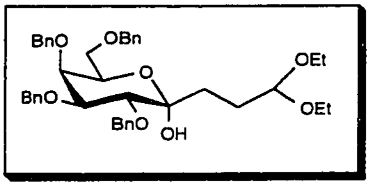
The lactone **88**[200] (1.00 g, 1.86 mmol) was dissolved in dry tetrahydrofuran (50 ml) and cooled to -79°C. To this solution was added a tetrahydrofuran solution (-79^{0}C) containing the enolate of *tert* -butylacetate[201] (7.44 mmol). The mixture was kept at -79^{0}C for 1 h. A saturated solution of ammonium chloride (10 ml) was added and the temperature was raised to room temperature slowly (1 h). The mixture was concentrated under reduced pressure and water (40 ml) and dichloromethane (40 ml) were added. The aqueous layer was back-extracted with dichloromethane (2 x 20 ml), dried (Na_2SO_4) and concentrated. Chromatographic purification using 4% ethyl acetate in toluene as the eluent gave **89** (1.02 g, 84%) as a colourless oil. The product **89** was characterized as follows: R_f 0.56 ethyl acetate/toluene, 1:10; ^{1}H NMR (360 MHz, CD_3Cl) δ: 7.40-7.20 (m, 20H, aromatics), 5.43 (s, br., 1H, OH), 4.95 (d, 1H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.91 (d, 1H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.74 (ABq, 2H, J_{gem} = 11.7 Hz, $OC\underline{H}_2Ph$), 4.65 (d, 1H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.58 (d, 1H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.41 (ABq, 2H, J_{gem} = 11.8 Hz, $OC\underline{H}_2Ph$), 4.15 (m, 1H, H-7), 4.08 (dd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 2.8 Hz, H-5), 4.00 (dd, 1H, $J_{6,7}$ = 1.2 Hz, H-6), 3.75 (d, 1H, H-4), 3.60 (dd, 1H, $J_{7,8a}$ = 7.7 Hz, $J_{8a,8b}$ = 9.2 Hz, H-8a), 3.45 (dd, 1H, $J_{7,8b}$ = 5.6 Hz, H-8b), 2.75 (d, 1H, $J_{2a,2b}$ = 15.6 Hz, H-2a), 2.33 (d, 1H, H-2b), 1.38 (s, 9H, $C(C\underline{H}_3)_3$); HRMS (ES) $[M+H]^+$ calcd. 655.32709, found 655.32735. The signal at 5.43 disappeared by addition of D_2O (20 μl) to the ^{1}H NMR sample.

5,6,7,9-Tetra-*O*-benzyl-2,3-dideoxy-α-D-*galacto*-non-2-yne-ulopyranose diethyl acetal (**90**)



The lactone **88**[200] (0.10 g, 0.18 mmol) was dissolved in dry tetrahydrofuran (5 ml) and cooled to -79^{0}C. To this solution, a tetrahydrofuran solution (2 ml, -79^{0}C) containing the lithium salt of propiolaldehyde diethylacetal[202] (4 equiv.) was added. After keeping the mixture for 1 h at -79^{0}C, a saturated solution of ammonium chloride (1 ml) was added and the temperature was raised to room temperature slowly. The mixture was concentrated under reduced pressure and water (5 ml) and dichloromethane (4 ml) were added. The aqeous layer was extracted with dichloromethane (2 x 3 ml), dried (Na_2SO_4) and concentrated. Chromatographic purification using 12% ethyl acetate in toluene as the eluent gave **90** (86,40 mg, 72%) as an oil; R_f 0.15 ethyl acetate/toluene, 1:10; ^{1}H NMR (360 MHz, CD$_3$Cl) revealed the presence of the open and closed form (open/closed 1:2). Characteristic data for **90** (closed) δ: 5.25 (s, 1H, C$\underline{H}$(OEt)$_2$), 3.90 (d, 1H, $J_{5,6}$ = 9.9 Hz, H-5), 3.78 (dd, 1H, $J_{6,7}$ = 9.7 Hz, $J_{7,8}$ = 2.8 Hz, H-7); MS (ES) [M+H]$^+$ 667.3.

5,6,7,9-Tetra-O-benzyl-2,3-dideoxy-α-D-galacto-nonulopyranose diethyl acetal (**91**)

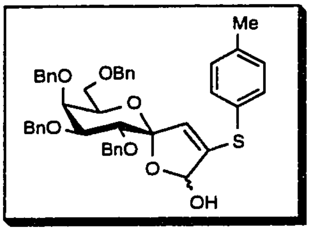


Compound **91** (0.12 g, 0,18 mmol) was dissolved in methanol (3 ml). Platiniumdioxide (20 mg) was added and the mixture was hydrogenated for 3 h and filtered (celite). The mixture was concentrated and chromatographically purified using 11% ethylacetate in toluene as the eluent. Compound **91** (45 mg, 38%) was obtained as a colourless oil; R_f 0.18 ethyl acetate/toluene, 1:8; ^{1}H NMR (360 MHz, CD$_3$Cl) δ: 7.55-7.20 (m, 20H, benzyl), 4.95 (d, 1H, J_{gem} = 11.1 Hz, OC$\underline{H}_2$Ph), 4.93 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.70 (ABq, 2H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.67 (d, 1H, J_{gem} = 11.1 Hz, OC$\underline{H}_2$Ph), 4.60 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.45-4.39 (m, 3H, 2 x OC$\underline{H}_2$Ph, C$\underline{H}$(OEt)), 4.28 (s, br., 1H, OH), 4.11 (m, 1H, H-8), 3.99 (dd, 1H, $J_{6,7}$ = 2.8 Hz, $J_{7,8}$ = 1.1 Hz, H-7), 3.95 (dd, 1H, $J_{5,6}$ = 9.5 Hz, H-6), 3.78 (d, 1H, H-5), 3.65-3.39 (m, 6H, H-8a, H-8b, 2 x OC$\underline{H}_2$), 1.97-1,90 (m, 2H), 1.68-1.60 (m, 2H), 1.10-1.05 (2 x t, 6H, CH$_3$, ethyl); ^{13}C NMR (75.5 MHz, CD$_3$Cl) δ: 139.04, 138.69, 138.47, 138.23 (4 x C aromatic), 128.49-127.48 (C-H aromatics), 103.04 (C-H, $\underline{C}$H(OEt)$_2$), 98.12 (C-OH), 81.22 (C-H), 79.56 (C-H), 75.57 (C-H$_2$), 74.59 (C-H), 74.48 (C-H$_2$), 73.40 (C-H$_2$), 72.57 (C-H_2), 69.81 (C-H), 69.00 (C-H_2), 61.47 (C-H_2), 61.30 (C-H_2), 33.08 (C-H_2), 26.57 (C-H_2), 15.25, 15.14 (2 x C-H_3); MS (ES) M+H]$^+$ 671.3.

Attempted addition of nitromethane to **89** and **91**

Compound **89** or **91** (35 μmol) was dissolved in tetrahydrofuran (2 ml) and 1,8 diazabicyclo[5.4.0]undec-7-ene (70 μmol), 2-hydroxypyridine (70 μmol), nitromethane (150 μl, 3.5 mmol) and molecular sieves (AW 300, 200 mg) were added and stirred for 5 days. The reaction mixture was neutralized with Amberlite resin (IRC 50 H$^+$, 200 mg), filtered and concentrated. Chromatographic purification using 4% (**89**) or 11% (**91**) ethyl acetate in toluene as the eluent gave the unreacted starting materials in 90% recovered yields.

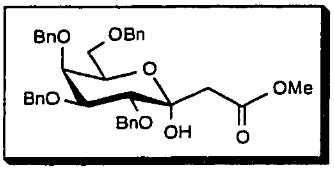
5,6,7,9-Tetra-*O*-benzyl-2,3-dideoxy-2-*p*-thiocresyl-α-D-galacto-2-en-4-nonos-4-ulo-4,8-pyranose (**93**)



Compound **90** (10 mg, 15 μmol), was dissolved in a (1:1) mixture of dichloromethane and trifluoroacetic acid. After stirring for 1 h, toluene (2 ml) was added and the mixture was concentrated and codistilled with toluene (2 x 3 ml). p-Thiocresol (37.3 mg, 0.33 mmol) and diisopropylethylamine (5.2 μl, 30 μmol) were then added. The mixture was stirred for 3 h and then concentrated under reduced pressure and chromatographically purified using 6% ethyl acetate in toluene as the eluent. The ^{1}H NMR (360 MHz, CD_3Cl) revealed the presence of four stereoisomers (ratio: 4:1:1:1) due to the presence of four singlet signals for the methyl group of thiocresol. The major isomer **93** showed the following data: R_f 0.60 ethyl acetate/toluene, 1:4; ^{1}H NMR (360 MHz, CD_3Cl) δ: 7.40-7.20 (m, 24H, aromatic), 5.72 (dd, br., 1H, $J_{1,OH}$ = 10.6 Hz, H-1), 5.28

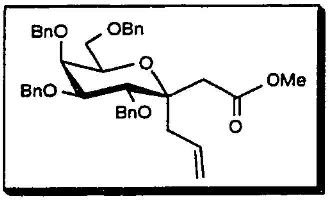
(d, 1H, $J_{1,3}$ = 1.3 Hz, H-3), 4.99 (d, 1H, J_{gem} = 10.1 Hz, OC<u>H</u>$_2$Ph), 4.98 (d, 1H, J_{gem} = 11.8 Hz, OC<u>H</u>$_2$Ph), 4.75 (d, 1H, J_{gem} = 10.1 Hz, OC<u>H</u>$_2$Ph), 4.72 (ABq, 2H, J_{gem} = 11.9 Hz, OC<u>H</u>$_2$Ph), 4.61 (d, 1H, J_{gem} = 11.8 Hz, OC<u>H</u>$_2$Ph), 4.42 (ABq, 2H, J_{gem} = 11.9 Hz, OC<u>H</u>$_2$Ph), 4.32 (d, 1H, $J_{5,6}$ = 9.9 Hz, H-5), 4.15 (m, 1H, H-8), 4.05 (dd, 1H, $J_{6,7}$ = 2.8 Hz, $J_{7,8}$ = 1.6 Hz, H-7), 3.97 (dd, 1H, H-6), 3.60 (dd, 1H, $J_{8,9}$ = 8.3 Hz, $J_{9a,9b}$ = 9.0 Hz, H-9a), 3.53 (dd, 1H, H-9b), 3.30 (d, br., OH), 2.35 (s, 3H, CH_3). Addition of D_2O (20 µl) to the ^{1}H NMR sample changed the signal at 5.72 to a (d, $J_{1,3}$ = 1.3 Hz) and led to the disappearance of the signal at 3.30; MS (ES) $[M+H]^+$ 717.2.

Methyl (4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-3-octulopyranosid)onate (**94**)



The acetal **89** (1.00 g, 1.52 mmol) was dissolved in a (1:1) mixture containing of dichloromethane and trifluoroacetic acid (1:1, 25 ml) and was stirred for 2 h at 0^{0}C. Toluene (30 ml) was added and the mixture was concentrated under reduced pressure and codistilled with toluene (2 x 10 ml). The residue was dissolved in dry *N,N*-dimethylformmide (30 ml) and cesium carbonate (743 mg, 2.28 mmol) and methyl iodide (284 µl, 4.56 mmol) were added. The mixture was stirred for 4 h. The reaction flask was opened and left in the fume hood overnight to remove excess methyl iodide. The residue was concentrated under reduced pressure and water (40 ml) and dichloromethane (40 ml) were added. The aqeous layer was extracted with dichloromethane (2 x 20 ml). The combined organic layer were dried (Na_2SO_4) and concentrated. Chromatographic purification using 5% ethyl acetate in toluene as the eluent gave **94** (766 mg, 82%) as a colourless oil; R_f 0.40 ethyl acetate/toluene, 1:5; ^{1}H NMR (360 MHz, CD_3Cl) δ: 7.40-7.20 (m, 20H, aromatics), 5.43 (s, br., 1H, OH), 4.95 (d, 1H, J_{gem} = 11.5 Hz, OC<u>H</u>$_2$Ph), 4.91 (d, 1H, J_{gem} = 11.6 Hz, OC<u>H</u>$_2$Ph), 4.74 (ABq, 2H, J_{gem} = 11.7 Hz, OC<u>H</u>$_2$Ph), 4.65 (d, 1H, J_{gem} = 11.5 Hz, OC<u>H</u>$_2$Ph), 4.58 (d, 1H, J_{gem} = 11.5 Hz, OC<u>H</u>$_2$Ph), 4.41 (ABq, J_{gem} = 11.8 Hz, OC<u>H</u>$_2$Ph), 4.15 (m, 1H, H-7), 4.08 (dd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 2.8 Hz, H-5), 4.00 (dd, 1H, $J_{6,7}$ = 1.2 Hz, H-6), 3.75 (d, 1H, H-4), 3.60 (s, 3H, OCH_3), 3.59 (dd, 1H, $J_{7,8a}$ = 7.7 Hz, $J_{8a,8b}$ = 9.2 Hz, H-8a), 3.45 (dd, 1H, $J_{7,8b}$ = 5.6 Hz, H-8b), 2.75 (d, 1H, $J_{2a,2b}$ = 15.6 Hz, H-2a), 2.33 (d, 1H, H-2b). The signal at 5.43 disappeared by addition of D_2O; HRMS (ES) $[M+H]^+$ calcd. 613.28014, found 613.28033.

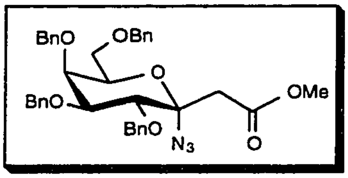
Methyl (3-*C*-allyl-3,7-anhydro-4,5,6,8 tetra-*O*-benzyl-2-deoxy D-*glycero*-L-*gluco*-oct)onate (**95**)



The acetal **94** (20 mg, 32.6 μmol) was dissolved under an inert argon atmosphere in dry acetonitrile (1 ml). Allyl trimethylsilane (16 μl, 97.5 μmol) and Lewis acid (boron trifluoride diethyl etherate or TMSOTf, 3 equiv.) were added at 0°C. The temperature was raised to room temperature and the mixture was stirred for an additional 1 h. Dichloromethane (2 ml) and saturated sodium bicarbonate solution (2 ml) were added. The aqeous layer was extracted with dichloromethane (2 x 2 ml) and the combined organic layer were dried (Na_2SO_4), concentrated and chromatographically purified using 5% ethyl acetate in toluene as the eluent. Compound **95** was obtained as a colourless syrup; R_f 0.65 ethyl acetate/toluene, 1:5; ^1H NMR (360 MHz, CD_3Cl) δ: 7.40-7.20 (m, 20H, aromatic), 5.86-5.73 (m, 1H, $CH_2C\underline{H}CH_2$), 5.15-5.05 (m, 2H, $CH_2CHC\underline{H}_2$), 5.03 (d, 1H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.95 (d, 1H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.74 (d, 1H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.73 (d, 1H, J_{gem} = 11.7 Hz, $OC\underline{H}_2Ph$), 4.63 (d, 1H, J_{gem} = 11.7 Hz, $OC\underline{H}_2Ph$), 4.52 (d, 1H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.43 (ABq, 2H, J_{gem} = 11.8 Hz, $OC\underline{H}_2Ph$), 4.40 (d, 1H, $J_{2,3}$ = 9.9 Hz, H-2), 3.99 (d, 1H, $J_{3,4}$ = 2.3 Hz, H-4), 3.79 (dd, 1H, H-3), 3.73-3.68 (m, 1H, H-5), 3.58 (dd, 1H, $J_{5,6a}$ = 7.6 Hz, $J_{6a,6b}$= 9.1 Hz, H-6a), 3.48 (dd, 1H, $J_{5,6b}$ = 5.4 Hz, H-6b), 3.43 (s, 3H, OCH_3), 2.62 (dd, 1H, J_{gem} = 15.3 Hz, $C\underline{H}_2CHCH_2$, J_{viic}= 6.2 Hz, $C\underline{H}_2CHCH_2$), 2.57 (s, 2H, $C\underline{H}_2COOMe$), 2.44 (dd, 1H, J_{vic} =

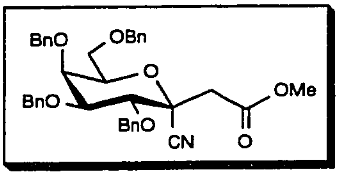
8.3 Hz, $C\underline{H}_2CHCH_2$); The axial positon of the allyl group was deduced from the hetero coupling constant $J_{H2,\underline{C}H2COOMe}$< 3Hz and $J_{H4,\underline{C}H2CHCH2}$~ 5Hz; MS (FAB) $[M+H]^+$ 637.3.

Methyl (3-Azido 4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-3-octulopyranosid)onate **(96)**



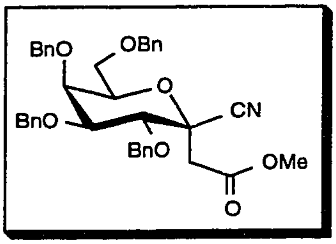
The acetal **94** (20 mg, 32.60 μmol) was dissolved under an inert argon atmosphere in dry acetonitrile (1 ml). Azidotrimethylsilane (13 μl, 98 μmol) and TMSOTf (19 μl, 98 μmol) were added at 0°C. The temperature was raised to room temperature and the mixture was stirred for an additional 4 h. Dichloromethane (2 ml) and saturated sodium bicarbonate solution (2 ml) were added. The aqeous layer was extracted with dichloromethane (2 x 2 ml) and the combined organic layers were dried (Na_2SO_4), concentrated and chromatographically purified using 5% ethyl acetate in toluene as the eluent. Compound **96** (15.5 mg, 75%) was obtained as a colourless syrup; R_f 0.70 ethyl acetate/toluene, 1:5; ^{1}H NMR (360 MHz, CD_3Cl) δ: 7.40-7.20 (m, 20H, aromatic), 4.99 (d, 1H, J_{gem} = 11.3 Hz, $OC\underline{H}_2Ph$), 4.93 (d, 1H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.73 (d, 1H, J_{gem} = 11.4 Hz, $OC\underline{H}_2Ph$), 4.70 (ABq, 2H, J_{gem} = 11.7 Hz, $OC\underline{H}_2Ph$), 4.55 (d, 1H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.44 (ABq, 2H, J_{gem} = 11.7 Hz, $OC\underline{H}_2Ph$), 4.28 (d, 1H, $J_{2,3}$ = 9.7 Hz, H-2), 4.02-3.98 (m, 2H, H-6, H-7), 3.92 (dd, 1H, $J_{5,6}$ = 2.6 Hz, H-5), 3.59 (dd, 1H, $J_{7,8a}$ = 7.8 Hz, $J_{8a,8b}$= 9.3 Hz, H-8a), 3.55 (s, 3H, OCH_3), 3.52 (dd, 1H, $J_{7,8b}$ = 5.5 Hz, H-8b), 2.90 (s, 2H, $C\underline{H}_2COOMe$); The axial positon of the azido group was deduced from the hetero coupling constants $J_{H4,CH2COOMe}$~ 3Hz; ^{1}H NMR 500 MHz, CD_3Cl); MS (FAB) $[M+H]^+$ 638.2; IR (cm^{-1}) 2120, 1742.

Methyl (3,7-anhydro-4,5,6,8 tetra-*O*-benzyl-3-cyano-2-deoxy-D-*glycero*- L-*manno*-oct)onate (**97**α)



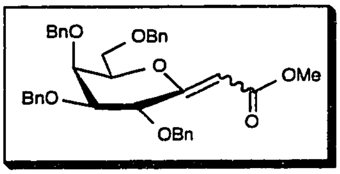
The acetal **94** (20 mg, 32.6 μmol) was dissolved under an inert argon atmosphere in dry acetonitrile (1 ml). TMSCN (13 μl, 97.5 μmol) and TMSOTf (19 μl, 98 μmol) were added at 0°C. The temperature was raised to room temperature and the mixture was stirred for an additional 8 h. Dichloromethane (2 ml) and saturated sodium bicarbonate solution (2 ml) were added. The aqeous layer was extracted with dichloromethane (2 x 2 ml) and the combined organic layer were dried (Na_2SO_4), concentrated and chromatographically purified using 5% ethyl acetate in toluene as the eluent. Compound **97**α (15 mg, 75%) was obtained as a colourless syrup; $[\alpha]_D$= 37.0 (c 1.8, $CDCl_3$); R_f 0.63 ethyl acetate/toluene, 1:5; ^{1}H NMR (500 MHz, CD_3Cl) δ: 7.40-7.20 (m, 20H, aromatic), 5.05 (d, 1H, J_{gem} = 11.7 Hz, $\mathrm{OC\underline{H}_2Ph}$), 4.90 (d, 1H, J_{gem} = 11.3 Hz, $\mathrm{OC\underline{H}_2Ph}$), 4.71 (ABq, 2H, J_{gem} = 11.4 Hz, $\mathrm{OC\underline{H}_2Ph}$), 4.70 (d, 1H, J_{gem} = 11.7 Hz, $\mathrm{OC\underline{H}_2Ph}$), 4.55 (d, 1H, J_{gem} = 11.3 Hz, $\mathrm{OC\underline{H}_2Ph}$), 4.44 (ABq, 2H, J_{gem} = 11.8 Hz, $\mathrm{OC\underline{H}_2Ph}$), 4.07-4.03 (m, 2H, H-6, H-7), 4.01 (d, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 3.92 (dd, 1H, $J_{4,5}$ = 2.6 Hz, H-5), 3.59 (dd, 1H, $J_{7,8a}$ ~ 8.0 Hz, $J_{8a,8b}$ = 9.3 Hz, H-8a), 3.59 (s, 3H, OCH_3), 3.53 (dd, 1H, $J_{7,8b}$ = 5.5 Hz, H-8b), 2.94 (d, 1H, $J_{2a,2b}$ = 15.2 Hz, H-2a), 2.64 (d, 1H, H-2b); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 176.86 (C=O), 138.51, 137.79 x 2, 137.58 (C, aromatic), 128.60-127.66 (C-H, aromatic), 116.52 (CN), 81.94 (C-H), 76.63 (C-H), 76.19 (C), 75.37 (C-H_2), 75.18 (C-H), 74.85 (C-H_2), 74.49 (C-H_2), 73.43 (C-H), 72.80 (C-H_2), 67.72 (C-H_2), 52.04 (OC-H_3), 41.21 (C-H_2); The axial positon of the allyl group was deduced from the hetero coupling constant $J_{\mathrm{H4,CN}}$ = 7.3 Hz and $J_{\mathrm{H4,\underline{C}H2COOMe}}$= 3.6 Hz); HRMS (ES) $[M+H]^+$ calcd. 622.28047, found 622.28095.

Benzyl (3,7-anhydro-4,5,6,8 tetra-*O*-benzyl-3-cyano-2-deoxy-D-*glycero*- L-*gluco*-oct)onate (**97**β)



Compound **97**β (2 mg, 9%) was obtained as the slower moving fraction of the chromatographic purification of **97**α as a liquid; R_f 0.47 ethyl acetate/toluene, 1:5; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 20H, aromatic), 4.90 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.89 (d, 1H, J_{gem} = 11.4 Hz, OC$\underline{H}_2$Ph), 4.73 (ABq, 2H, J_{gem} = 11.4 Hz, OC$\underline{H}_2$Ph), 4.67 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.62 (dt, 1H, $J_{6,7}$ = 1.2 Hz, $J_{7,8}$ = 6.4 Hz, H-7), 4.58 (d, 1H, J_{gem} = 11.4 Hz, OC$\underline{H}_2$Ph), 4.53 (dd, 1H, $J_{5,6}$ = 2.7 Hz, $J_{4,5}$ = 9.8 Hz, H-5), 4.41 (ABq, 2H, J_{gem} = 12.4 Hz, OC$\underline{H}_2$Ph), 4.05 (dd, 1H, H-6), 4.04 (d, 1H, H-4), 3.50 (s, 3H, OCH_3), 3.43 (d, 2H, J = 6.5 Hz, H-8a, H-8b), 2.62 (d, 1H, J_{gem} = 18.1 Hz, H-2a), 2.45 (d, 1H, H-2b); MS (ES) $[M+Na]^+$ 644.3 (15%), $[M-HCN]^+$ 617.3 (100 %).

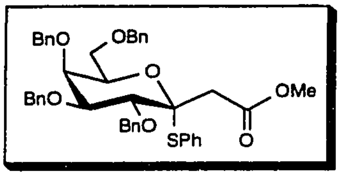
Methyl (3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-2-deoxy-D-galacto-oct-2-en-pyranosid)onate (**98**)



The acetal **94** (20 mg, 33 μmol) was dissolved under an argon atmosphere in dry acetonitrile (1 ml). Trimethylsilyl urea (12.9 mg, 97.5 μmol) and TMSOTf (18.90 μl, 97.50 μmol) were added at 0^0C. The temperature was raised to room temperature and the mixture was stirred for an additional 8 h. Dichloromethane (2 ml) and saturated sodium bicarbonate solution (2 ml) was added. The water layer was extracted with dichloromethane (2 x 2ml). The combined organic layers were dried (Na_2SO_4), concentrated and chromatographically purified using 5% ethyl acetate in toluene as the eluent. Compound **98** (10.6 mg, 55%) was obtained as a colourless syrup; R_f 0.5 ethyl acetate/toluene, 1:5; ^{1}H NMR (500 MHz,

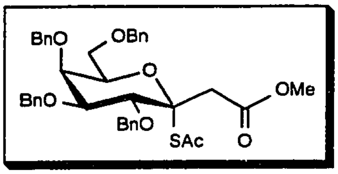
$CDCl_3$) δ: 7.40-7.20 (m, 20H, aromatic), 5.58 (d, 1H, $J_{2,4}$ = 1.6 Hz, H-2), 4.75 (ABq, 2H, J_{gem} = 11.4 Hz, $OC\underline{H}_2Ph$), 4.72 (ABq, 2H, J_{gem} = 11.1 Hz, $OC\underline{H}_2Ph$), 4.68 (s, 2H, $OC\underline{H}_2Ph$), 4.48 (ABq, 2H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.41 (dd, 1H, $J_{4,5}$ = 9.2 Hz, H-4), 4.10 (dd, 1H, $J_{5,6}$ = 2.6 Hz, $J_{6,7}$ = 1.5 Hz, H-6), 3.98 (dt, 1H, $J_{7,8}$ = 6.6 Hz, H-7), 3.76 (d, 2H, H-8a, H-8b), 3.70 (dd, 1H, H-5), 3.62 (s, 3H, OCH_3); MS (ES) $[M+H]^+$ 595.1.

Methyl (4,5,6,8-tetra-*O*-benzyl-2-deoxy-3-*S*-phenyl-3-thio-α-D-*galacto*-oct-3-ulopyranosid)onate (**99**)



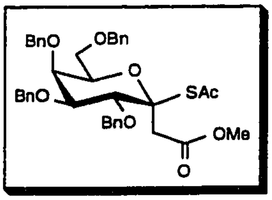
The acetal **94** (20 mg, 32.6 μmol) was dissolved under an argon atmosphere in dry acetonitrile (1 ml). Thiophenol (9.10 μl , 97.50 μmol) and TMSOTf (18.90 μl, 97.50 μmol) were added at 0^{0}C. After the addition, the temperature was raised to room temperature and the mixture was stirred for an additional 2 h. Dichloromethane (2 ml) and saturated sodium bicarbonate solution (2 ml) were added. The aqeous layer was extracted with dichloromethane (2 x 2 ml). The combined organic layers were dried (Na_2SO_4), concentrated and chromatographically purified using 5% ethyl acetate in toluene as the eluent. Compound **99** (21.7 mg, 95%) was obtained as a colourless syrup; R_f 0.5 ethyl acetate/toluene, 1:5; ^{1}H NMR (500 MHz, CD_3Cl) δ: 7.55-7.20 (m, 25H, aromatic), 5.10 (d, 1H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 5.00 (ABq, 2H, J_{gem} = 11.8 Hz, $OC\underline{H}_2Ph$), 4.83 (d, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 4.78 (ABq, 2H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.59 (d, 1H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.54 (dt, 1H, $J_{6,7}$ = 1.2 Hz, $J_{7,8}$ = 7.0 Hz, H-7), 4.49 (ABq, 2H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.15 (dd, 1H, $J_{5,6}$ = 2.6 Hz, H-5), 4.09 (dd, 1H, H-6), 3.65 (dd, 1H, $J_{7,8a}$ = 6.9 Hz, $J_{8a,8b}$ = 11.6 Hz, H-8a), 3.59 (dd, 1H, $J_{7,8b}$ = 6.9 Hz, H-8b), Hz, $OC\underline{H}_2Ph$), 3.44 (s, 3H, OMe), 3.01 (d, 1H, $J_{2a,2b}$ = 13.9 Hz, H-2a), 2.69 (d, 1H, H-2b); The axial orientation of the thiophenyl group was deduced from the hetero coupling constant $J_{\underline{H}4,\underline{C}H2COOMe}$~ 3.0 Hz; MS (ES) $[M+H]^+$ 705.1.

Methyl (3-*S*-acetyl-4,5,6,8-tetra-*O*-benzyl-2-deoxy-3-thio-α-D-*galacto*-oct-3-ulopyranosid)onate (**100α**)



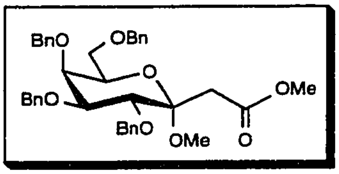
The acetal **94** (20 mg, 32.5 μmol) was dissolved under an inert argon atmosphere in dry acetonitrile (1 ml). Thiolacetic acid (8 μl , 97.5 μmol) and borontrifluoride diethyl etherate (12.00 μl, 97.50 μmol) were added at 0°C. The temperature was raised to room temperature immediatelly and the mixture was stirred for an additional 2 h. Dichloromethane (2 ml) and saturated sodium bicarbonate solution (2 ml) were added. The aqeous layer was extracted with dichloromethane (2 x 2 ml). The combined organic layers were dried (Na_2SO_4), concentrated and chromatographically purified using 6% ethyl acetate in toluene. Compound **100α** (14.80 mg, 68%) was obtained as a colourless syrup; R_f 0.56 ethyl acetate/toluene, 1:5; ^{1}H NMR (500 MHz, CD_3Cl) δ: 7.40-7.20 (m, 20H, aromatic), 4.90 (ABq, 2H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.74 (ABq, 2H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.66 (ABq, 2H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.59 (d, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 4.43 (ABq, 2H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.00-3.95 (m, 2H, H-6, H-7), 3.79 (dd, 1H, $J_{5,5}$ = 2.7 Hz, H-5), 3.63 (t, 1H, J = 8.8 Hz, H-8a), 3.57 (d, 1H, $J_{2a,2b}$ = 13.9 Hz, H-2a), 3.48 (dd, 1H, $J_{7,8b}$ = 5.0 Hz, $J_{8a,8b}$ = 9.0 Hz, H-8b), 3.45 (s, 3H, OCH_3), 3.12 (d, 1H, H-2b), 2.25 (s, 3H, SCH_3); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 193.67 (C=O), 169.18 (C=O), 139.13, 138.57, 138.24, 138.09 (C, aromatics), 128.44-127.21 (C-H, aromatics), 95.48 (C), 81.47 (C-H), 77.85 (C-H), 75.44 (C-H_2), 74.41 (C-H_2),73.62 (C-H_2), 73.63 (C-H), 73.28 (C-H), 72.39 (C-H_2), 68.14 (C-H_2), 51.64 (OC-H_3), 42.13 (C-H_2), 32.52 (C-H_3); The axial orientation of the thioacetyl group was deduced from the hetero coupling constant $J_{H4,\underline{C}H2COOMe}$~ 3.8 Hz; MS FAB $[M+H]^+$ 671.2.

Methyl (3-acetyl 4,5,6,8-tetra-*O*-benzyl-2-deoxy-3-thio-β-D-*galacto*-oct-3-ulopyranosid)onate (**100β**)



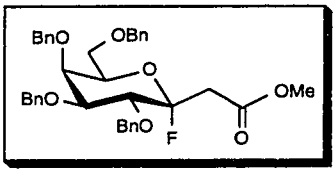
Compound **100β** (2 mg, 9%) was obtained as the lower moving fraction in the chromatographic purification of **100α** (as a liquid); R_f 0.62 ethyl acetate/toluene, 1:5; ^{1}H NMR (360 MHz, CD_3Cl) δ: 7.4-7.2 (m, 20H, aromatic), 4.93 (ABq, 2H, J_{gem} = 11.7 Hz, OCH_2Ph), 4.77 (d, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 4.73 (ABq, 2H, J_{gem} = 11.4 Hz, $OC\underline{H}_2Ph$), 4.70 (ABq, 2H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.43 (ABq, 2H, J_{gem} = 12.0 Hz, $OC\underline{H}_2Ph$), 4.07 (dt, 1H, $J_{6,7}$ ~ 0.8Hz, H-7), 4.05 (dd, 1H, $J_{5,6}$ = 2.7 Hz, H-5), 4.00 (dd, 1H, H-6), 3.52 (dd, 1H, $J_{7,8a}$ = 7.4 Hz, $J_{8a,8b}$ = 9.5 Hz, H-8a), 3.43 (s, 3H, OCH_3), 3.39 (dd, 1H, $J_{7,8b}$ = 5.9 Hz, H-8b), 3.00 (d, 1H, $J_{2a,2b}$ = 13.9 Hz, H-2a), 2.80 (d, 1H, H-2b), 2.30 (s, 3H, SCH_3); MS (ES) MS $[M+H]^+$ 671.2.

Methyl (methyl 4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-oct-3-ulopyranosid)onate (**101**)



The acetal **94** (20 mg, 32.5 µmol) was dissolved under an argon atmosphere in dry acetonitrile (1 ml). Methanol (13 µl, 325 µmol) and boron trifluoride diethyl etherate (12.00 µl, 97.50 µmol) were added at 0°C. The temperature was raised to room temperature and the mixture was stirred for an additional 8 h. Dichloromethane (2 ml) and saturated sodium bicarbonate solution (2 ml) were added. The aqeous layer was extracted with dichloromethane (2 x 2 ml). The combined organic layers were dried (Na_2SO_4) and concentrated. Compound **101** (20 mg, quant.) was obtained as a colourless liquid; R_f 0.62 ethyl acetate/toluene, 1:4; ^{1}H NMR (360 MHz, CD_3Cl) δ: 7.40-7.20 (m, 20H, aromatic), 4.97 (d, 1H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.96 (d, 1H, J_{gem} = 11.5 Hz, OC$\underline{\text{H}}_2$Ph), 4.73 (d, 1H, J_{gem} = 11.5 Hz, OC$\underline{\text{H}}_2$Ph), 4.70 (ABq, 2H, J_{gem} = 11.0 Hz, OC$\underline{\text{H}}_2$Ph), 4.53 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{\text{H}}_2$Ph), 4.44 (ABq, 2H, J_{gem} = 11.8 Hz, OC$\underline{\text{H}}_2$Ph), 4.33 (d, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 4.03 (dd, 1H, $J_{5,6}$ = 2.7 Hz, H-5), 3.97 (dd, 1H, $J_{6,7}$ = 1.2 Hz, H-6), 3.75-3.70 (m, 1H, H-7), 3.58 (t, 1H, J = 7.5 Hz, H-8a), 3.53 (dd, 1H, $J_{7,8b}$ = 5.9 Hz, $J_{8a,8b}$ = 9.2 Hz, H-8b), 3.45 (s, 3H, OCH_3), 3.26 (s, 3H, OCH_3), 2.80 (d, 1H, $J_{2a,2b}$ = 13.7 Hz, H-2a), 2.70 (d, 1H, H-2b); MS (MALDI) $[M+Na]^+$ = 650.0. The axial orientation of the methoxy group was deduced from the hetero coupling constant $J_{H2,\underline{C}H2COOMe}$ ~3.5 Hz.

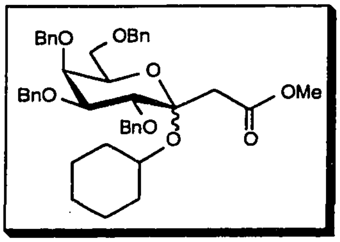
Methyl (4,5,6,8-tetra-*O*-benzyl-2-deoxy-3-fluoro-α-D-*galacto*-oct-3-ulopyranosyl)onate **(102)**



The thiophenyl glycoside **99** (22 mg, 31.2 μmol) was dissolved in dichloromethane (2 ml) at -15°C. HF/pyridine (70:30) complex (10 μl), and *N*-bromosuccinimide (8.7 mg, 43 μmol) were added[176]. The mixture was stirred at -15°C for 2 h . Water (2 ml) was added and the organic layer was separated, washed with sodium thiosulfate, dried (Na_2SO_4) and concentrated. Chromatographic purification using 3% ethyl acetate in toluene as the eluent gave the galactosyl fluoride **102** (12.5 mg, 65%) as a white powder; R_f 0.62 ethyl acetate/toluene, 1:5; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 20H, aromatic), 4.99 (d, 1H, J_{gem} = 11.3 Hz, OC$\underline{\text{H}}_2$Ph), 4.96 (d, 1H, J_{gem} = 11.5 Hz, OC$\underline{\text{H}}_2$Ph), 4.76-4.66 (m, 3H, 3x OC$\underline{\text{H}}_2$Ph), 4.58 (d, 1H, J_{gem} = 11.5 Hz, OC$\underline{\text{H}}_2$Ph), 4.44 (ABq, 2H, J_{gem} = 11.9 Hz, OC$\underline{\text{H}}_2$Ph), 4.28 (dd, 1H, $J_{4,5}$ = 9.9 Hz, $J_{4,3F}$ = 23.3 Hz, H-4), 4.09 (m, 1H, H-7), 4.06 (dd, 1H, $J_{5,6}$ = 2.6 Hz, $J_{6,7}$ = 1.2 Hz, H-6), 3.97 (dd, 1H, H-5), 3.61 (dd, 1H, $J_{7,8a}$ = 5.3 Hz, $J_{8a,8b}$ = 9.2 Hz, H-8a), 3.56 (s, 3H, OCH_3), 3.53 (dd, 1H, H-8b), 2.88 (dd, 1H, $J_{2a,2b}$ = 14.3 Hz, $J_{2a,3F}$ = 12.4 Hz, H-2a), 2.80 (d, 1H, $J_{2b,3F}$ = 10.7

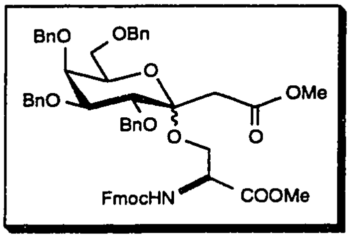
Hz, H-2b); No heteronuclear coupling ($J_{H4,C2}$~ 0 Hz) was observed; MS (MALDI) $[M+Na]^+$ 637.5.

Methyl (cyclohexyl 4,5,6,8-tetra-*O*-benzyl-2-deoxy-α(β)-D-*galacto*-oct-3-ulo-pyranosid)onate (**103**)



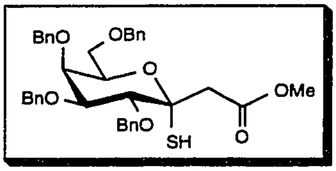
The thioglycoside **99** (22 mg, 31.2 μmol), cyclohexanol (10 μl, 93 μmol) and molecular sieves (4 Å) were dissolved in dry dichloromethane (2 ml) under an inert argon atmosphere at 0°C. *N*-bromosuccinimide (8.3 mg, 46.8 μmol) was added and the temperature was slowly raised to room temperature. The stirring continued for an additional 8 h under an argon atmosphere. Water (2 ml) was added and the organic layer was washed with sodium thiosulfate, dried (Na_2SO_4) and concentrated. Chromatographic purification using 3% ethyl acetate in toluene as the eluent gave compound **103** (6.5 mg, 30%) as a colourless liquid; R_f 0.57 ethyl acetate/toluene, 1:10; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 20H, aromatic), 4.99 (d, 1H, J_{gem} = 11.8 Hz, OC$\underline{H}_2$Ph), 4.96 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.79 (d, 1H, J_{gem} = 11.8 Hz, OC$\underline{H}_2$Ph), 4.70 (ABq, 2H, J_{gem} = 11.5 Hz, OC$\underline{H}_2$Ph), 4.52 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.46 (ABq, 2H, J_{gem} = 11.9 Hz, OC$\underline{H}_2$Ph), 4.20 (d, 1H, $J_{4,5}$ = 9.2 Hz, H-4), 4.04-3.96 (m, 3H, H-5, H-6, H-7), 3.80-3.70 (m, 1H, C$\underline{H}$($CH_2)_2$), 3.59 (dd, 1H, $J_{7,8a}$= 7.7 Hz, $J_{8a,8b}$ = 9.2 Hz, H-8a), 3.50 (dd, 1H, $J_{7,8b}$ = 5.5 Hz, H-8b), 3.47 (s, 3H, OCH_3), 2.84 (d, 1H, $J_{2a,2b}$ = 13.1 Hz, H-2a), 2.76 (d, 1H, H-2b), 1.90-1.1 (m, 10H, cyclohexyl); MS (MALDI) $[M+Na]^+$ = 717.2.

Methyl [(Fmoc)N-L-Ser (OMe) 4,5,6,8-tetra-*O*-benzyl-2-deoxy-α(β)-D-*galacto*-oct-3-ulopyranosid]onate (**105**)



The thioglycoside **99** (22 mg, 31.2 μmol), L-MeOO Ser NHFmoc (12.70 mg, 37.40 μmol) and molecular sieves (4 , 50 mg) were suspended in dry dichloromethane (2 ml) under an argon atmosphere at 0°C. *N*-bromosuccinimide (8.3 mg, 46.80 μmol) was added and the temperature was slowly raised to room temperature. The stirring continued to stirr for an additional 4 h under an argon atmosphere. Water (2 ml) was added and the organic layer was washed with sodium thiosulfate, dried (Na_2SO_4) and concentrated. Chromatographic purification using 3% ethyl acetate in toluene as the eluent gave compound **105** (15.20 mg, 50%) as a colourless liquid; R_f 0.76 ethyl acetate/toluene, 1:5; Characteristic data: ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.80-7.20 (m, 28H, aromatic), 6.15 (d, br. 1H, $J_{NH,H\alpha Ser}$ = 6.7 Hz, NH), 4.20 (d, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.65 (s, 3H, OCH_3), 3.40 (s, 3H, OCH_3), 2.75 (d, 1H, $J_{2a,2b}$ = 13.7 Hz, H-2a), 2.66 (d, 1H, H-2b); MS (MALDI) $[M+Na]^+$ = 958.3.

Methyl (4,5,6,8-tetra-*O*-benzyl-2-deoxy-3-thio-α-D-*galacto*-oct-3-ulopyranosid)onate (**106**)

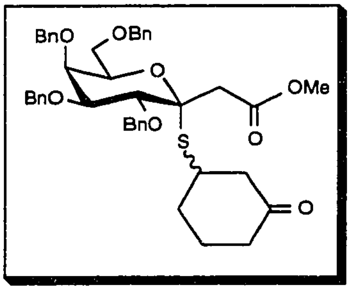


The thioacetate **100**α (14 mg, 20.8 μmol) was dissolved in dry methanol (1 ml) and a sodium methoxide (30 μmol) was added at 0° C. After stirring the reaction mixture for 30 min at 0° C, Amberlite (IRC 50, 15 mg) was added to neutralize the reaction. The solution was filtered through celite and concentrated to yield a colourless oil **106** (14 mg) which was of sufficient purity for further reactions; R_f 0.63 ethyl acetate/toluene, 1:5; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 20H, aromatic), 4.85 (ABq, 2H, J_{gem} = 11.5 Hz, OC$\underline{H}_2$Ph), 4.77 (ABq, 2H, J_{gem} = 11.4 Hz, OC$\underline{H}_2$Ph), 4.69 (ABq, 2H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.43 (ABq, 2H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.27-4.21 (m, 1H, H-7), 4.22 (d, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 4.00 (dd, 1H, $J_{5,6}$ = 2.7 Hz, $J_{6,7}$ = 1.1 Hz, H-6), 3.93 (dd, 1H, H-5), 3.60 (dd, 1H, $J_{7,8a}$ = 7.7 Hz, $J_{8a,8b}$ = 9.2 Hz, H-8a), 3.53 (s, 3H. OCH$_3$), 3.49 (dd, 1H, $J_{7,8b}$ = 5.5 Hz, H-8b), 3.02 (d, 1H, $J_{2a,2b}$ = 14.6 Hz, H-2b), 2.92 (d, 1H, H-2b), 2.48 (s, 1H, SH); MS (ES) $[M+Na]^+$ 651.2. Addition of D_2O (20 µl) led to the disappearance of the signal at 2.48 ppm.

General procedure[203] for the conjugate addition and alkylation of **106**.

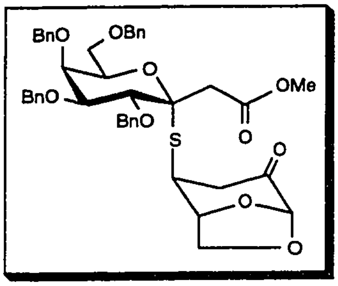
The thiol **106** (12.5 mg, 20 µmol) was dissolved in dry benzene (1.5 ml) under an inert argon atmosphere. Triethylamine (24 µmol) and Michael acceptor or alkylating reagent (80 µmol) were added. After 1 h Michael acceptor (80 µmol) and triethylamine (24 µmol) was added and the mixture was stirred for a specified time under an inert argon atmosphere. The mixture was concentrated and chromatographically purified using ethyl acetate (10-5%) in toluene:

Methyl (3,7 anhydro-4,5,6,8-tetra-*O*-benzyl-3-*S*-(cyclohex-3-one-1yl)-2-deoxy-3-thio-α-D-*galacto*-oct-3-ulopyranosid)onate (**107**)



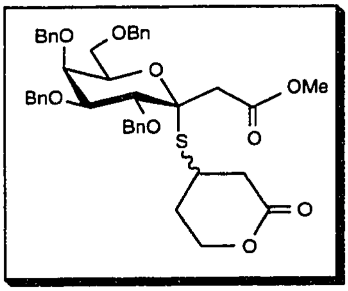
Compound mixture **107** (2.5 mg, 17 %): R_f 0.50 ethyl acetate/toluene, 1:5; MS (ES) $[M+Na]^+$ 747.2. The isomeric mixture was determined by integration of the methyl ester (OCH$_3$, δ: 3.45 and 3.42).

1,6-Anhydro-3-deoxy-4-*S*-(methyl (4,5,6,8-tetra-*O*-benzyl-2-deoxy-3-thio-α-D-*galacto*-3-octulopyranosid)onate)-D-glycero-hexo-pyranos-2-ulose (**108**)



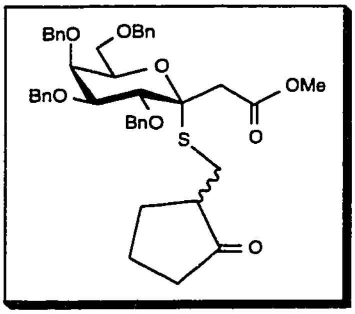
Compound **108** (9.8 mg, 65 %, transparent oil); R_f 0.39 ethyl acetate/toluene, 1:5; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 20H, aromatic), 5.02 (s, br. 1H, H-1), 4.70 (ABq, 2H, J_{gem} = 11.7 Hz, $OC\underline{H}_2Ph$), 4.73 (ABq, 2H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.68 (s, 2H, $OC\underline{H}_2Ph$), 4.65 (d, 1H, $J_{4',5'}$ = 9.7 Hz, H-4'), 4.62-4.59 (m, 1H, H-5), 4.38 (ABq, 2H, J_{gem} = 11.1 Hz, $OC\underline{H}_2Ph$), 4.04-4.00 (m, 1H, H-7'), 3.98 (dd, 1H, $J_{5',6'}$ = 2.7 Hz, H-5'), 3.84 (dd, 1H, $J_{6',7'}$ = 1.1 Hz, H-6'), 3.60 (dd, 1H, $J_{7',8'a}$ = 7.8 Hz, $J_{8'a,8'b}$ = 10.0 Hz, H-8'a), 3.49 (s, 3H, OCH_3), 3.44 (dd, 1H, $J_{7',8'b}$ = 3.6 Hz, H-8'b), 3.43-3.37 (m, 3H, H-4, H-6a, H-6b), 3.04 (d, 1H, $J_{2'a,2'b}$ = 13.8 Hz, H-2'a), 3.01 (dd, 1H, $J_{3a,3b}$ = 16.8 Hz, $J_{3a,4}$ = 7.7 Hz, H-3a), 2.72 (d, 1H, H-2'b), 2.48 (d, br. 1H, H-3b); MS (MALDI) $[M+Na]^+$ 778.0.

Methyl (3,7 anhydro-4,5,6,8-tetra-*O*-benzyl-2-deoxy-3-*S*-(tetrahyropyran-2-on-4-yl)-3-thio-α-D-*galacto*-oct-3-ulopyranosid)onate (**109**)



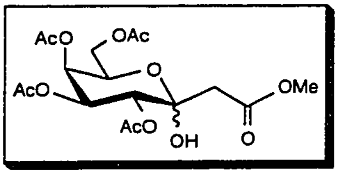
Compound mixture **109** (oil, 2.9 mg, 20 %): R_f 0.53 ethyl acetate/toluene, 1:5; MS (ES) $[M+Na]^+$ 749.4. The isomeric ratio (1:1.5) was determined by integration of the methyl ester (OCH_3, δ: 3.47 and 3.43).

Methyl (3,7 anhydro-4,5,6,8-tetra-*O*-benzyl-2-deoxy-3-*S*-(2-methylene-cyclopentanone)-3-thio-α-D-*galacto*-oct-3-ulopyranosid)onate (**110**)



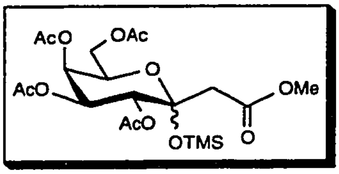
Compound **110** (oil, 3.00 mg, 21 %): R_f 0.55 ethyl acetate/toluene, 1:5; MS (ES) $[M+Na]^+$ 747.40. The isomeric ratio (1:1) was determined by integration of the methyl ester (OCH_3, δ: 3.45 and 3.42).

Methyl (4,5,6,8-tetra-*O*-acetyl-2-deoxy-α-D-*galacto*-oct-3-ulopyranosid)onate (**111**)



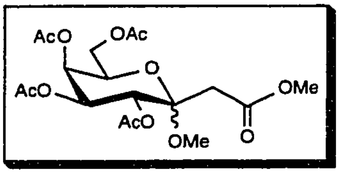
The acetal **94** (225 mg, 367.00 µmol) was dissolved in dry methanol (10 ml). Palladium hydroxide ($Pd(OH)_2$ /C), 54 mg) was added and the reaction was hydrogenated for 5 h. The mixture was filtered over a millipore filter, concentrated and acetylated using 33% pyridine in acetic anhydride overnight. The tlc showed a number of spots. Extensive chromatography using a (1:1) mixture of ethyl acetate and toluene gave **111** (70 mg, ~65% purity) as an oil; R_f 0.63 ethyl acetate/toluene, 4:1; ^1H NMR (360 MHz, $CDCl_3$) δ: 5.67 (d, br. 1H, OH), 5.43 (dd, 1H, $J_{5,6}$ = 3.4 Hz, $J_{6,7}$ = 1.4 Hz, H-6), 5.38 (dd, 1H, $J_{4,5}$ = 10.6 Hz, H-5), 5.12 (dd, 1H, $J_{OH.H4}$ = 1.4 Hz, H-4), 4.41 (dt, 1H, $J_{7,8}$ = 6.7 Hz, H-7), 4.30 (t, 2H, H-8a, H-8b), 3.71 (s, 3H, OCH_3), 2.70 (d, 1H, $J_{2a,2b}$ = 14.5 Hz, H-2a), 2.60 (d, 1H, H-2b), 2.10, 2.08, 2.00, 1.90 (4 x s, 12H, 4 x OAc); D_2O addition led to the disappearance of the signal at 5.67 and changed the signal at 5.12 to a dublet; MS (MALDI) $[M+Na]^+$ 443.0.

Methyl (4,5,6,8-tetra-*O*-acetyl-2-deoxy-3-*O*-trimethylsilyl-α-D-*galacto*-oct-3-ulopyranosid)onate (**112**)



The crude mixture **111** (15 mg, 36.00 μmol) was dissolved in dry acetonitrile (2 ml) under an inert argon atmosphere. TMSCN (15 μl, 108 μmol) and TMSOTf (21 μl, 108 μmol) were added at 0^{0}C. The temperature was raised to room temperature. After stirring the reaction mixture for 10 h, saturated sodium bicarbonate solution (2 ml) was added and dichloromethane (5 ml) were added and the organic layers were separated, dried (Na_2SO_4) and concentrated. The crude product was chromatographically purified using 25% ethyl acetate in toluene as the eluent. The major product (oil, 70 %) was identified as **112**; R_f 0.70 ethyl acetate/toluene, 4:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 5.57 (d, 1H, $J_{4,5}$ = 10.4 Hz, H-4), 5.39 (dd, 1H, $J_{5,6}$ = 3.3 Hz, $J_{6,7}$ = 1.4 Hz, H-6), 5.18 (dd, 1H, H-5), 4.23 (dt, 1H, $J_{7,8}$ = 6.7 Hz, H-7), 4.07 (dd, 1H, $J_{7,8a}$ = 6.5 Hz, $J_{8a,8b}$ = 11.2 Hz, H-8a), 4.03 (dd, 1H, $J_{7,8b}$ = 7.0 Hz, H-8b), 3.66 (s, 3H, OCH_3), 2.70 (s, 2H, H-2a H-2b), 2.10, 2.08, 2.00, 1.90 (4 x s, 12H, 4 x OAc), 0.2 (s, 9H, TMS); ^{13}C NMR (75.5 MHz, CD_3Cl) δ: 170.39, 170.22, 170.05, 169.70, 168.05 (5 x C=O), 98.14 (C), 69.70, 69.45, 68.05, 67.56 (4 x C-H), 61.66 (C-H_2), 51.76 (C-H_3), 43.02 (C-H_2), 20.89, 20.69 x 2, 20.45 (4 x C-H_3, OAc), 1.70 (C-H_3, TMS); MS (MALDI) $[M+Na]^+$ 515.7.

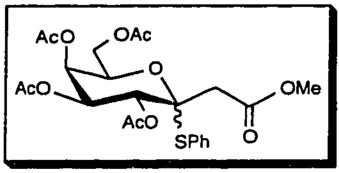
Methyl (methyl 4,5,6,8-tetra-*O*-acetyl-2-deoxy-α-D-*galacto*-oct-3-ulopyranosid)onate (**113**)



The acetal **101** (200 mg, 0.32 mmol) was dissolved in dry methanol (10 ml). Palladium hydroxide ($Pd(OH)_2$ /C), 54 mg) was added and the reaction was hydrogenated for 12 h. The mixture was filtered through a millipore filter, concentrated and acetylated using 33%

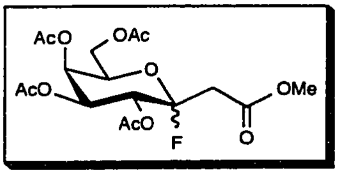
pyridine in acetic anhydride (10 ml) overnight. Removal of the solvent under reduced pressure gave crude **113** (132.00 mg, 95%) as a colourless oil; R_f 0.2 ethyl acetate/toluene, 4:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 5.57 (d, 1H, $J_{4,5}$ = 10.5 Hz, H-4), 5.40 (dd, 1H, $J_{5,6}$ = 3.3 Hz, $J_{6,7}$ = 1.0 Hz, H-6), 5.29 (dd, 1H, H-5), 4.10-4.00 (m, 3H, H-7, H-8a, H-8b), 3.65 (s, 3H, OCH_3), 3.35 (s, 3H, OCH_3), 2.75 (s, 2H, H-2a H-2b), 2.10, 2.05, 2.03, 1.90 (4 x s, 12H, 4 x OAc); MS (MALDI) $[M+Na]^+$ 457.0.

Methyl (4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-2-deoxy-3-*S*-phenyl-3-thio-α-D-*galacto*-oct-3-ulopyranosid)onate (**114**)



The methylglycoside **113** (20 mg, 46 μmol) was dissolved in dry dichloromethane (2 ml) under an argon atmosphere. Thiophenol (20 μl, 182 μmol) and tin tetrachloride (14 μl, 138 μmol) were added at 0°C and the mixture allowed to warm to room temperature. The stirring continued overnight. Then saturated sodium bicarbonate solution (2 ml) and dichloromethane (5 ml) were added. The organic layers were separated, dried (Na_2SO_4) and concentrated. Chromatographic purification using 16% ethyl acetate in toluene as the eluent gave **114** (9.6 mg, 41%) as an oil; R_f 0.28 ethyl acetate/toluene, 1:4; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.50-7.30 (m, 5H, aromatics), 6.14 (d, 1H, $J_{4,5}$ = 10.6 Hz, H-4), 5.51 (dd, 1H, $J_{5,6}$ = 3.3 Hz, $J_{6,7}$ = 1.3 Hz, H-6), 5.39 (dd, 1H, H-5), 4.83 (dt, 1H, $J_{7,8}$ = 6.4 Hz, H-7), 4.10 (d, 2H, H-8a, H-8b), 3.61 (s, 3H, OCH_3), 2.75 (s, 2H, H-2a, H-2b), 2.20, 2.15, 2.10, 1.95 (4 x s, 12H, 4 x OAc); MS (MALDI) $[M+Na]^+$ 535.7.

Methyl (4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-2-deoxy-3-fluoro-α-D-*galacto*-oct-3-ulopyranosyl)onate (**115**)



The thioglycoside **114** (25 mg, 49 μmol), *N*-bromosuccinimide (12.5 mg, 67 μmol) and HF/pyridine (70:30, 20 μl) were dissolved in dry dichloromethane (5 ml) under an inert argon atmosphere at 0°C. The mixture was then allowed to warm to room temperature while stirring. After 90 min, saturated sodium bicarbonate solution (2 ml) and dichloromethane (5 ml) were added and the organic layer was separated, dried (Na_2SO_4) and concentrated. Chromatographic purification using 20% ethyl acetate in toluene gave **115** (15 mg, 73%) as an oil; R_f 0.44 ethyl acetate/toluene, 1:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 5.53 (dd, 1H, $J_{4,5}$ = 10.5 Hz, $J_{5,4F}$ = 22.5 Hz, H-4), 5.48 (dd, 1H, $J_{5,6}$ = 3.2 Hz, $J_{6,7}$ = 1.3 Hz, H-6), 5.28 (dd, 1H, H-5), 4.38 (dt, 1H, $J_{7,8}$ = 6.7 Hz, H-7), 4.10 (d, 2H, H-8a, H-8b), 3.68 (s, 3H, OCH_3), 2.75 (d, 2H, $J_{2,3F}$ = 20.2 Hz, H-2a, H-2b), 2.18, 2.10, 2.05, 1.95 (4 x s, 12H, 4 x OAc); MS (MALDI) $[M-F]^+$ 404.0.

Attempted C-glycosylation of **115**

The fluoro compound **115** (22 mg, 52 μmol), trimethysilyl cyanide (21 μl, 156 μmol) and boron trifluoride diethyl etherat (19 μl, 156 μmol) were dissolved at 0°C under an inert argon atmosphere. The reaction was worked up after 5 min by addition of saturated sodium bicarbonate solution (1 ml). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. A mixture (two isomers) **116** (20 mg, 90%) was obtained as an oil

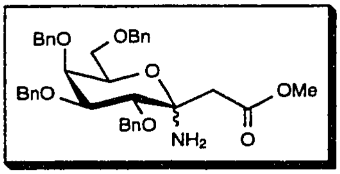
116 (mixture 1:1); ^{1}H NMR (360 MHz, $CDCl_3$) δ: 5.65 (dd, 1H, J= 3.4 Hz, J = 7.4 Hz), 5.41 (d, 1H, J = 3.5 Hz), 5.27 (dd, 1H, J = 2.1 Hz, J = 4.3 Hz), 5.12 (dd, 1H, J = 4.5 Hz, J = 6.2 Hz), 4.86 (d, 1H, J = 7.7 Hz), 4.74 (d, 1H, J = 6.2 Hz), 4.30 (t, 1H, 6.4

Hz), 4.23 (dt, 1H, J = 2.2 Hz, J = 6.6 Hz), 4.12-4.02 (m, 4H), 3.72 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.14 (d, 1H, J = 14.4 Hz), 2.95 (d, 1H, J = 14.4 Hz), 2.87 (d, 1H, J = 14.1 Hz), 2.68 (d, 1H, J = 14.1 Hz), 2.1-1.75 (8 x s, 24H, 8 x Me); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ: 170.42, 170.38, 169.82 x 2, 169.77, 169.5, 167.89, 167.77 (8 x C=O), 117.50 (CN), 118.05 (CN), 106.39 ($C(O)_2$), 106.19 ($C(O)_2$), 99.16 x 2 (C), 76.41, 74.15, 69.89, 69.70, 68.89, 68.60, 65.49, 64.61 (8 x C-H), 61.43, 61.31 (2 x C-H_2), 52.19, 52.08 (2 x C-H_3), 43.82, 41.54 (2 x C-H_2), 27.41, 27.05, 20.70, 20.63 x 2, 20.58, 20.46, 20.39 (8 x C-H_3); MS (MALDI) $[M+H]^+$ 429.7.

Attempted thioglycosylation of **115** and **113**.

Compound **115** or **113** (40 μmol) and thiolacetic acid (240 μmol) were dissolved under an argon atmosphere in dry dichloromethane (3 ml) at at 0°C. Boron trifluoride diethyl etherate (110 μmol) was added and the reaction was allowed to warm to room temperature. After stirring the mixture for 2 h, saturated sodium bicarbonate solution (1 ml) was added and the organic layer were separated, dried (Na_2SO_4) and concentrated. ^{1}H NMR of the crude product revealed that compound **111** was formed in nearly quantitative yield.

Methyl (4,5,6,8-tetra-*O*-acetyl-3-amino-2-deoxy-α-D-*galacto*-oct-3-ulopyranosid)onate (**117**)



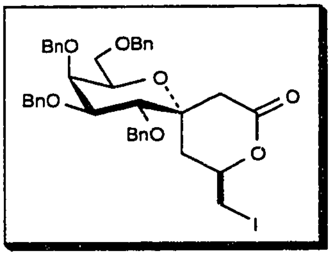
The azido compound **96** (20.7 mg, 32.5 μmol) and PtO_2 (4 mg) were added to dry methanol (1 ml) and hydrogenated (1 atm) for 15 min. The solution was filtered through a millipore filter and concentrated to give **117** (18 mg, 88 %) as an oil; R_f 0.38 ethyl acetate/toluene, 1:5; <u>Immediate</u> ^{1}H NMR analysis of **117** (~80 % purity) (360 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 20H, aromatic), 4.98 (d, 1H, J_{gem} = 11.3 Hz, $OC\underline{H}_2Ph$), 4.89 (d, 1H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.67 (ABq, 2H, J_{gem} = 11.7 Hz, $OC\underline{H}_2Ph$), 4.60 (d, 1H,

J_{gem} = 11.3 Hz, OC$\underline{H}_2$Ph), 4.57 (d, 1H, J_{gem} = 11.5 Hz, OC$\underline{H}_2$Ph), 4.41 (ABq, 2H, J_{gem} = 11.8 Hz, OC$\underline{H}_2$Ph), 4.28 (m, 1H, H-7), 3.96 (dd, 1H, $J_{5,6}$ = 2.8 Hz, $J_{6,7}$ = 1.2 Hz, H-6), 3.95 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-5), 3.83 (d, 1H, H-4), 3.58 (dd, 1H, $J_{7,8a}$ = 7.9 Hz, $J_{8a,8b}$ = 9.3 Hz, H-8a), 3.53 (s, 3H, OMe), 3.47 (dd, 1H, $J_{7,8b}$ = 5.5 Hz, H-8b), 2.65 (d, 1H, $J_{2a,2b}$ = 14.0 Hz, H-2a), 2.54 (d, 1H, H-2b), 2.08-2.0 (s, br., 2H, NH_2); The signal at 2.08 disappeared by addition of D_2O (20 µl); MS (MALDI) $[M+Na]^+$ 634.3.

Attempted acylation of **117** with Fmoc-L-Ala-OPfp

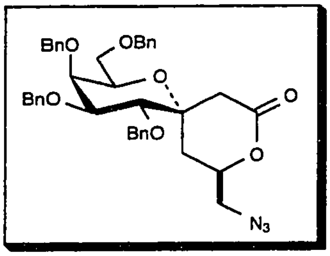
The amine **117** (20 mg, 32.7 µmol), 1-hydroxybenzotriazole (13 mg, 96 µmol) and Fmoc-L-Ala-OPfp (45.8 mg, 96 µmol) were dissolved in a (1:1) mixture of *N,N*-dimethylformamide and dry dichloromethane and stirred for 48 h. The solvent was removed under reduced pressure and the product was chromatographically purified using 16% ethyl acetate in toluene. No coupling product was formed; Isolated product corresponded to **94**; MS (ES) $[M+Na]^+$ 613.2. Addition of D_2O (15 µl) led over a periode of 24 h to the disappearance of the two H-2 protons (H-D exchange).

3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-2-deoxy-3-*C*-[3-iodo-2(R)-hydroxypropan]-D-*glycero*-L-*gluco*-octonic acid lactone (**118**)



The methyl ester **95** (130 mg, 204 µmol), iodide (78 mg, 307 µmol) were dissolved at 0°C in a (1:1) mixture of dichloromethane and diethylether (6 ml). The temperature was raised to room temperature and the mixture was stirred for 16 h. The organic layer was washed with saturated sodium bicarbonate solution (2 ml) sodium thiosulfate solution (2 ml) water (2 ml) and dried (Na_2SO_4). Chromatographic purification using 5% ethyl acetate in toluene as the eluent gave product **118** (70 mg, 46%) as an oil together with unreacted starting material (~ 20%). Compound **118**; R_f 0.66 ethyl acetate/toluene, 1:3; ^{1}H NMR (360 MHz, CDCl$_3$) δ: 7.40-7.20 (m, 20H, aromatics), 4.96 (d, 1H, J_{gem} = 11.1 Hz, OC$\underline{\mathrm{H}}_2$Ph), 4.92 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{\mathrm{H}}_2$Ph), 4.70 (ABq, 2H, J_{gem} = 11.4 Hz, OC$\underline{\mathrm{H}}_2$Ph), 4.63 (d, 1H, J_{gem} = 11.0 Hz, OC$\underline{\mathrm{H}}_2$Ph), 4.59 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{\mathrm{H}}_2$Ph), 4.43 (ABq, 2H, J_{gem} = 11.7 Hz, OC$\underline{\mathrm{H}}_2$Ph), 4.37-4.31 (m, 1H, CH$_2$C$\underline{\mathrm{H}}$(CH$_2$I)O), 3.97 (d, 1H, $J_{5,6}$ = 2.0 Hz, H-6), 3.79 (d, 1H, $J_{4,5}$ = 10.0 Hz, H-4), 3.67 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-5), 3.59 (t, br., 1H, $J_{7,8}$ ~ 6.6 Hz, H-7), 3.52 (dd, 1H, $J_{7,8a}$ = 6.9 Hz, $J_{8a,8b}$ = 9.0 Hz, H-8a), 3.47 (dd, 1H, $J_{7,8b}$ = 6.1 Hz, H-8b), 3.34 (dd, 1H, J_{gem} = 10.4 Hz, J_{vic} = 4.5 Hz, C-$\underline{\mathrm{H}}_2$I), 3.26 (dd, 1H, J_{vic} = 6.8 Hz, C-$\underline{\mathrm{H}}_2$I), 2.70 (d, 1H, $J_{2a,2b}$ = 17.4 Hz, H-2$_{ax}$), 2.50 (dd, 1H, $^4J_{H\text{-}2b(eq),CH2(eq)}$= 1.9 Hz, H-2$_{eq}$), 2.34 (ddd, 1H, J_{vic} = 2.9 Hz, J_{gem} = 14.8 Hz, C$\underline{\mathrm{H}}_{2(eq)}$CH(O)CH$_2$I), 1.88 (dd, 1H, J_{vic} = 12.0 Hz, C$\underline{\mathrm{H}}_{2(ax)}$CH(O)CH$_2$I); MS (MALDI) [M+Na]$^+$ 771.3.

3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-2-deoxy-3-C-[3-azido-2(R)-hydroxypropan]-D-*glycero*-L-*gluco*-octonic acid lactone (**119**)



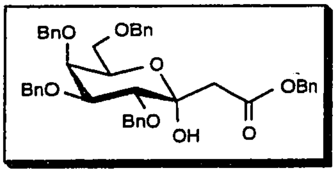
The iodo compound **118** (42.8 mg, 57.1 μmol) and sodium azide (12 mg, 185 μmol) were dissolved in dry *N*,*N*-dimethylformamide (6 ml) and warmed to 80°C. After 80 min, the mixture was allowed to room temperature and concentrated under reduced pressure. Chromatographic purification using 6% ethyl acetate in toluene as the eluent gave product **119** (36 mg, 94%) as an oil; δ: 7.40-7.20 (m, 20H, aromatics), 4.96 (d, 1H, J_{gem} = 11.1 Hz, OC$\underline{\mathrm{H}}_2$Ph), 4.92 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{\mathrm{H}}_2$Ph), 4.70 (ABq, 2H, J_{gem} = 11.4 Hz, OC$\underline{\mathrm{H}}_2$Ph), 4.63 (d, 1H, J_{gem} = 11.0 Hz, OC$\underline{\mathrm{H}}_2$Ph), 4.59 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{\mathrm{H}}_2$Ph), 4.56-4.48 (m, 1H, CH$_2$C$\underline{\mathrm{H}}$(CH$_2$I)O), 4.43 (ABq, 2H, J_{gem} = 11.7 Hz, OC$\underline{\mathrm{H}}_2$Ph), 3.94 (d, 1H, $J_{5,6}$ = 2.4 Hz, H-6), 3.77 (d, 1H, $J_{4,5}$ = 10.0 Hz, H-4), 3.64 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-5), 3.55-3.44 (m, 4H, H-7, H-8a, H-8b, C$\underline{\mathrm{H}}_2$N$_3$), 3.41 (dd, 1H, J_{gem} = 13.0 Hz, J_{vic} = 5.0 Hz, C-$\underline{\mathrm{H}}_2$N$_3$), 2.70 (d, 1H, $J_{2a,2b}$

= 17.4 Hz, H-2_{ax}), 2.52 (d, br., 1H, H-2_{eq}), 1.98 (s, br., 2H, $C\underline{H}_2CH(O)CH_2I$); IR 2104 cm^{-1} (s); MS (MALDI) $[M+Na]^+$ 687.9.

Attempted addition of the enolate of *tert* butylacetate to **119**

The lactone **119**[200] (20 mg, 30 μmol) was dissolved in dry tetrahydrofuran (3 ml) and cooled to -79°C. To this solution was added a tetrahydrofuran solution (-79°C) containing the enolate of *tert* -butylacetate[201] (120 μmol). The mixture was kept for 1 h at -79°C. A saturated solution of ammonium chloride (10 ml) was added and the temperature was warmed to room temperature slowly. The mixture was concentrated under reduced pressure and water (2 ml) and dichloromethane (2 ml) was added. The aqeous layer was extracted with dichloromethane (2 x 2 ml) dried (Na_2SO_4) and concentrated. The starting material (16 mg) was recovered. Traces of a new spot were detected (tlc) but not isolated.

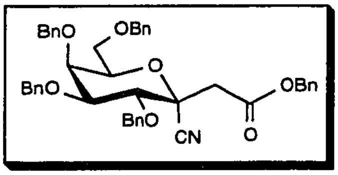
Benzyl-(4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-oct-3-ulopyranosid)onate (**120**)



The acetal **89** (1.07 g, 1.63 mmol) was dissolved in a (1:1) mixture containing dichloromethane and trifluoroacetic acid. After stirring for 2 h, toluene (30 ml) was added and the mixture was concentrated under reduced pressure. The residue was codistilled with toluene (2 x 10 ml). The mixture was dissolved in dry *N,N*-dimethylformamide (40 ml), cesium carbonate (743 mg, 2.28 mmol) and benzyl bromide (542 μl, 4.56 mmol) were added and stirred for 2 h. The residue was concentrated under reduced pressure, water (40 ml) and dichloromethane (40 ml) was added. The aqeous layer was extracted with dichloromethane (2 x 20 ml), dried (Na_2SO_4) and concentrated. Chromatographic purification using 5% ethyl acetate in toluene as the eluent gave **120** (809 mg, 72%) as a colourless oil. The product **120** was characterized as follows: R_f 0.43 ethyl acetate/toluene, 1:5; ^1H NMR

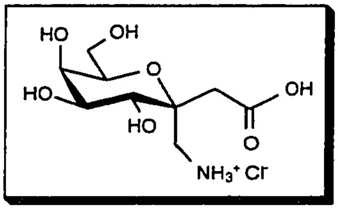
(360 MHz, CD_3Cl) δ: 7.40-7.20 (m, 25H, aromatic), 5.24 (s, br., 1H, OH), 5.08 (ABq, 2H, J_{gem} = 12.3 Hz, OC$\underline{H}_2$Ph), 5.00 (d, 1H, J_{gem} = 11.4 Hz, OC$\underline{H}_2$Ph), 4.95 (d, 1H, J_{gem} = 11.5 Hz, OC$\underline{H}_2$Ph), 4.77 (ABq, 2H, J_{gem} = 11.7 Hz, OC$\underline{H}_2$Ph), 4.67 (d, 1H, J_{gem} = 11.4 Hz, OC$\underline{H}_2$Ph), 4.61 (d, 1H, J_{gem} = 11.5 Hz, OC$\underline{H}_2$Ph), 4.44 (ABq, 2H, J_{gem} = 11.8 Hz, OC$\underline{H}_2$Ph), 4.17 (m, 1H, H-7), 4.10 (dd, 1H, $J_{4,5}$ = 9.8 Hz, $J_{5,6}$ = 2.7 Hz, H-5), 4.00 (dd, 1H, $J_{6,7}$ = 1.2 Hz, H-6), 3.82 (d, 1H, H-4), 3.60 (dd, 1H, $J_{7,8a}$ = 6.2 Hz, $J_{8a,8b}$ = 9.2 Hz, H-8a), 3.43 (dd, 1H, $J_{7,8b}$ = 5.5 Hz, H-8b), 2.87 (d, 1H, $J_{2a,2b}$ = 14.6 Hz, H-2a), 2.43 (d, 1H, H-2b). The signal at 5.24 disappeared by addition of D_2O (15 μl). HRMS (ES) $[M+Na]^+$ calcd. 711.29338, found 711.29395.

Benzyl (3,7-anhydro-4,5,6,8 tetra-*O*-benzyl-3-cyano-2-deoxy D-*glycero*- L-*gluco*-oct)onate (**121α**)



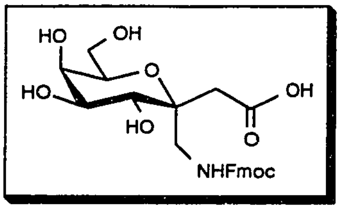
The acetal **120** (99.7 mg, 144.8 μmol) was dissolved under an argon atmosphere in dry acetonitrile (3 ml). Trimethylsilyl cyanide (58 μl, 435 μmol) and TMSOTf (87 μl, 449 μmol) were added at 0°C. After the addition the temperature was raised to room temperature and the mixture was stirred for an additional 6 h. Dichloromethane (6 ml) and saturated sodium bicarbonate solution (4 ml) were added. The aqeous laxer was extracted with dichloromethane (2 x 5 ml) and the combined organic layers were dried (Na_2SO_4), concentrated and chromatographically purified using 3% ethyl acetate in toluene as the eluent. The chromatographed product (67 mg, 66%, α/β mixture ~ 7:1) was purified by crystallization (chloroform). Compound **121α** (white powder), R_f 0.83 ethyl acetate/toluene, 1:5; $[\alpha]_D$= 32.5 (c 1.1, $CHCl_3$); ^{1}H NMR (500 MHz, CD_3Cl) δ: 7.40-7.20 (m, 25H, aromatics), 5.06 (ABq, 2H, J_{gem}= 12.3 Hz, OC$\underline{H}_2$Ph), 5.0 (d, 1H, J_{gem} = 12.6 Hz, OC$\underline{H}_2$Ph), 4.90 (d, 1H, J_{gem} = 11.3 Hz, OC$\underline{H}_2$Ph), 4.71 (ABq, 2H, J_{gem} = 11.5 Hz, OC$\underline{H}_2$Ph), 4.70 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.55 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.43 (ABq, 2H, J_{gem} = 11.8 Hz, OC$\underline{H}_2$Ph), 4.07-4.02 (m, 2H, H-6, H-7), 4.03 (d, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 3.92 (dd, 1H, $J_{4,5}$ = 2.7 Hz, H-5), 3.56 (dd, 1H, $J_{7,8a}$ ~ 7.9 Hz, $J_{8a,8b}$ = 9.2 Hz, H-8a), 3.45 (dd, 1H, $J_{7,8b}$ = 5.2 Hz, H-8b), 3.00 (d, 1H, $J_{2a,2b}$ = 15.1 Hz, H-2a), 2.70 (d, 1H, H-2b); ^{13}C NMR (75.5 MHz, CD_3OD) δ: 116.3 (CN). The axial orientation of the cyano group was deduced from the hetero coupling constant $J_{H4,\underline{C}N}$= 8.5 Hz and $J_{H4,\underline{C}H2COOMe}$= 3.8 Hz); HRMS (ES) $[M+H]^+$ calcd. 698.31177, found 698.31283.

3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-2-deoxy-3-*C*-(aminomethyl)-D-*glycero*-L-*gluco*-octonic acid hydrochloride (**122**)



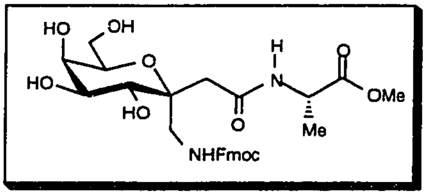
The cyanide **121** (10 mg, 14.30 μmol), hydrochloric acid (20 μmol) and palladium hydroxide ($(PdOH)_2$/C, 8.5 mg) were added to a dry methanol (3 ml) and hydrogenated (1 atm) for 16 h. The residue was filtered through a millipore filter and concentrated under reduced pressure. A mixture containing **122** and **155** was obtained (ratio **122**:**155**, 4:1). ^{13}C NMR (75.5 MHz, CD_3OD, for the mixture) δ: 174.19 (C=O), 172.41 (C=O), 78.54 (C), 77.42 (C), 75.04, 74.99, 73.80, 73.55, 72.00, 71.78, 70.77 x 2 (8x C-H), 63.20, 63.12 (2 x $C\text{-}H_2$), 52.61 ($C\text{-}H_3$), 42.59, 42.31, 39.24, 38.74 (4 x $C\text{-}H_2$). MS (ES) 234.1 $[\mathbf{122}\text{-}H_2O]^+$, 252.1 $[\mathbf{122}]^+$, 266.1 $[\mathbf{155}]^+$.

3,7-Anhydro-2-deoxy-3-C-(aminomethyl(*N*-(9-fluorenylmethoxycarbonyl)))-D-*glycero*-L-*gluco*-octonic acid (**123**)



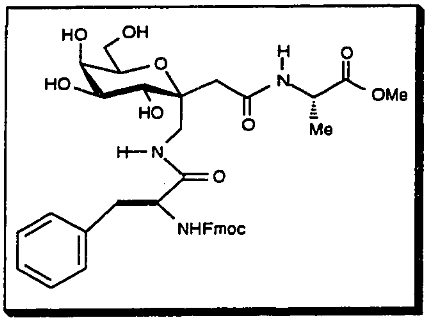
A mixture of **122**/**125** (~2:1, 122 mg) was dissolved in a mixture of water (5 ml) and acetone (10 ml), sodium bicarbonate powder (61 mg) and Fmoc pentafluorophenylester[144] (Fmoc-OPfp, 450 mg) were added. After stirring the reaction for 12 h, formic acid (0.5 ml) was added. The reaction was stirred for 10 minutes. Then water (10 ml) was added and ethyl acetate (3 x 10 ml) was used to extract the product. Product **123** was purified with an ethyl acetate equilibrated colomn using ethyl acetate/methanol/water, 12:2:1 as the eluent. The product **123** (57 mg, 42%) was obtained as an oil; R_f 0.2 ethyl acetate/methanol/water, 12:2:1, ^{1}H NMR (360 MHz, CD_3OD/CD_3Cl, 1:1) δ: 7.78 (d, 2H, J ~ 7Hz, Fmoc), 7.65 (d, 2H, J ~ 7 Hz, Fmoc), 7.40-7.26 (m, 4H, Fmoc), 4.36 (d, 2H, J = 6.9 Hz, OC$\underline{H}_2$, Fmoc), 4.20 (t, 1H, J = 6.9 Hz, C$\underline{H}$CH$_2$, Fmoc), 4.15 (d, 1H, $J_{4,5}$ = 10.0 Hz, H-4), 3.82 (d, 1H, $J_{5,6}$ = 2.8 Hz, H-6), 3.78-3.53 (m, 6H), 2.70 (d, 1H, $J_{2a,2b}$ = 15.1 Hz, H-2a), 2.62 (d, 1H, H-2b); MS (MALDI) $[M+Na]^+$ 496.7.

3,7-Anhydro-2-deoxy-3-*C*-(aminomethyl(*N*-(9-fluorenylmethoxycarbonyl)))-D-*glycero*-L-*gluco*-octon-(L-Ala-OMe)amide (**124**)



The carboxylic acid **123** (6 mg, 12.7 µmol), *N,N'* 1,3 -dicyclohexylcarbodiimide (16 mg, 78 µmol), L-alanine methyl ester hydrochloride (12 mg, 85 µmol) and 1-hydroxybenzotriazole (3 mg, 22 µmol) were dissolved in dry *N,N*-dimethylformamide (1.5 ml) and stirred for 48 h. The urea precipitation was filtered (celite) and the residue was concentrated under reduced pressure. The residue was chromatographed twice using 10% methanol in ethyl acetate as the eluent. Product **124** (5 mg, 72%) was obtained as a white powder; R_f 0.8 ethyl acetate/methanol/water, 4:2:1; Characteristic data: ^{1}H NMR (360 MHz, $CDCl_3/CD_3OD$, 7:1); δ: 7.81-7.75 (d, br., 1H, J ~ 7Hz, NH(Ala)), 7.70 (d, 2H, J = 7.6 Hz, aromatic, Fmoc), 7.53 (d, 2H, J = 7.5 Hz, aromatic, Fmoc), 7.33 (t, 2H, 7.6 Hz, aromatic, Fmoc), 7.23 (t, 2H, 7.6 Hz, aromatic, Fmoc), 6.10-6.00 (t, br., 1H, J ~ 6 Hz, NH(Fmoc), 4.15 (t, 1H, J = 6.7 Hz, C-H, Fmoc), 3.86 (d, 1H, $J_{5,6}$ = 3.0 Hz, H-6), 3.84 (d, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.66 (s, 3H, OCH_3), 3.52 (dd, 1H, J_{gem} = 14.8 Hz, J_{vic} = 5.8 Hz, $C\underline{H}_2NH$), 3.38 (dd, 1H, J_{vic} = 6.2 Hz, $C\underline{H}_2NH$), 2.55 (d, 1H, $J_{2a,2b}$ = 15.1 Hz, H-2a), 2.47 (d, 1H, H-2b); 1.33 (d, 3H, $C-H_3$, Ala); The signal at 7.81 and 6.10 exchanged under this conditions extremely slow (~3d); MS (MALDI) $[M+Na]^+$ 581.3.

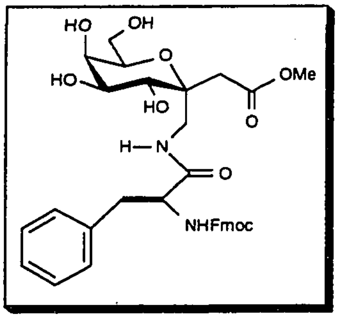
3,7-Anhydro-2-deoxy-3-*C*-(aminomethyl(*N*-L-Phe-N^α(Fmoc)amide))-D-*glycero*- L-*gluco*-octon(L-Ala-OMe)amide (**125**)



The glycodipeptide **124** (5.2 mg, 9.1 µmol) was dissolved in dry dimethylformamide (1.5 ml). Morpholine (1 ml) was added and the reaction was stirred for 1 h at room temperature. The solvent was removed under reduced pressure and codistilled with toluene (2 x 3 ml). The residue was dissolved in dry *N*,*N*-dimethylformamide (1.5 ml), Fmoc-L-Phe-OPfp (14.9 mg, 27 µmol) and 1-hydroxybenzotriazole (3 mg, 22 µmol) were added. The reaction was stirred for 14 hours at room temperature. The solvent was removed under reduced pressure and the poduct **125** was purified by chromatography using 9% methanol in ethyl acetate. The product **125** (5.8 mg, 92%) was obtained as a white powder; R_f 0.25 ethyl acetate/methanol/water, 11:1; ^{1}H NMR (360 MHz, CD_3OD) δ: 7.75 (d, 2H, J = 7.5 Hz, aromatic, Fmoc), 7.58 (d, 2H, J = 7.5 Hz, aromatic, Fmoc), 7.37 (t, 2H, J = 7.6 Hz, aromatic, Fmoc), 7.28 (t, 2H, J = 7.5 Hz, aromatic, Fmoc), 7.25-7.14 (m, 5H, Phe), 4.37 (dd, 1H, J = 6.0 Hz, 9.0 Hz), 4.33-4.20 (m, 3H, H-αAla, $OC\underline{H}_2$, Fmoc)), 4.14 (t, 1H, $C\underline{H}CH_2$, Fmoc), 4.00 (d, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 3.84 (d, 1H, $J_{5,6}$ = 3.1 Hz, H-5), 3.75-3.68 (m, 2H), 3.66 (s, 3H, OMe), 3.65-3.42 (m, 4H), 3.12 (dd, 1H, J_{gem} = 13.7 Hz, J_{vic} = 5.6 Hz, $C\underline{H}_2$(Phe), 2.90 (dd, 1H, J_{vic} = 10.2 Hz, $C\underline{H}_2$(Phe), 2.50 (s, 2H, H-2a, H-2b), 1.28 (d, 3H, Me(Ala)); ^{13}C NMR (75.5 MHz,

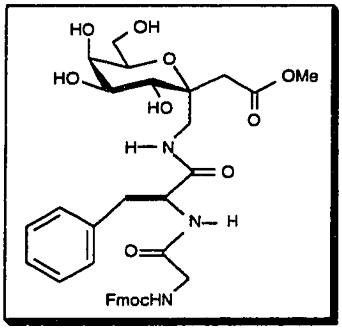
CD_3OD) δ: 174.92, 174.47, 172.70 (2x) (4 x C=O), 145.26, 145.10, 142.54 x 2, 138.60 (5x C, aromatics), 130.39-120.87 (C-H, aromatics), 79.56 (C), 74.51, 72.45, 71.93, 71.07 (4 x C-H), 68.13 (C-H_2), 63.29 (C-H_2), 58.17, 52.77, 49.65, 48.33 (3 x C-H, 1 x C-H_3), 42.98, 38.81, 38.64 (3 x C-H_2), 17.26 C-H_3); HRMS (ES) $[M+H]^+$ calcd. 706.29758, found 706.29773.

Methyl [3,7-anhydro-2-deoxy-3-C-(aminomethyl(*N*-L-Phe-*N*$^\alpha$(Fmoc)amide))-D-*glycero*-L-*gluco*-oct]onate (**126**)



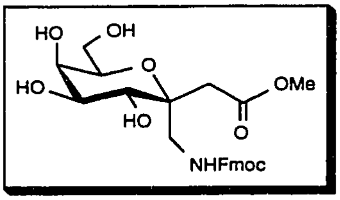
The Cyanide **97**α (50 mg, 80 μmol) HCl /H_2O (110 μmol) and palladium hydroxide/C (50 mg) were added to a dry methanol (8 ml) and hydrogenated for 48 h. The residue was filtered through a millipore filter and concentrated under reduced pressure. The residue was redissolved in dry methanol and hydrochloric acid (110 μmol) and palladium(II)hydroxide/C (50 mg) were added. The hydrogenation continued for an additional 48 h. Then the reaction mixture was filtered through a millipore filter and concentrated under reduced pressure. The product **155** (23 mg, 90%) was directly used for the coupling reaction. Compound **155** (23 mg, 76 μmol), sodium bicarbonate (9.5 mg, 113.40 μmol) and Fmoc-L-Phe-OPfp (126 mg, 227 μmol) were dissolved in a mixture of 20% water in acetone and stirred for 13 h. The solvent was removed under reduced pressure and the residue was purified by chromatography using 7% methanol in ethyl acetate as the eluent on an ethyl acetate equilibrated silica coloumn. Product **126** (31.2 mg, 65%) was obtained as a white powder; R_f 0.33 ethyl acetate/methanol, 7:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.70 (d, 2H, J = 7.5 Hz, aromatic, Fmoc), 7.53 (d, 2H, J = 7.4 Hz, aromatic, Fmoc), 7.33 (t, 2H, aromatic, Fmoc),), 7.23 (t, 2H, aromatic, Fmoc), 7.3-7.15 (m, 5H, Ph), 7.05-6.95 (t, br., 1H, J ~ 6.5 Hz, NH), 6.04-5.96 (d, br. 1H, NH(Phe)), 4.37 (t, 2H, J = 6.7 Hz, CHC$\underline{H}_2$, Fmoc), 4.10 (t, 1H, J = 6.7 Hz, C$\underline{H}$CH$_2$ Fmoc), 4.12-4.08 (m, 1H, Hα(Phe)), 3.86 (d, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.78 (d, 1H, $J_{5,6}$ = 3.0 Hz, H-6), 3.65-3.32 (m, 9H), 3.05-2.87 (m, 2H, C$\underline{H}_2$Ph), 2.80-2.60 (s, br., 4H, 4 x OH), 2.50 (d, 1H, $J_{2a,2b}$ = 15.4 Hz, H-2a), 2.37 (d, 1H, H-2b). The signals at 7.05, 6.10 and 2.80 disappeared by addition of D_2O); MS (MALDI) $[M+Na]^+$ 657.6.

Methyl [3,7-anhydro-2-deoxy-3-*C*-(aminomethyl(*N*-L-Phe-Gly-*N*$^{\alpha}$(Fmoc)amide))-D-*glycero*-L-*gluco*-oct]onate (**128**)



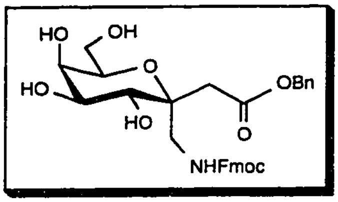
Compound **126** (46 mg, 76 μmol), tritylchloride resin (160 mg, capacity 950 μmol/g ~ 152.00 μmol) were added to a dry dichloromethane solution (2 ml) containing diisopropylethylamine (380 μmol, 66 μl). The mixture was stirred for 2 h while the thin layer chromatogram showed the complete disappearance of the starting material. The solvent was removed by filtration and the resin was washed with diisopropylethylamine/methanol/dichloromethane, 2:1:17 (3 x 10 ml), *N*,*N*-dimethylformamide 2 x 5 ml), dichloromethane (2 x 10 ml) and methanol (2 x 10 ml) before it was dried under vacuum overnight. A portion of this resin **127** (35 mg, ~ 16.60 μmol) was taken and suspended in a mixture of dichloromethane/morpholine, 1:1 (2 ml) for 2 h. The solvent was removed by filtration and the resin was washed with *N*,*N*-dimethylformamide (2 x 10 ml), dichloromethane (2 x 10 ml), methanol (2 x 10 ml) and dichloromethane (2 x 10 ml) before it was dried under vacuum overnight. The free amino resin (~ 30 mg), Fmoc-Gly-OPfp (23 mg, 49.6 μmol) and 1-hydroxybenzotriazole (3 mg, 22 μmol) were added to a solution of dry *N*,*N*-dimethylformamide (1.5 ml) and stirred for 7 h. The resin was washed with diisopropylethylamine/methanol/dichlormethane, 2:1:17 (3 x 10 ml), dimethylformamide (2 x 5 ml), dichloromethane (2 x 10 ml) and methanol (2 x 10 ml) before it was dried under vacuum overnight. Cleavage of the product **128** from the resin was achieved using 0.5% trifluoroacetic acid in dry dichloromethane for 5 min. After filtration and solvent removal **128** (6.8 mg, ~60%) was obtained as a white powder; ^{1}H NMR (360 MHz, CD$_3$OD) δ: 7.80 (d, 2H, J = 7.5 Hz, aromatic, Fmoc), 7.66 (d, 2H, J = 7.4 Hz, aromatic, Fmoc), 7.38 (t, 2H, J = 7.5 Hz, aromatic, Fmoc),), 7.31 (t, 2H, J = 7.5 Hz, aromatic, Fmoc), 7.27-7.10 (m, 5H, Ph), 4.65 (dd, 1H, J = 5.5 Hz, J = 9.1 Hz, Hα(Phe)), 4.35 (d, 2H, J = 7.0 Hz, CH$_2$, (Fmoc), 4.21 (t. 1H, J = 7.0 Hz, CH, Fmoc), 4.07 (d, 1H, J$_{4,5}$ = 9.9 Hz, H-4), 3.78 (d, 1H, J$_{5,6}$ = 3.1 Hz, H-6), 3.77-3.48 (m, 11H including s at 3.6 (~3H, OCH$_3$)), 3.17 (d, 1H, J$_{gem}$ = 13.7 Hz, C$\underline{H}_2$Ph), 2.93 (dd, 1H, C$\underline{H}_2$Ph), 2.64 (d, 1H, J$_{2a,2b}$ = 14.8 Hz, H-2a), 2.52 (d, 1H, H-2b); MS (MALDI) [M+Na]$^+$ 714.2.

Methyl [3,7-anhydro-2-deoxy-3-*C*-(aminomethyl(*N*-(9-fluorenylmethoxycarbonyl)))-D-*glycero*-L-*gluco*-oct]onate (**129**)



Compound **123** (55 mg, 182.3 μmol), FmocPfp (156 mg, 384 μmol) and sodium bicarbonate (30.6 mg, 364.6 μmol) were dissolved in a solvent mixture containing water (5.5 ml) and acetone (10 ml). After stirring the mixture for 4 h, the solvent was removed under reduced pressure. The residue was purified with a gradient silica chromatography using 9% to 12.5% methanol in ethyl acetate as eluent. The product **129** (57 mg, 64%) was obtained as a white powder; R$_f$ 0.42 ethyl acetate/methanol, 7:1; ^{1}H NMR (360 MHz, CD$_3$OD) δ; 7.78 (d, 2H, J ~ 7.0 Hz, aromatic, Fmoc), 7.65 (d, 2H, J ~ 7 Hz, aromatic, Fmoc), 7.40-7.26 (m, 4H, aromatic, Fmoc), 4.36 (d, 2H, J = 6.9 Hz, CHC$\underline{H}_2$, Fmoc), 4.20 (t, 1H, J = 6.9 Hz, C$\underline{H}$CH$_2$, Fmoc), 4.15 (d, 1H, J$_{4,5}$ = 10.0 Hz, H-4), 3.82 (d, 1H, J$_{5,6}$ = 2.8 Hz, H-6), 3.78-3.53 (m, 9H, including s at 3.64, (~3H, OCH$_3$)), 2.70 (d, 1H, J$_{2a,2b}$ = 15.1 Hz, H-2a), 2.62 (d, 1H, H-2b); MS (MALDI) [M+Na]$^+$ 510.5.

Benzyl [3,7-anhydro-2-deoxy-3-*C*-(aminomethyl(*N*-(9-fluorenylmethoxycarbonyl)))-D-*glycero*-L-*gluco*-oct]onate (**130**)



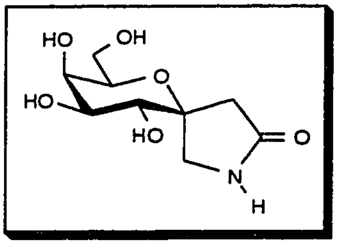
Compound **123** (8 mg, 17 μmol), benzyl bromide (50 μl, 170 μmol) and sodium bicarbonate (16.80 mg, 0.20 mmol) were dissolved in dimethylsulfoxid (1 ml) and stirred for 4 h. Filtration (celite) and chromatographic purification with a gradient silica chromatography using 9% to 12.5% methanol in ethyl acetate as the eluent gave product **130** (5 mg, 50%) as a white powder; R$_f$ 0.47 ethyl acetate/methanol, 7:1; ^{1}H NMR (360 MHz, CD$_3$OD) δ; 7.78 (d, 2H, J ~ 7Hz, aromatic, Fmoc), 7.65 (d, 2H, J ~ 7 Hz, aromatic, Fmoc), 7.40-7.24 (m, 9H, aromatic, Fmoc, Ph), 5.10 (ABq, 2H, J$_{gem}$ = 12.0 Hz, OC$\underline{H}_2$Ph), 4.35 (d, 2H, J = 6.9 Hz, CHC$\underline{H}_2$, Fmoc), 4.19 (t, 1H, J = 6.9 Hz, C$\underline{H}$CH$_2$, Fmoc), 4.18 (d, 1H, J$_{4,5}$ = 9.0 Hz, H-4), 3.83 (d, 1H, J$_{5,6}$ = 2.7 Hz, H-6), 3.78-3.57 (m, 6H), 2.74 (d, 1H, J$_{2a,2b}$ = 14.6 Hz, H-2a), 2.66 (d, 1H, H-2b); MS (MALDI) [M+Na]$^+$ 586.5.

Immobilization of **129** or **130** on the trityl resin and attempted solid phase synthesis of **131** and **132**.

Compound **129** or 130 (8 μmol) and tritylchloride resin (17 mg, capacity 950 μmol/g ~ 17.8 μmol) were added to dry *N*,*N*-dimethylformamide (1 ml) and stirred for 2 h. The thin layer chromatogram showed the complete disappearance of the compounds **129** or **130**. The solvent was removed by filtration and the resin was washed with diisopropylethylamine/methanol/dichloromethane, 2:1:17 (3 x 2 ml), *N*,*N*-dimethylformamide 2 x 2 ml), dichloromethane (2 x 2 ml) and methanol (2 x 2 ml). The residue was dried under vacuum overnight to give the resins **131** or **132**, respectively. These resins were directly used for further coupling reactions. The resins **131** or **132** were dissolved in a (1:1) mixture of dimethylformamide/morpholine (1.5 ml) for 0.5 h.

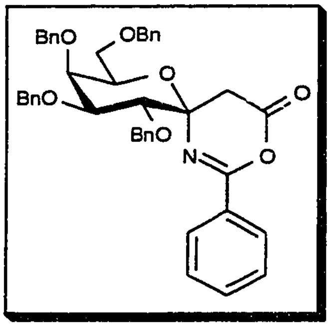
The solvent was removed by filtration and the resins were washed with *N*,*N*-dimethylformamide (2 x 10 ml), dichloromethane (2 x 10 ml), methanol (2 x 10 ml) and dichloromethane (2 x 10 ml) before beeing dried under vacuum for two hours. The resins were suspended in *N*,*N*-dimethylformamide (1.0 ml) and 1-hydroxybenzotriazole (12 µmol) and Fmoc-L-Phe-OPfp (32 µmol) were added. The reaction was stirred overnight. Cleavage of the resin was done with 0.5% trifluoroacetic acid in dry dichloromethane for 5 minutes. The residue was filtered (celite) and concentrated to yield **133** (~ 3.5 mg) as a white solid.

3,7 Anhydro-2-deoxy-3-*C*-(aminomethyl)-D-*glycero*-L-*gluco* -octonic acid lactam (**133**)



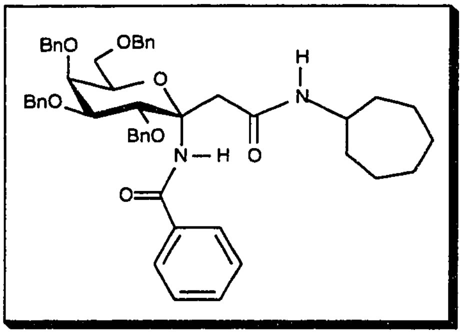
^{1}H NMR (360 MHz, CD_3OD) δ: 3.85 (d, 1H, $J_{5,6}$ = 3.0 Hz, H-6), 3.75 (d, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 3.68-3.65 (m, 3H, H-7, H-8a, H-8b), 3.66 (d, 1H, $C\underline{H}_2NH$), 3.46 (d, 1H, $C\underline{H}_2NH$), 3.42 (dd, 1H, H-5), 2.78 (d, 1H, $J_{2a,2b}$ = 17.5 Hz, H-2a), 2.37 (d, 1H, H-2b). MS (MALDI) $[M+H]^+$ 234.5. Identical samples (MS, ^{1}H-NMR) were independently prepared by treatment of compounds **129** and **130** with a (1:1) mixture of morpholine and *N*,*N*-dimethylformamide for 0.5 h.

(1S) 2,3,4,6-Tetra-*O*-benzyl-3'phenylspiro[1,5 anhydro-D-galactitiol-1,4'-[1,3] dihydrooxazin-6-one] (**134**)



The acetale **89** (50 mg, 76.3 µmol) and benzonitrile (78 µl, 763 µmol) were dissolved in dry dichloromethane (3 ml). At 0° C TMSOTf (44 µl, 229 µmol) was added and the reaction was stirred for 10 min. Saturated sodium bicarbonate solution (1.5 ml) was added and the organic layer was separated, dried (Na_2SO_4) and concentrated. Chromatographic purification using 2% ethyl acetate in toluene as the eluent gave product **134** (48 mg, 92%) as a white powder; R_f 0.75 in ethyl acetate/toluene 1:30; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 8.07-8.03 (m, 2H, aromatic), 7.54-7.48 (m, 1H, aromatic), 7.47-7.40 (m, 2H, aromatic), 7.35-7.18 (m, 20H, aromatic), 4.97 (d, 1H, J_{gem} = 11.4 Hz, $OC\underline{H}_2Ph$), 4.85 (d, 1H, J_{gem} = 11.9 Hz, $OC\underline{H}_2Ph$), 4.70 (d, 2H, J_{gem} = 11.4 Hz, $OC\underline{H}_2Ph$), 4.67 (ddd, 1H, $J_{4,5}$ ~ 1Hz, $J_{5,6a}$ = 7.9 Hz, $J_{5,6b}$ = 5.5 Hz, H-5), 4.64 (d, 1H, J_{gem} = 11.4 Hz, $OC\underline{H}_2Ph$), 4.60 (d, 1H, J_{gem} = 11.4 Hz, $OC\underline{H}_2Ph$), 4.48 (ABq, 2H, J_{gem} = 11.9 Hz, $OC\underline{H}_2Ph$), 4.12 (dd, 1H, $J_{3,4}$ = 2.4 Hz, H-4), 4.06 (dd, 1H, $J_{2,3}$ = 9.9 Hz, H-3), 4.02 (d, 1H, H-2), 3.65 (dd, 1H, $J_{6a,6b}$ = 9.1 Hz, H-6a), 3.56 (dd, 1H, H-6b), 2.89 (d, 1H, $J_{5'a,5'b}$ = 16.3 Hz, H-5'a), 2.73 (d, 1H, H-5'b): ^{13}C NMR (75 MHz, $CDCl_3$) δ: 164.93 (C=O), 152.34 (C=N), 138.70, 138.15, 137.90, 137.38 (4 x C), 132.15 (C-H), 130.35 (C), 128.47-127.57 (C-H, aromatic), 88.89 (C), 80.54 (C-H), 80.45 (C-H), 75.11 ($C\text{-}H_2$), 74.93 ($C\text{-}H_2$), 74.77 (C-H), 73.66 ($C\text{-}H_2$), 72.05 ($C\text{-}H_2$), 70.99 (C-H), 68.67 ($C\text{-}H_2$), 38.25 ($C\text{-}H_2$); IR (CH_2Cl_2): 1798, 1674, 1199, 1099 cm^{-1}; MS (ES) $[M+Na]^+$ calcd. 706.27807, found 706.27842.

N^1-Cycloheptyl (3-amino-3-*N*-benzoyl-4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-oct-3-ulopyranosid)onamide (**135**)

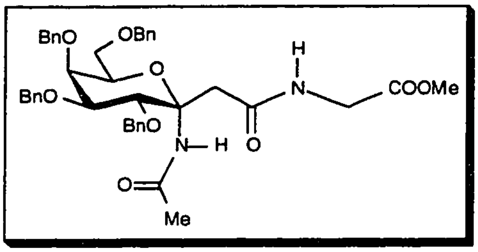


Compound **134** (20 mg, 29 μmol) was dissolved in dry dichloromethane (3 ml). Cycloheptylamine (37 μl, 292 μmol) was added and the reaction mixture was stirred for 1 hour. The solvent was removed under reduced pressure and the residue was purified by gradient chromatography using 9% to 16% ethyl acetate in toluene. Product **135** (21 mg, 90%) was obtained as a white powder; $[\alpha]_D = 62.5^0$ (c 2.4, $CHCl_3$); R_f 0.41 in toluene/ethyl acetate; ^1H NMR (600 MHz, $CDCl_3$) δ: 7.60-7.20 (m, 25H, aromatic), 6.75 (s, br., 1H, N-H), 6.57 (d, br., 1H, $J_{N\text{-}H,CHc\text{-}heptyl}$ = 7.2 Hz, N-H), 4.96 (s, 2H, OC$\underline{H}_2$Ph), 4.72 (ABq, 2H, J_{gem} = 11.3 Hz, OC$\underline{H}_2$Ph), 4.68 (ABq, 2H, J_{gem} = 11.7 Hz, OC$\underline{H}_2$Ph), 4.47 (ABq, 2H, J_{gem} = 11.5 Hz, OC$\underline{H}_2$Ph), 4.21 (d, 1H, $J_{4,5}$ = 11.3Hz, H-4), 4.08 (d, br., 1H, $J_{5,6}$ = 2.4 Hz, $J_{6,7} < 1.0$ Hz, H-6), 3.89 (m, 1H, H-7), 3.77 (m, 1H, C-$H_{c\text{-}heptyl}$), 3.74-3.71 (m, 2H, H-5, H-8a), 3.69 (d, 1H, $J_{2a,2b}$ = 14.8 Hz, H-2a), 3.65 (dd, 1H, $J_{7,8b}$ = 5.0 Hz, $J_{8a,8b}$ = 8.8 Hz, H-8b), 2.70 (d, 1H, H-2b), 1-84-1.80 (m, 12H, c-heptyl): ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.30 (C=O), 166.71 (C=O), 138.57, 138.05, 137.89, 137.77, 134.69 (5 x C, aromatic), 131.89-126.91 (C-H, aromatic), 87.36 (C), 80.47 (C-H), 76.88 (C-H), 76.17, 75.16, 73.65 (3x C-H_2), 73.52 (C-H), 72.16 (C-H_2), 71.14 (C-H), 67.93 (C-H_2), 50.63 (C-H), 43.02 (C-H_2), 34.79, 34.65, 27.95, 27.90, 24.21, 24.16 (6 x C-H_2); HRMS (ES) $[M+Na]^+$ calcd. 819.39852, found 819.40140. The axial orientation of the singlet NH was determined by one dimensional TROESY experiment. Excitation by a 240ms selective e-burp1 pulse[192] showed inter-proton effects to H-5 and H-7 (2.5 and 0.5%, respectively of the amide resonance integral). Addition of D_2O (10 µl) led to the disappearance of the signals at 6.75 and 6.57.

General synthesis of compounds **136-140**

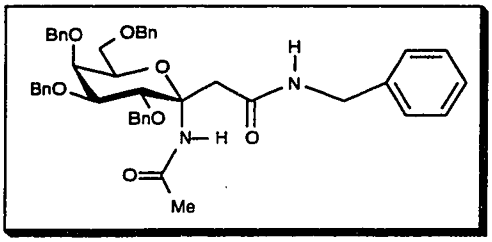
Compound **89** (25 mg, 38.10 µmol) was dissolved in a (1:1) mixture of dry dichloromethane and trifluoroacetic acid (10 ml) under an argon atmosphere. The reaction was stirred for 2 h at 0°C. Toluene (10 ml) was added and the solvent was removed under reduced pressure. The residue was codistilled with toluene (3 x 5 ml) and dried in high vacuum. The residue was dissolved in dry dichloromethane (2 ml) and the nitrile component (380 µmol) was added under an argon atmosphere. The reaction was cooled to 0°C and TMSOTf (22 µl, 114.3 µmol) was added. After stirring the reaction mixture for 1 hour, the amine component (380 µmol) was added at rt and the reaction was stirred for an additional 1h. The solvent was removed under reduced pressure and the product was purified with gradient chromatography using ethyl acetate (9% to 20%) in toluene as the eluent. All products (**136-140**) were obtained in 60-70% yield as single stereoisomers. The axial position of the singlet NH proton was determined by a one dimensional TROESY experiment. Excitation by a 240ms selective e-burp1 pulse[192] showed inter-proton effects to H-5 and H-7 (usually, 2.5 and 0.5%, respectively of the amide resonance integral).

N^1-Gly-OMe (3-amino-3-*N*-acetyl-4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-oct-3-ulopyranosid)onamide (**136**)



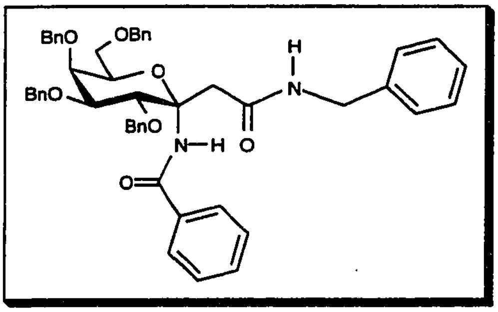
Compound **136** was obtained as a white powder; $[\alpha]_D = 58.9^0$ (c 0.8, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.40-7.14 (m, 21H, 20H, aromatic + t, 1H, NH), 6.06 (s, br., 1H, N-H), 4.89 (d, 1H, J_{gem} = 10.4 Hz, $OC\underline{H}_2Ph$), 4.87 (d, 1H, J_{gem} = 10.5 Hz, $OC\underline{H}_2Ph$), 4.77 (d, 1H, J_{gem} = 12.0 Hz, $OC\underline{H}_2Ph$), 4.75 (d, 1H, J_{gem} = 11.4 Hz, $OC\underline{H}_2Ph$), 4.67 (d, 1H, J_{gem} = 11.7 Hz, $OC\underline{H}_2Ph$), 4.58 (d, 1H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.48 (d, 1H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.46 (d, 1H, J_{gem} = 10.4 Hz, $OC\underline{H}_2Ph$), 4.35 (d, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 4.1 (s, br., 1H, H-6), 3.88-3.75 (m, 3H, H-7, H-8a, H-2a), 3.67-3.62 (m, 2H, H-5, H-8b), 3.56 (s, 3H, OCH_3), 3.45 (dd, 1H, $J_{Ha(Gly),Hb(Gly)}$ = 17.8Hz, $J(_{Ha(Gly)},H_{N-H(Gly)})$ = 5.0 Hz, $H_{a(Gly)}$), 3.34 (dd, 1H, $J_{Hb(Gly),HNH(Gly}$ = 6.0 Hz, $H_{b(Gly)}$), 2.68 (d, 1H, $J_{2a,2b}$ = 16.1 Hz, H-2b), 1.95 (s, 3H, CH_3); MS (ES) $[M+Na]^+$ calcd. 733.31010, found 733.31350. Addition of D_2O (10 μl) led to the disappearance of one proton at 7.40-7.14 and the signal at 6.06.

N^1-Benzyl (3-amino-3-*N*-acetyl-4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-oct-3-ulopyranosid)onamide (**137**)



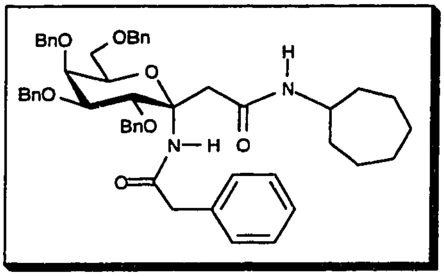
Compound **137** was obtained as a white powder; $[\alpha]_D = 34.4^0$ (c 0.8, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.40-7.00 (m, 26H, (25H aromatic, + t, 1H, NH)), 6.05 (s, br., 1H, N-H), 4.86 (m, 2H, $\mathrm{OC\underline{H}_2Ph}$), 4.80 (d, 1H, J_{gem} = 10.9 Hz, $\mathrm{OC\underline{H}_2Ph}$), 4.67 (ABq, 2H, J_{gem} = 11.6 Hz, $\mathrm{OC\underline{H}_2Ph}$), 4.46 (d, 1H, J_{gem} = 10.2 Hz, $\mathrm{OC\underline{H}_2Ph}$), 4.45 (d, 1H, J_{gem} = 10.8 Hz, $\mathrm{OC\underline{H}_2Ph}$), 4.39 (dd, 1H, $J_{\mathrm{Ha(benzyl),Hb(benzyl}}$ = 14.8 Hz, $J_{\mathrm{Ha(benzyl),HN\text{-}H(benzyl)}}$ = 6.8 Hz, $\mathrm{(NHC\underline{H}_2C_6H_5)}$), 4.35 (d, 1H, J_{gem} = 11.6 Hz, $\mathrm{OC\underline{H}_2Ph}$), 4.16 (d, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 4.06 (d, 1H, $J_{5,6}$ = 2.4 Hz, H-6), 3.80 (dd, 1H, $J_{7,8a}$ = 4.8 Hz, $J_{8a,8b}$ = 8.7 Hz), 3.74 (dd, 1H, $J_{\mathrm{Hb(benzyl),N\text{-}H(benzyl)}}$ = 4.4 Hz, $\mathrm{(NHC\underline{H}_2C_6H_5)}$), 3.69 (d, 1H, $J_{2a,2b}$ = 15.4 Hz, H-2a), 3.63 (dd, 1H, H-5), 3.57 (t, 1H, H-7), 3.49 (dd, 1H, $J_{7,8b}$ = 5.0 Hz, H-8b), 2.70 (d, 1H, H-2b), 1.93 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ; 169.92 (C=O), 168.69 (C=O), 138.42 x 3, 137.95, 137.70 (5 x C, aromatics), 133.35-127.00 (C-H, aromatics), 86.76 (C), 80.92 (C-H), 76.90 (C-H), 74.48 (C-H_2), 74.87 (C-H_2), 73.68 (C-H_2), 73.12 (C-H), 72.28 (C-H_2), 70.80 (C-H), 67.66 (C-H_2), 43.21 (C-H_2), 42.95 (C-H_2), 24.68 (C-H_3); HRMS (ES) $[M+Na]^+$ calcd. 751.33592, found 751.33750. Addition of D_2O (10 μl) led to the disappearance of one proton (t) at 7.40-7.10 and the signal at 6.05.

N^1-Benzyl (3-amino-3-N-benzoyl-4,5,6,8-tetra-O-benzyl-2-deoxy-α-D-*galacto*-oct-3-ulopyranosid)onamide (**138**)



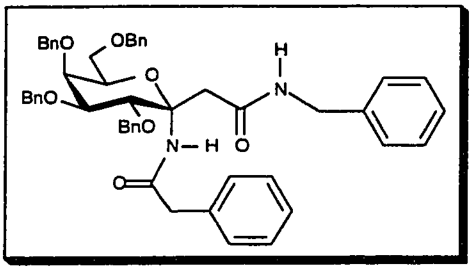
Compound **137** was obtained as a white powder; $[\alpha]_D = 61.9^0$ (c 1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ; 7.62-7.16 (m, 28 H, aromatics), 7.09 (dd, br., 1H, N-H(benzyl)), 7.08-7.05 (m, 2H, aromatic), 6.85 (s, br. 1H, N-H), 4.96 (ABq, 2H, J_{gem} = 10.7 Hz, $OC\underline{H}_2Ph$), 4.85 (d, 1H, J_{gem} = 10.8 Hz, $OC\underline{H}_2Ph$), 4.71 (ABq, 2H, J_{gem} = 11.7 Hz, $OC\underline{H}_2Ph$), 4.49 (d, 1H, J_{gem} = 10.8 Hz, $OC\underline{H}_2Ph$), 4.47 (d, 1H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.43 (dd, 1H, $J_{Ha(benzyl),Hb(benzyl}$ = 14.7 Hz, $J_{Ha(benzyl),HN(benzyl)}$ = 6.8 Hz, $NHC\underline{H}_2C_6H_5$), 4.37 (d, 1H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.31 (d, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 4.10 (d, 1H, $J_{5,6}$ = 2.4 Hz, H-6), 3.89 (dd, 1H, $J_{7,8a}$ = 8.8 Hz, H-7), 3.83(d, 1H, $J_{2a,2b}$ = 15.2 Hz, H-2a), 3.80 (dd, 1H, $J_{Ha(benzyl),HN(benzyl)}$ = 4.6 Hz, $NHC\underline{H}_2C_6H_5$), 3.74 (dd, 1H, H-5), 3.63 (t, 1H, $J_{8a,8b}$ = 8.8 Hz, H-8a), 3.55 (dd, 1H, H-8b), 2.80 (d, 1H, H-2b); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 168.65 (C=O), 166.67 (C=O), 138.45 x 2, 137.87 x 2, 137.73, 134.66 (6 x C, aromatic), 131.90-126.91 (C-H, aromatic), 87.12 (C), 80.67 (C-H), 77.17 (C-H), 76.24 ($C-H_2$), 74.86 ($C-H_2$), 73.65 ($C-H_2$), 73.01 (C-H), 72.09 ($C-H_2$), 71.05 (C-H), 67.69 ($C-H_2$), 43.23 ($C-H_2$), 43.03 ($C-H_2$); HRMS (ES) $[M+Na]^+$ calcd. 813.35157, found 813.35290. Addition of D_2O (10 μl) led to the disappearance of the signals at 7.09 and 6.85.

N^1-Cycloheptyl (3-amino-3-*N*-benzoyl-4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-oct-3-ulopyranosid)onamide (**139**)



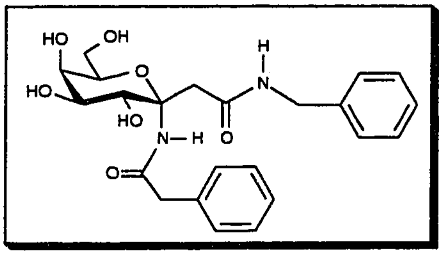
Compound **139** was obtained as a white powder; $[\alpha]_D = 39.8^0$ (c 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ: 7.40-7.07 (m, 25H, aromatic), 6.50 (d, br., 1H, $J_{HN\text{-}H(c\text{-}heptyl),H(c\text{-}heptyl)}$ = 7.0 Hz, N-$H_{c\text{-}heptyl}$), 6.04 (s, br., 1H, N-H), 4.82 (d, 1H, J_{gem} = 11.2 Hz, $OC\underline{H}_2Ph$), 4.67 (s, 2H, $OC\underline{H}_2Ph$), 4.53-4.42 (m, 5H, $OC\underline{H}_2Ph$), 3.97 (d, 1H, $J_{4,5}$ = 9.5 Hz, H-4), 3.91 (d, 1H, $J_{5,6}$ = 2.6 Hz, H-6), 3.73 (m, 1H, CH, c-heptyl), 3.70-3.63 (m, 2H, H-7, H-8a), 3.59 (dd, 1H, $J_{7,8b}$ = 4.8 Hz, $J_{8a,8b}$ = 8.3 Hz, H-8b), 3.54-3.46 (m, 3H, 2 x $COC\underline{H}_2Ph$, H-2a), 3.22 (dd, 1H, H-5), 2.48 (d, 1H, $J_{2a,2b}$ = 15.0 Hz, H-2b), 1.80-0.96 (m, 12H, c-heptyl); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.58 (C=O), 167.16 (C=O), 138.49, 138.01, 137.93, 137.74, 134.66 (5 x C, aromatics), 129.16-127.49 (C-H, aromatics), 86.87 (C), 80.45 (C-H), 76.54 (C-H), 75.56 (C-H_2), 75.04 (C-H_2), 73.72 (C-H), 73.58 (C-H_2), 72.39 (C-H_2), 71.03 (C-H), 67.87 (C-H_2), 50.56 (C-H), 45.13 (C-H_2), 42.80 (C-H_2), 34.73 (C-H_2), 34.59 (C-H_2), 27.87 (C-H_2), 28.81 (C-H_2), 24.16 (C-H_2), 24.12 (C-H_2); HRMS (ES) $[M+Na]^+$ calcd. 833.41417, found 833.41430. Addition of D_2O (10 μl) led to the disappearance of the signals at 6.50 and 6.04.

N^1-Benzyl (3-amino-3-*N*-phenacetyl-4,5,6,8-tetra-*O*-benzyl-2-deoxy-α-D-*galacto*-oct-3ulopyranosid)onamide (**140**)



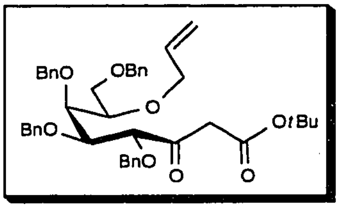
Compound **140** was obtained as a white powder; $[\alpha]_D = 34.2^0$ (c 1, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$) δ: 7.36-7.01 (m, 30H, aromatic), 6.99 (dd, br., 1H, N-H(benzyl)), 6.09 (s, br., 1H, N-H), 4.79 (d, 1H, J_{gem} = 10.9 Hz, OC$\underline{H}_2$Ph), 4.77 (ABq, 2H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.54 (d, 1H, J_{gem} = 11.9 Hz, OC$\underline{H}_2$Ph), 4.46 (d, 1H, J_{gem} = 11.5 Hz, OC$\underline{H}_2$Ph), 4.45 (d, 1H, J_{gem} = 10.2 Hz, OC$\underline{H}_2$Ph), 4.43 (d, 1H, J_{gem} ~ 11.0 Hz, OC$\underline{H}_2$Ph), 4.36 (dd, 1H, $J_{Ha(benzyl),Hb(benzyl)}$ = 14.7 Hz, $J_{Ha(benzyl),HN(benzyl)}$ = 6.7 Hz, H-a(benzyl)), 4.35 (d, 1H, J_{gem} = 11.6 Hz, OC$\underline{H}_2$Ph), 4.06 (d, 1H, $J_{4,5}$ = 9.5 Hz, H-4), 3.94 (d, 1H, $J_{5,6}$ = 2.4 Hz, H-6), 3.76 (dd, 1H, $J_{Hb(benzyl),HN(benzyl)}$ = 4.6 Hz, H-b(benzyl)), 3.67 (dd, 1H, $J_{7,8a}$ = 8.7 Hz, $J_{7,8b}$ = 5.4 Hz, H-7), 3.63 (d, 1H, $J_{2a,2b}$ = 15.4 Hz, H-2a), 3.54 (t, 1H, $J_{8a,8b}$ ~ 9 Hz, H-8a), 3.53-3.46 (m, 3H, H-8b, C$\underline{H}_2$CO), 3.24 (dd, 1H, H-5), 2.68 (d, 1H, H-2b); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.59 (C=O), 168.55 (C=O), 138.37 x 2, 137.87 x 2, 137.70, 134.64 (6 x C, aromatics), 129.17-126.97 (C-H aromatics), 86.64 (C), 80.57 (C-H), 76.88 (C-H), 75.69 (C-H_2), 74.73 (C-H_2), 73,60 (C-H_2), 73.17 (C-H), 72.24 (C-H_2), 70.95 (C-H), 67.65 (C-H_2), 45.12 (C-H_2), 43.18 (C-H_2), 42.77 (C-H_2); HRMS (ES) $[M+Na]^+$ calcd. 827.36722, found 827.36779. Addition of D_2O (10 μl) led to the disappearance of the signals at 6.99 and 6.09.

N^1-Benzyl (3-amino-3-*N*-phenacetyl-2-deoxy-α-D-*galacto*-oct-3-ulopyranosid)onamide (**141**)



Compound **140** (45.3 mg, 56.3 μmol) was dissolved in a (1:1) mixture of ethyl acetate and methanol and was hydrogenated (1 atm) with palladium-(II)-hydroxide/C (16 mg) for 4 h. The solution was filtered (celite) and evaporated to dryness to give the product **141** (23.7 mg, 95%) as a white powder. ^{1}H NMR (360 MHz, CD_3OD) δ: 7.70-7.40 (m, 10H, aromatics), 4.37 (d, 1H, J = 15.0 Hz, PhC$\underline{H}_2$C=O), 4.27 (d, 1H, PhC$\underline{H}_2$C=O), 3.89 (d, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 3.94 (dd, 1H, $J_{5,6}$ = 2.4 Hz, $J_{6,7}$ < 1 Hz, H-6), 3.66 (dd, 1H, $J_{7,8a}$ = 6.8 Hz, $J_{8a,8b}$ = 11.3 Hz, H-8a), 3.58 (dd, 1H, $J_{7,8b}$ = 5.2 Hz, H-8b), 3.54 (s, 2H, PhC$\underline{H}_2$NH), 3.52 (ddd, 1H, H-7), 3.47 (dd, 1H, H-5), 3.16 (d, 1H, $J_{2a,2b}$ = 14.4 Hz, H-2a), 2.98 (d, 1H, H-2b); HRMS (ES) $[M+Na]^+$ calcd. 467.17942, found 467.17979.

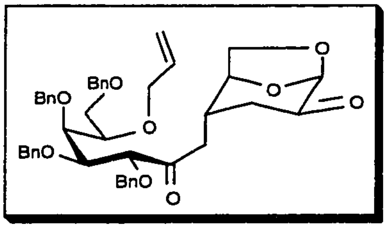
tert-Butyl (7-*O*-allyl-4,5,6,8 tetra-*O*-benzyl-2-deoxy-D-*galacto*-3-octulos)onate (**142**)



The acetal **89** (57 mg, 87 μmol), allyl bromide (75 μl, 780 μmol) were dissolved in dry dimethylformamide (4 ml). At 0°C sodium hydride (20 mg, 833 μmol) was added and the reaction was stirred for 1 h at 0°C. A (1:1) mixture of acetic acid and methanol (0.5 ml) was slowly added at 0°C and the temperature was raised to room temperature. The solvent was removed under reduced pressure and the residue **142** was purified by chromatography using 1% ethyl acetate in toluene as the eluent. Unreacted starting material (28 mg) was recovered. Product **142** (14 mg, 25%) was obtained as a colourless liquid and existed predominantly in the keto form (>75%, $CDCl_3$); R_f 0.66 ethyl acetate/toluene, 1:10; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.40-7.12 (m, 20H, aromatics), 5.96-5.81(m, 1H,

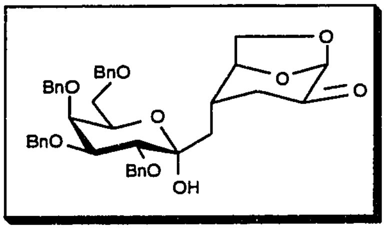
$OCH_2C\underline{H}CH_2$), 5.18 (m, 1H, J_{vic} = 16.1 Hz, $OCH_2CHC\underline{H}_2$), 5.07 (m, 1H, J_{vic} = 10.4 Hz, $OCH_2CHC\underline{H}_2$), 4.64 (d, 1H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.57 (d, 1H, J_{gem} = 12.1 Hz, $OC\underline{H}_2Ph$), 4.55 (s, 2H, $OC\underline{H}_2Ph$), 4.52 (d, 1H, J_{gem} ~ 11.8 Hz, $OC\underline{H}_2Ph$), 4.43 (s, 2H, $OC\underline{H}_2Ph$), 4.37 (d, 1H, J_{gem} = 11.5 Hz, $OC\underline{H}_2Ph$), 4.30 (d, 1H, J = 3.3 Hz, H-4), 4.22-4.12 (m, 2H), 4.05-3.95 (m, 2H), 3.91-3.84 (m, 1H), 3.65 (dd, 1H, $J_{7,8a}$ = 5.5 Hz, $J_{8a,8b}$ = 9.5 Hz, H-8a), 3.57 (dd, 1H, $J_{7,8b}$ = 5.5 Hz, H-8b), 3.52 (d, 1H, $J_{2a,2b}$ = 16.2 Hz, H-2a), 3.46 (d, 1H, H-2b), 1.40 (s, 9H, *t* -Bu); MS (ES) $[M+Na]^+$ 717.3.

1,6-Anhydro-D-*glycero*-hexo-pyranos-2-ulose, 4-*C*-[6'-*O*-allyl-3',4',5',7' tetra-*O*-benzyl-1-deoxy-α-D-*galacto*-hept-2-ulose]-3,4-dideoxy (**144**)



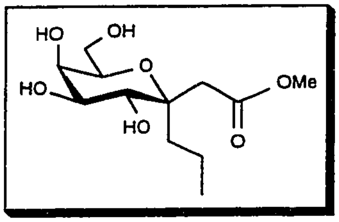
Compound **142** (22 mg, 31.6 µmol),.levoglucosenone (10 µl, ~3 equiv.) were dissolved in dry *N*,*N*-dimethylformamide (1 ml). Potassium carbonate (5 mg, 35 µmol) was added and the reaction was stirred for 2 hours. The solvent was removed under reduced pressure and the crude material was purified by chromatography using 3% ethyl acetate in toluene as the eluent. The diastereomeric mixture **143** (ratio 2:1, 18.6 mg 72%) was obtained as a colourless liquid; MS (ES) $[M+Na]^+$ 843.3. The mixture **143** (12 mg, 14.6 µmol) was dissolved in a (1:2) mixture of dichloromethane and trifluoroacetic acid (1.5 ml) at 0^0 C. The temperature was raised to room temperature and the reaction was stirred for 2 h. Toluene (2 ml) was added and the mixture was evaporated to dryness and codistilled with toluene (2 x 3 ml). The residue was dissolved in toluene (1 ml) and the mixture was heated up to 70^0C for 40 minutes. The flask was cooled and the mixture was concentrated under reduced pressure. Chromatographic purification using 4% ethyl acetate in toluene as the eluent gave the product **144** (8 mg, 75%), as an oil; R_f 0.44 toluene/ethyl acetate, 10:1; ^1H NMR (360 MHz, $CDCl_3$) δ: 7.4-7.12 (m, 20H, aromatics), 5.87 (m, 1H, $OCH_2C\underline{H}CH_2$), 5.22 (m, 1H, J_{vic} = 17.3 Hz, $OCH_2CHC\underline{H}_2$), 5.10 (m, 1H, J_{vic} =10.4 Hz, 1x $OCH_2CHC\underline{H}_2$), 4.93 (s, br., 1H, $J_{1,3eq}$ < 1 Hz, H-1), 4.64-4.35 (m, 8H, $OC\underline{H}_2Ph$), 4.18 (m, 1H, $OC\underline{H}_2CHCH_2$), 4.10-3.95 (m, 4H), 3.92-3.84 (m, 2H), 3.78-3.72 (m, 2H), 3.68 (dd, 1H, J = 5.3 Hz, J = 9.8 Hz), 3.60 (dd, 1H, J = 5.4 Hz, J = 9.8 Hz), 2.76 (dd, 1H, $J_{4,1'a}$ = 6.5 Hz, $J_{1'a,1'b}$ = 19.0 Hz, H-1'a), 2.60-2.47 (m, 2H, H-3_{ax}, H-5b), 2.15 (q, br., 1H, J ~ 7.2 Hz, H-4), 1.81 (d, br., 1H, $J_{3ax,3eq}$ = 16.7 Hz, H-3_{eq}); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 208.43 (C=O), 200.26 (C=O), 138.47, 138,24, 138,09, 137.15 (4 x C, aromatics), 135.23 (C-H), 128.57-127.59 (C-H, aromatics), 116.70 (C-H_2), 101.42 (C-H), 85.69 (C-H), 79.75 (C-H), 78.69 (C-H), 77.48 (C-H), 76.28 (C-H), 74.04 (C-H_2), 73.64 (C-H_2), 73.52 (C-H_2), 73.40 (C-H_2), 72.01 (C-H_2), 69.71 (C-H_2), 67.82 (C-H_2), 42.12 (C-H_2), 37.10 (C-H_2), 35.85 (C-H); MS (ES) $[M+Na]^+$ 743.3.

1,6 Anhydro-D-*glycero*-hexo-pyranos-2-ulose, 4-*C*-[3',4',5',7' tetra-*O*-benzyl-1-deoxy-α-D-*galacto*-pyranos-2-heptulose]-3,4-dideoxy (**145**)



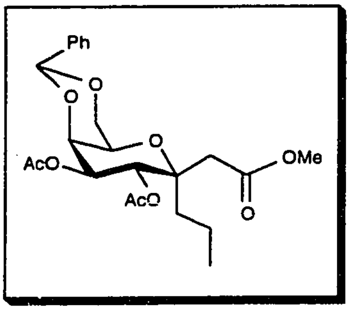
Compound **144** (6.6 mg, 9 μmol) was dissolved in dry methanol (1 ml). Palladium-(II)-chloride (1.7 mg, 9.1 μmol) was added under argon and the reaction was stirred at room temperature for 70 min. Saturated sodium bicarbonate solution (1 ml) and dichloromethane (4 ml) was added and the organic layer was separated, dried (Na_2SO_4) and concentrated. The thin layer chromatogramm showed the presence of unreacted starting material as well as a second unidentified product ($[M+Na]^+$ 717.3, (~15%). The product **145** (3.7 mg, 60%) was obtained as a liquid; ^1H NMR (600 MHz, $CDCl_3$) δ: 7.40-7.20 (m, 20H, aromatics), 4.98 (s, 1H, H-1), 4.93 (d, 1H, J_{gem} = 11.2 Hz, $OC\underline{H}_2Ph$), 4.89 (d, 1H, J_{gem} = 12.6 Hz, $OC\underline{H}_2Ph$), 4.82 (m, 1H, H-5), 4.75 (d, 1H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.70 (d, 1H, J_{gem} = 11.1 Hz, $OC\underline{H}_2Ph$), 4.64 (d, 1H, J_{gem} = 11.6 Hz, $OC\underline{H}_2Ph$), 4.56 (d, 1H, J_{gem} = 11.3 Hz, $OC\underline{H}_2Ph$), 4.40 (s, 2H, OC$\underline{H}_2$Ph), 4.07 (m, 1H, $J_{5',6'}$ ~1Hz, $J_{6',7'a}$ = 7.4 Hz, $J_{6',7'b}$ = 5.8 Hz, H-6'), 4.01 (dd, 1H, $J_{4',5'}$ = 2.6 Hz, H-5'), 3.84 (dd, 1H, $J_{3',4'}$ = 9.4 Hz, H-4'), 3.78-3.72 (m, 3H, H6a, H6b, H-3'), 3.55 (dd, 1H, $J_{7'a,7'b}$ = 9.1 Hz, H-7'a), 3.51 (dd, 1H, H-7'b), 2.83 (s, br., 1H, OH), 2.57 (dd, 1H, $J_{3ax,3eq}$ = 16.4 Hz, $J_{3ax,4}$ = 8.9 Hz, H-3_{ax}), 2.19-2.15 (m, 1H, H-4), 2.05 (dd, 1H, $J_{4,1'a}$ = 9.0 Hz, $J_{1'a,1'b}$ = 14.2 Hz, H-1'a), 1.97 (d, br., 1H, $J_{3eq,H1}$ < 1Hz, H-3_{eq}), 1.58 (dd, 1H, $J_{4,1'b}$ = 4.7 Hz, H-1'b); MS (ES) $[M+Na]^+$ 703.3. Addition of D_2O (10 ml) led to the disappearance of the signal at 2.83.

Methyl (3,7-anhydro-2-deoxy-3-*C*-propyl-D-*glycero*-L-*gluco*-oct)onate (**146**)



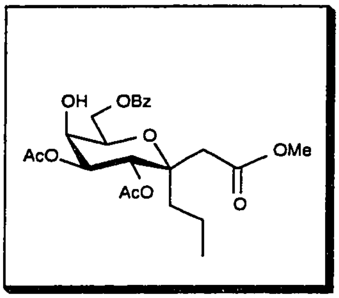
The acetal **95** (1 g, 1.57 mmol) was dissolved in a (1:2) mixture of ethyl acetate and methanol. Palladium-(II)-hydroxide/C (300 mg) was added and the reaction was hydrogenated (1 atm) for 8 h. The solution was filtered (celite) and evaporated to dryness to give **146** (415 mg, 95%) as a white powder; $[\alpha]_D$ = 102.8^0 (c 1.6, CH_3OH); ^{1}H NMR (360 MHz, CD_3OD) δ: 3.97 (d, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 3.83 (d, 1H, $J_{5,6}$ = 3.3 Hz, H-6), 3.67-3.60 (m, 6H, OCH_3, H-5, H-8a, H-8b), 3.52 (t, 1H, J ~ 6.7 Hz, H-7), 2.68 (d, 1H, $J_{2a,2b}$ = 13.8 Hz, H-2a), 2.55 (d, 1H, H-2b), 1.78-1.61 (m, 2H, C$\underline{H}_2$CH$_2$CH$_3$), 1.46-1.33 (m, 2H, CH$_2$C$\underline{H}_2$CH$_3$), 0.95 (t, 3H, J = 7.5 Hz, CH_3); MS (ES) $[M+Na]^+$ 301.1.

Methyl (3,7-anhydro-4,5 di-*O*-acetyl-6,8-*O*-benzylidene-2-deoxy-3-*C*-propyl-D-*glycero*-L-*gluco*-oct)onate (**147**)



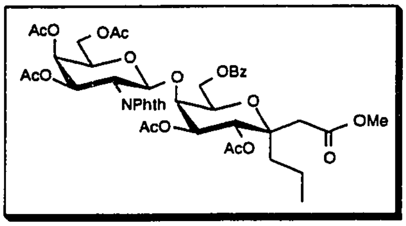
Compound **146** (380 mg, 1.36 mmol), p-toluensulfonic acid (26 mg, 0.14 mmol) and benzaldehyde dimethyl acetal (265 µl, 1.76 mmol) were dissolved in dry acetonitrile (30 ml) and stirred in an argon atmosphere for 30 minutes. Triethylamine (380 µl, 2.72 mmol) was added and the solvent was removed under reduced pressure. The solid residue was directly acetylated using a (1:1) mixture of pyridine and acetic anhydride (25 ml). Chromatographic purification using 25% ethyl acetate in toluene as eluent gave product **147** (478 mg, 78%) as an oil; $[\alpha]_D = 99.8^0$ (c 2.0, $CHCl_3$); R_f 0.72 in toluene/ethyl acetate, 1:2; ^1H NMR (360 MHz, CD_3OD) δ: 7.50-7.30 (m, 5H, aromatics), 5.78 (d, 1H, $J_{4,5} = 9.5$ Hz, H-4), 5.44 (s, 1H, $PhC\underline{H}(O)_2$), 5.15 (dd, 1H, $J_{5,6} = 3.6$ Hz, H-5), 4.35 (dd, 1H, $J_{6,7} < 1$ Hz, H-6), 4.18 (dd, 1H, $J_{7,8a} = 1.4$ Hz, $J_{8a,8b} = 12.3$ Hz, H-8a), 3.97 (dd, 1H, $J_{7,8b} = 1{,}7$ Hz, H-8b), 3.57 (s, 3H, OCH_3), 3.47 (s, br., 1H, H-7), 2.70 (d, 1H, $J_{2a,2b} = 14.8$ Hz, H-2a), 2.57 (d, 1H, H-2b), 2.03, 2.01 (2 x s, 6H, OAc), 1.90-1.77 (m, 2H, $C\underline{H}_2CH_2CH_3$), 1.50-1.27 (m, 2H, $CH_2C\underline{H}_2CH_3$), 0.95 (t, 3H, J = 7.3 Hz, CH_3); MS (ES) $[M+Na]^+$ 473.4. Anal. calcd. for $C_{23}H_{30}O_9$: C 61.32, H 6.71 found: C 61.56, H 6.82.

Methyl (3,7-anhydro-4,5 di-*O*-acetyl-6-*O*-benzoyl-2-deoxy-3-*C*-propyl-D-*glycero*-L-*gluco*-oct)onate (**148**)



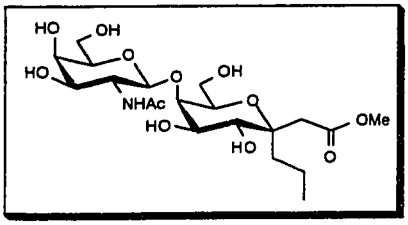
Compound **147** (50 mg, 111 μmol) was dissolved in a (1:1) mixture of ethyl acetate and methanol (4 ml) and hydrogenated (1 atm) with palladium-(II)-hydroxide/C (20 mg) for 1 h. The solvent was filtered (celite) and concentrated. The residue was dissolved in dry pyridine (2.0 ml) and benzoylchloride (14.8 μl, 133.2 μmol) was added slowly. The reaction was stirred for 4 hours at 0^0C and then methanol (1 ml) was added. The stirring continued for an additional 30 min. The solvent was removed under reduced pressure and the residue was purified by chromatography using 20% ethyl acetate in toluene as the eluent. Product **148** (31.2 mg, 60%) was obtained as an oil; R$_f$ 0.67 in ethyl acetate/toluene, 2:1; ^{1}H NMR (360 MHz, CDCl$_3$) δ: 8.01-7.97 (m, 2H, aromatics), 7.51-7.57 (m, 1H, aromatics), 7.44-7.37 (m, 2H, aromatics), 5.95 (d, 1H, J$_{4.5}$ = 10.4 Hz, H-4), 5.07 (dd, 1H, J$_{5.6}$ = 2.8 Hz, H-5), 4.44 (dd, 1H, J$_{7.8a}$ = 5.9 Hz, J$_{8a.8b}$ = 11.5 Hz, H-8a), 4.40 (dd, 1H, J$_{7.8b}$ = 4.9 Hz, H-8b), 4.10-4.04 (s, br., 1H. H-6), 3.92 (t, 1H, J ~ 6.0 Hz, H-7), 3.68 (s, 3H, OMe), 3.80-3.70 (d, br., 1H, OH), 2.72 (d, 1H, J$_{2a.2b}$ = 17.2 Hz, H-2a), 2.35 (d, 1H, H-2b), 2.07, 2.02 (2 x s, 6H, 2 x OAc), 1.83 (dd, 1H, J$_{vic}$ ~ 6.0 Hz, J$_{gem}$ = 11.1 Hz, C$\underline{H}_2$CH$_2$CH$_3$), 1.47-1.37 (m, 2H), 1.30-1.23 (m, 1H), 0.87 (t, 3H, CH$_3$); The signal at 3.80 disappeared and the siganal at 4.10 changed to a dublet (J = 2.8 Hz) by addition of D$_2$O (10 μl) to the ^{1}H NMR sample. HRMS (ES) [M+Na]$^+$ calcd. 489.17366, found 489.17385.

Methyl [3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranosyl-(1→4) (3,7-anhydro-4,5 di-*O*-acetyl-8-*O*-benzoyl-2-deoxy-3-*C*-propyl D-*glycero*-L-*gluco*-oct)]onate (**149**)



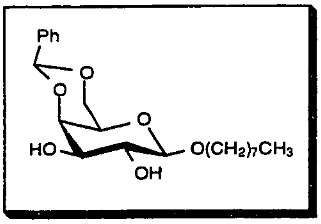
Compound **148** (25 mg, 53.6 μmol), compound **26** (32.4 mg, 69.60 μmol) and molecular sieves (4 Å, 150 mg) were added to dry dichloromethane (5 ml) under argon. The mixture was stirred for 30 min. After cooling the reaction mixture to 0^0C *N*-iodosuccinimide (16.8 mg, 74 μmol) and trifluoromethanesulfonic acid (5 μmol) were added. The mixture was stirred for additional 30 min at 0^0C, filtered (celite) and saturated sodium bicarbonate solution (2 ml) was added. The organic layer was separated, washed with saturated sodium thiosulfate solution (3 ml) and water (2 ml), dried (Na_2SO_4) and concentrated. Chromatographic purification using 14% ethyl acetate in toluene as the eluent gave product **149** (40 mg, 84%) as an oil; R_f 0.31 in ethyl acetate/toluene, 2:1; $[\alpha]_D = 27.4^0$ (c 1.0, $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$) δ: 8.05-7.10 (m, 9H, aromatics), 5.90 (dd, 1H, $J_{2',3'}$ = 11.5 Hz, $J_{3',4'}$ = 3.4 Hz, H-3'), 5.45 (d, 1H, H-4'), 5.33 (d, 1H, $J_{1',2'}$ = 8.5 Hz, H-1'), 5.06 (s, 2H, H-6'a, H-6'b), 4.55 (dd, 1H, $J_{7,8a}$ = 5.0 Hz, $J_{8a,8b}$ = 11.7 Hz, H-8a), 4.55 (dd, 1H, H-2'), 4.36 (dd, 1H, $J_{7,8b}$ = 7.1 Hz, H-8b), 4.16 (s, 1H, H-5'), 4.10 (dd, 1H, $J_{4,5}$ = 9.0 Hz, $J_{5,6}$ = 4.0 Hz, H-5), 4.04 (d, 1H, H-4), 4.02 (d, 1H, H-6), 3.88 (dd, 1H, H-7), 3.60 (s, 3H, OCH_3), 2.40 (s, 2H, H-2a, H-2b), 2.10, 2.01, 2.00, 1.88, 1.70 (5 x s, 15H, 5 x OAc), 1.7-1.59 (m, 2H, $OC\underline{H}_2CH_2CH_3$), 1.30-1.20 (m, 2H, $OC\underline{H}_2CH_2CH_3$), 0.92 (t, 3H, CH_3); MS (ES) $[M+Na]^+$ 906.3. Anal. calcd. for $C_{43}H_{49}O_{19}N$: C 58.43, H 5.58, N 1.58 found C 58.32, H 5.75, N 1.55.

Methyl [2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→6)-(3,7-anhydro-2-deoxy-3-*C*-propyl-D-*glycero*-L-*gluco*-oct)]onate (**150**)



Compound **149** (25 mg, 28.3 μmol) was dissolved in dry methanol (3 ml) and a solution of sodium methoxide (0.1 M) was added (pH ~9). After stirring the mixture for 24 h, Amberlite (IRC 50) was added to neutralize the solution. At neutral pH, the solution was filtered (celite) and the solvent was removed under reduced pressure. The solid residue was dissolved in methanol (5 ml) and hydrazine acetate (90 mg, 97.7 μmol) was added. After refluxing the mixture for 10 h, it was cooled and concentrated under reduced pressure. The residue was acetylated in a (1:1) mixture of pyridine and acetic anhydride and purified by column chromatography using 20% toluene in ethyl acetate as eluent. The residue (8 mg) was dissolved in methanol and deacetylated (sodium methoxide, pH ~ 9) to give **150** as a white powder; $[\alpha]_D = 46.2^0$ (c 0.5, H_2O); Characteristic data; ^{1}H NMR (360 MHz, CD_3OD) δ: 4.63 (d, 1H, $J_{1',2'} = 8.5$ Hz, H-1'), 3.97 (d, 1H, $J_{2,3} = 9.9$ Hz, H-2), 3.62 (s, 3H, OCH_3), 2.63 (d, 1H, $J_{2a,2b} =$ 13.7 Hz, H-2a), 2.47 (d, 1H, H-2b), 2.07 (s, 3H, NHAc), 1.75-1.25 (m, 4H, propyl), 0.95 (t, 3H, CH_3, propyl); MS (FAB) $[M+Na]^+$ 503.7.

Octyl 4,6-*O*-benzylidene-β-D-galactopyranoside (**154**)



Compound **154** was obtained as white powder; $[\alpha]_D = -23.8^0$ (c 1.0, H_2O); R_f 0.40 in ethyl acetate/hexane, 6:1; ^{1}H NMR (360 MHz, $CDCl_3$) δ: 7.55-7.35 (m, 5H, aromatics), 5.55 (s, 1H, PhC<u>H</u>$(O)_2$), 4.35 (dd, 1H, $J_{5,6a} = 1.5$ Hz, $J_{6a,6b} = 12.5$ Hz, H-6a), 4.30 (d, 1H, $J_{1,2} =$ 7.5 Hz, H-1), 4.10 (dd, 1H, $J_{3,4} = 3.4$ Hz, $J_{4,5} < 1$ Hz, H-4), 3.97 (dt, 1H, $J_{gem} = 9.9$ Hz, $J_{vic} = 6.8$ Hz, OC<u>H</u>$_2$CH$_2$, octyl), 3.78-3.63 (m, 2H, $J_{2,3}=$ H-2, H-3), 3.57-3.44 (m, 2H, H-5, OC$\underline{H}_2$CH$_2$, octyl), 2.60-2.50 (2 x s, br., 2 x OH), 1.7-1.2 (m, 12H, 6 x CH$_2$, octyl), 0.85 (t, 3H, J_{vic} = 7.5 Hz, CH$_3$ octyl); Anal. calcd. for $C_{21}H_{32}O_6$: C 66.62, H 8.47 found: C 66.98, H 8.32.

Inhibition studies

Purified[204] and biotinylated[137] pili were gratefully provided by Dr. R. T. Irvin, Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, AB T6G 2G2, Canada. Compound **9** and compound **132** was generously provided by Dr. O. P. Srivastava (Alberta Research Council). Compound **152** was purchased from Sigma.

Direct Binding Assay:

Polystyrene microtitre plates (Costar, Cambridge, MA) were coated with 100 µl/well of asialo GM$_1$ (Sigma, 5 µg/ml) in methanol. The solvent was evaporated at room temperature inside a fumehood. Non-specific binding sites in the wells were blocked with 100 µl/well of 5% (wt/vol) BSA in PBS. After incubating at 37°C for 1.5 h, the wells were washed three times with PBS (250 µl) supplemented with 0.05% (wt/vol) BSA (Buffer A). Aliquots (100 µl) of various amounts of biotinylated *P. aeruginosa* PAK pili (0, 0.10, 0.30, 0.80, 2.00, 4.00 µg/ml) were added to each well. After 1.5 h incubation at 37°C, the wells were washed three times with 250 µl of Buffer A. One hundred microliters of streptavidin-alkaline phosphatase conjugate (Gibco BRL) at 1:1500 dilution with Buffer A was added to each well. After incubation for 1.5 h at 37°C, the plate was washed three times with 250 µl/well of Buffer A. Then, 125 µl of p-nitrophenyl phosphate substrate solution (1 mg/ml in 10% diethanolamine, pH 9.8) was added to each well. The optical density readings at 405 nm were recorded after 1 and 2 h incubation at room temperature.

Competitive inhibition of *P. aeruginosa* Pili binding to immobilized asialo-GM_1

Polystyrene microtitre plates (Costar, Cambridge, MA) were coated with 100 µl/well of asialo GM_1 (Sigma, 5 µg/ml) in methanol. The solvent was evaporated at room temperature inside a fumehood. Non-specific binding sites in the wells were blocked with 100.00 µl/well of 5% (wt/vol) BSA in PBS. After incubating at 37°C for 1.5 h, the wells were washed three times with PBS (250 µl) supplemented with 0.05% (wt/vol) BSA (Buffer A). Aliquots (100 µl) of biotinylated *P. aeruginosa* PAK pili (0.88 µg/ml, diluted 1:1000 in Buffer A) containing varying concentration of sugar analogs (in the range of 0.6 µM to 300 µM) were added to each well. After 1.5 h incubation at 37°C, the wells were washed three times with 250 µl of Buffer A. One hundred microliters of streptavidin-alkaline phosphatase conjugate (Gibco BRL) at 1:1500 dilution with Buffer A was added to each well. After incubation for 1.5 h at 37°C, the plate was washed three times with 250 µl/well of Buffer A. Then, 125 µl of p-nitrophenyl phosphate substrate solution (1.00 mg/ml in 10% diethanolamine, pH 9.8) was added to each well. The optical density readings at 405 nm were recorded after 1 and 2 h incubation at room temperature. The inhibition results were expressed as the percent inhibition resulting from the addition of sugar analogs as compared with the one without the sugar analog. All incubations were done in triplicates and at least four times repeated. Data are means ± standard deviations of 4-6 separate experiments.

E. Bibliography:

1. A. Varki, *Glycobiology*, **3** (1993) 97-130.
2. R. A. Dwek, *Cem. Rev.*, **96**, (1996) 683-720.
3. G. W. Hart, *Curr. Opin. Cell Biol.*, **4** (1992) 1017-1023.
4. N. Sharon and H. Lis, *Sci. Am.*, **268** (1993) 82-89.
5. B. K. Brandley, *Cell Biology*, **2** (1991) 281-287.
6. H. Schachter, *Trends Glycosci. Glycotech.*, **4** (1992) 241-250.
7. E. R. Vimr, *Trends Microbiol.*, **2** (1994) 271-277.
8. S. Tomlinson, L. C. Pontes de Carvalho and F. Vandekerckhove, *J. Immunol.*, **153** (1994) 3141-3147.
9. W. Colli, *FASEB J.*, **7** (1993) 1257-1264.
10. D. J. Miller, M. B. Macek and B. D. Shur, *Nature* **357** (1992) 589-593.
11. K. Mori, D. Daitoh and M. Irahara, *Am. J. Obstet. Gynecol.*, **161** (1989) 207-221.
12. S. Benoff, I. Hurley and G. W. Cooper, *Hum. Reprod.*, **8** (1993) 2141-2154.
13. M. Metzler, A. Gertz, M. Sarkar, H. Schachter, J. W. Schrader and J. D. Marth, *EMBO*, **13** (1994) 2056-2065.
14. E. Ioffe and P. Stanley, *Proc. Natl. Acad. Sci.*, **91** (1994) 728-732.
15. Q. Zhou and R. D. Cummings In *Cell Surface Carbohydrates and Cell Development*; M. Fukuda Ed.; CRC Press: Boca Raton, 1992; pp. 99-126.
16. R. Lotan In *Glycoconjugates: Composition, Structure, and Function*; H. Allen and E. Kaisailus Eds.; Marcel Dekker, Inc.: New York, 1990; pp. 635-671.
17. R. C. Hughes, *Biochem. Soc. Trans.*, **20** (1992) 279-284.
18. F. L. Harrison, *J. Cell Sci.*, **100** (1991) 9-14.

19. A. Raz and R. Lotan, *Cancer Res.*, **41** (1981) 3642-3647.

20. S. Borman, *Chem. Eng. News*, **71** (1993) 27-34.

21. J. Hodgson, *Biotechnology*, **7** (1991) 609-613.

22. L. A. Lasky, *Science*, **258** (1992) 964-969.

23. J. Travis, *Science*, **260** (1993) 906-908.

24. L. A. Lasky, M. S. Singer, D. Dowbenko, Y. Imai, W. J. Henzel, C. Grimely, C. Fennie, N. Gillet, S. R. Watson and S. D. Rosen, *Cell*, **69** (1992) 927-938.

25. Y. Imai, M. S. Singer, C. Fennie, L. A. Lasky and S.D. Rosen, *J. Cell. Biol.*, **113** (1991) 1213-1222.

26. T. Palabrica, R. Lobb, B. C. Furie, M. Aronovits, C. Benjamin, Y. M. Hsu, S. A. Sajer and B. Furie, *Nature*, **359** (1992) 848-851.

27. K. L. Moore, A. Varki and R.P. McEver, *J. Cell. Biol.*, **112** (1991) 491-499.

28. M. L. Phillips, E. Nudelman, F. C. A. Gaeta, M. Perez, A. K. Singhal, S. Hakomori and J. C. Paulson, *Science*, **250** (1990) 1130-1132.

29. G. Wals, A. Aruffo, W. Kolanus, M. Bevilacqua and B. Seed, *Science*, **250** (1990) 1132-1135.

30. Q. Zhou, K. L. Moore, D. F. Smith, A. Varki, R. McEver and R, Cummings, *J. Cell Biol.*, **115** (1991) 557-564.

31. G. Reuter, S. Kelm and R. Schauer, *Acta Histoch.*, Band XXXVI, (1988) 51-79.

32. M. Petitou and C. A. A. van Boeckel, *Prog. Chem. Org. Nat. Prod.*, **60** (1992) 143-152.

33. F. Goto and T. Ogawa, *Pure Appl. Chem.*, **65** (1993) 793-801.

34. L. Kjellen and U. Lindahl, *Ann. Rev. Biochem.*, **60** (1991) 443-475.

35. D. W. Ohannesian and R. Lotan In *Glycosciences*; H. -J. and S. Gabius Eds.; Chapman & Hall: Weinheim, 1997 pp. 459-469.

36. F. A. Quiocho, *Annu. Rev. Biochem.*, **55** (1986) 287-315.

37. R. U. Lemieux, *Chem. Soc. Rev.*, **18** (1989) 347-374.

38. R. U. Lemieux In *Complex Carbohydrates in Drug Research*, Alfred Benzon Symposium 36; K. Bock, H, Clausen, Eds.; Munksgaard: Copenhagen, 1994; pp. 188-197.

39. R. U. Lemieux In *Proceedings of the VIIIth International Symposium on Medicinal Chemistry* 27-31 Vol. 1; Swedish Pharmaceutical Press: Uppsala, 1985; pp. 329-351.

40. H. Beierbeck, L. T. J. Delbaere, M. Vandonselaar and R. U. Lemieux, *Can. J. Chem.*, **72** (1994) 463-470.

41. F. A. Quiocho, *Pure Appl. Chem.*, **61** (1989) 1293-1306.

42. L. T. J. Delbaere, M. Vandonselaar and L. Prasad, *Can. J. Chem.*, **68** (1990) 1116-1121.

43. R. U. Lemieux, L. T. J. Delbaere and H. Beierbeck In *Host-Guest Molecular Interactions; from Chemistry to Biology* , Ciba Foundation Symposium, 158; Wiley&Sons: Chichester, 1991; pp. 231-248.

44. B. W. Sigurskjold, E. Altman and D. R. Bundle, *Eur. J. Biochem.*, **197** (1991) 239-246.

45. B. W. Sigurskjold and D. R. Bundle, *J. Biol, Chem.*, **267** (1992) 8371-8376.

46. A. Imberty, Y. Bourne and C. Cambillau, *Adv. Biophys. Chem.*, **3** (1993) 71-117.

47 R. U. Lemieux, *Acc. Chem. Res.*, **29** (1996) 373-380.

48. L. Pauling In *The Nature of the chemical Bond*; Cornell University Press (3rd ed.): Ithaca NY, 1992.

49. I. P. Street, K. Rupitz and S. G. Withers, *Biochemistry*, **25** (1986) 6021-6027.

50. P. S. Vermesch, J. J. G. Tesmer and F. A. Quiocho, *J. Mol. Biol.*, **226** (1992) 923-929.

51. P. V. Nikrad, H. Beierbeck and R. U. Lemieux, *Can. J. Chem.*, **70**, (1992) 241-253.

52. F. Schweizer, H. Jiao, O. Hindsgaul, W. Y. Wong and R. T. Irvin, *Can. J. Microbiol.*, **44** (1998) 307-311.

53. R. U. Lemieux, M. -H. Du, and U. Spohr, *J. Am. Chem. Soc.*, **116** (1994) 9803-9804.

54. D. Loganathan, S. E. Osborne and G. D. Glick, *Arch. Biochem. Biophys.*, **299** (1992) 268-274.

55. G. Magnusson, A. Y. A. Chernyak and J. Kihlberg, in *Neoglycoconjugates; Preparation and Applications* (Y. C. Lee and R. T. Lee, eds.) (1994) pp. 53-143, San Diego, Academic Press.

56. K. G. Rice, P. Wu and L. Brand, *Curr. Opinion Struct. Biol.*, **3** (1993) 669-674.

57. M. A. Gallop, R. W. Barrett, W. J. Dower, S. P. A. Fodor and E. M. Gordon, *J. Med. Chem.*, **37** (1994) 1233-1251.

58. M. A. Gallop, R. W. Barrett, W. J. Dower, S. P. A. Fodor and E. M. Gordon, *J. Med. Chem.*, **37** (1994) 1385-1401.

59. N. K. Terrett, M. Gardner, D. W. Gordon, R. J. Kobylecki and J. Steele, *Tetrahedron*, **51** (1995) 8135-8173.

60. L. A. Thompson and J. A. Ellman, *Chem. Rev.*, **96** (1996) 555-600.

61. F. Balkenkohl, C. Bussche-Hunnefeld, A. Lansky and C. Zechel, *Angew. Chem. Int. Ed. Engl.*, **35** (1996) 2288-2299.

62. Z. -G. Wang and O. Hindsgaul, *Glycoimmunology*, **2** (1998) 219-236.

63. a) O. Kanie, F. Barresi, Y. Ding, J. Labbe, A. Otter, L.S. Forsberg, B. Ernst and O. Hindsgaul, *Angew. Chem. Int. Ed. Engl.*, **35** (1995) 2720-2722.

    b) Y. Ding, J. Labbe, O. Kanie and O. Hindsgaul, *Bioorg. Biomed. Chem.*, **4** (1996) 683-692.

64. R. Liang, L. Yan, J. Loebach, M. Ge, Y. Uozumi, K. Sekanina, N. Horan, J. Gildersleeve, C. Thompson, A. Smith, K. Biswas, W. C. Still and D. Kahne, *Science*, **274** (1996) 1520-1522.

65. G. J. Boons, B. Heskamp and F. Hout, *Angew. Chem. Int. Ed. Engl.*, **35** (1996) 2845-2847.

66. K. Heyns and H. Paulsen, *Chem. Ber.*, **88** (1955) 188-195

67. K. Isono, K. Asahi and S. Suzuki, *J. Am. Chem. Soc.*, **91** (1969) 7490-7494.

68. J. L. Fox, Y. Kuwada and K. A. Watanabe, *Tetrahedron Lett.*, **57** (1968) 6029-6032.

69. F. W. Lichtenthaler, T. Morino and H. M. Menzel, *Tetrahedron Lett.*, **1975** 665-668.

70. E. -F. Fuchs and J. Lehmann, *Chem. Ber.*, **108** (1975) 2254-2260.

71. E. -F. Fuchs and J. Lehmann, *J. Carbohydr. Res.*, **49** (1976) 267-273.

72. H. Kessler and E. Graf von Roedern, *Angew. Chem. Int. Ed. Engl.*, **33** (1994) 687-689.

73. O. Frey, M. Hoffmann and H. Kessler, *Angew. Chem. Int. Ed. Engl.*, **34** (1995) 2026-2028.

74. E. Graf von Roedern, E. Lohof, G. Hessler, M. Hoffmann and H. Kessler, *J. Am. Chem. Soc.*, **118** (1996) 10156-10167.

75. J. P. Mc Devitt and P. T. Jr. Lansbury, *J. Am. Chem. Soc.*, **118** (1996) 3818-3828.

76. J. Yoshimura, H. Ando, S. Tsuchida and H. Hashimoto, *Bull. Chem. Soc. Jap.*, **49** (1976) 2511-2514.

77. D. D. Apostopoulos, E. A. Couladouros and M. P. Georgiadis, *Liebigs Ann. Chem.*, **1994** 781-789.

78. L. Poitout, Y. Le Merrer and J. -C. Depezay, *Tetrahedron Lett.*, **36** (1995) 6887-6890.

79. C. Muller, E. Kitas and H. P. Wessel, *J. Chem. Soc. Chem. Commun.*, **1995** 2425-2426.

80. H. P. Wessel, C. M. Mitchell, C. M. Lobato and G. Schmid, *Angew. Chem. Int. Ed. Engl.*, **34** (1995) 2712-2713.

81. Y. Suhara, J. E. K. Hildreth and Y. Ichikawa, *Tetrahedron Lett.*, **37** (1996) 1575-1578.

82. Y. Suhara, M. Ichikawa J. E. K. Hildreth and Y. Ichikawa, *Tetrahedron Lett.*, **37** (1996) 2549-2552.

83. J. Gervay, T. M. Flaherty and C, Nguyen, *Tetrahedron Lett.*, **38** (1997) 1493-1496.

84. R. A. Jr. Goodnow, A. -R. Richou and S. Tam *Tetrahedron Lett.*, **38** (1997) 3195- 3198.

85. R. A. Jr. Goodnow, S. Tam, D. L. Pruess and W. W. McComas, *Tetrahedron Lett.*, **38** (1997) 3199-3202.

86. S. Sabesan, *Tetrahedron Lett.*, **38** (1997) 3127-3130.

87. P. S. Ramamoorthy and J. Gervay, *J. Org. Chem.*, **62** (1997) 7801-7805.

88. M. J. Sofia, R. Hunter, T. Y. Chan, A. Vaughan, R. Dulina, H. Wang and D. Gange, *J. Org. Chem.*, **63** (1998) 2802-2803.

89. K. C. Nicolaou, H. Floerke, M. Egan, T. Barth and V. A. Estevez, *Tetrahedron Lett.*, **36** 1995 1775-1778.

90. J. M. Kim and R. Roy, *Carbohydr. Lett.*, **1** (1996) 465-468.

91. I. Ugi, *Angew. Chem. Int. Ed. Engl.*, **21** (1982) 810-819.

92. D. P. Sutherlin, T. M. Stark, R. Hughes and R.W. Armstrong, *J. Org. Chem.*, **61** (1996) 8350-8354.

93. W. K. Park, M. Auer, H. Jaksche and C. H. Wong, *J. Am. Chem. Soc.*, **118** (1996) 10150-10155.

94. U. J. Nilsson, E. J. -L. Fournier and O. Hindsgaul, *Bioorg. Med. Chem.*, **6** (1998) 1563-1575.

95. R. Hirschmann, K. C. Nicolaou, S. Pietranico, E. M. Leahy, J. Salvino, B. Arison, M. A. Cichy, P. G. Spoors, W. C. Shakespeare, P. A. Sprengler, P. Hamely, A. B. III. Smith, T. Reisine, K. Raynor, L. Maechler, C. Donaldson, W. Vale, R. M. Freidinger, M. R. Cascieri and C. D. Strader, *J. Am. Chem. Soc.*, **115** (1993) 12550-12568.

96. P. Brazeau, W. Vale, R. Burgus, N. Ling. M. Butcher, J. Rovier and R. Guillemin, *Science*, **179** (1973) 77-79.

97. D. F. Veber, F. W. Holly, W. J. Paleveda, R. F. Nutt, S. J. Bergstrand, M. Torchiana, M. S. Glitzer, R., Saperstein and R. Hirschmann, *Proc. Natl. Acad. Sci. U.S.A.*, **75** (1978) 2636-2640.

98. B. H. Arison, R. Hirschmann and D. F. Veber, *Bioorg. Chem.*, **7** (1978) 447-451.

99. B. H. Arison, R. Hirschmann, W. J. Pavleda, S. F. Brady and D. F. Veber, *Biochem. Biophys. Res. Commun.*, **100** (1981) 1148-1153.

100. D. F. Veber, R. M. Freidinger, D. S. Perlow, W. J. Paleveda, F. W. Holly, R. G. Strachan, R. F. Nutt, B. H. Arison, C. Homnick, W. C. Randall, M. S. Glitzer, R. Saperstein and R. Hirschmann, *Nature*, **292** (1981) 55-58.

101. R. A. Garibaldi, M. R. Britt, M. L. Coleman, J. C. Reading and N. L. Pace, *J. Am. Med.*, **70** (1981) 677-680.

102. R. E. Woods, T. F. Boat and C. F. Doershuk, *Am. Rev. Respir. Dis.*, **113** (1976) 833-878.

103. B. G. McMillan, *Surg. Clin. N. Am.*, **60** (1980) 185-196.

104. H. H. Stone, *Ann. Surg.*, **163** (1966) 297-305.

105. R. G. Doggett In *Pseudomonas aeruginosa*; *Clinical Manifestations of Infection and Current Therapy*; R. G. Doggett Ed.; Academic Press: New York, 1979; pp 1-8.

106. N. Hoiby, *Acta Pathol. Microbiol .Scand. Sect. B*, **82** (1974) 551-558.

107. E. H. Beachey, *J. Inf. Dis.*, **143** (1981) 325-345.

108. D. E. Woods, D. C. Straus, W. G. Johanson Jr., V. K. Berry and J. A. Bass, *Infect. Immun.*, **29** (1980) 1146-1151.

109. P. Doig, T. Todd, P. A. Sastry, K. K. Lee, R. S. Hodges, W. Paranchych and R. T. Irvin, *Infect. Immun.*, **56** (1988) 1641-1646.

110. A. Linker and R. S. Jones, *J. Biol. Chem.*, **241** (1966) 3845-3851.

111. R. Ramphal and G. B. Pier, *Infect. Immun.*, **47** (1985) 1-4.

112. P. Doig, N. R. Smith, T. Todd and R. T. Irvin, *Infect. Immun.*, **55** (1987) 1517-1522.

113. N. R. Baker, V. Minor, C. Deal, M.S. Shahrabadi, D. A. Simpson and D. E. Woods, *Infect. Immun.*, **59** (1991) 2859-2863.

114. C. A. Lingwood, M. Cheng, H. C. Krivan and D. Woods, *Biochem. Biophys. Res. Commun.*, **175** (1991) 1076-1081.

115. L. Saiman, K. Ishimoto, S. Lory and A. Prince, *Infect. Immun.*, **57** (1989) 2764-2770.

116. R. Ramphal, L. Koo, K. S. Ishimoto, P. A. Totten, J. C. Lara and S. Lory, *Infect. Immun.*, **59** (1991) 1307-1311.

117. D. A. Simpson, R. Ramphal and S. Lory, *Infect. Immun.*, **60** (1992) 3771-3779.

118. J. Glick and N. Garber, *Gen. Microbiol.*, **129** (1983) 3085-3090.

119. D. W. McEachran and R. T. Ivin, *Can. J. Microbiol.*, **31** (1985) 563-569.

120. R. T. Irvin In *Pseudomonas aeruginosa as an oppurtunistic pathogen*; M. Campa Ed.; Plenum Press: New York, 1993; pp. 19-42.

121. R. T. Irvin, P. Doig, K. K. Lee, P. A. Sastry, B. Heller and W. Paranchych, *Microb. Ecol . Health Dis.*, **3** (1990) 39-47.

122. M. A. Farinha, B. D. Conway, L. M. G. Glasier, N. W. Ellert, R. T. Irvin, R. Sherburn and W. Paranchych, *Infect, Immun.*, **62** (1994) 4118-4123.

123. H. B. Sheth, K. K. Lee, W. Y. Wong, G. Srivastava, O. Hindsgaul, R. S. Hodges, W. Paranchych and R. T. Irvin, *Mol. Microbiol.*, **11** (1994) 715-723.

124. D. E. Bradley, *Genetical Res.*, **19** (1972) 39-51.

125. W. F. Folkhard, D. A. Marvin, T. H. Watts and W. Paranchych, *J. Mol. Biol.*, **149** (1981) 79-93.

126. W. Paranchych, P. A. Sastry, K. Volpel, B. A. Loh and D. P. Speert, *Clin. Invest. Med.*, **9** (1986) 113-118.

127. T. H. Watts, C. M. Kay and W. Paranchych, *Biochemistry*, **22** (1983) 3640-3646.

128. P. A. Sastry, J. R. Pearlstone, L. B. Smillie and W. Paranchych, *FEBS Lett.*, **151** (1983) 253-256.

129. W. Paranchych, B. L. Pasloke and P. A. Sastry In *Pseudomonas: Biotransformations, Pathogenesis and Evolving Biotechnology*; S. Sliver, A. M. Chakrabarty, B.B.H. Iglewski and S. Kaplan, Eds.; American Society of Microbiology: Washington, D. C., 1990; pp. 343-351.

130. W. Paranchych and L. S. Frost, *Adv. Microb. Physiol.*, **29** (1988) 53-113.

131. T. C. Elleman, *Microbiol. Rev.*, **52** (1988) 233-247.

132. R. Faast, M. A. Ogierman, U. H. Stroeher and P. A. Manning, *Gene*, **85** (1989) 227-231.

133. R. Ramphal, J. Sadoff, J. C. Pyle and J. D. Silipigni, *Infect. Immunol.*, **44** (1984) 38-40.

134. P. Doig, P. A. Hodges, R. S. Hodges, K. K. Lee, W. Paranchych and R. T. Irvin, *Infect. Immunol.*, **58** (1990) 124-130.

135. K. K. Lee, P. Doig, R. T. Irvin, W. Paranchych and R. S. Hodges, *Mol. Microbiol.*, **3** (1989), 1493-1499.

136. R. T. Irvin , P. Doig, K. K. Lee, P. A. Sastry, W. Paranchych, T. Todd and R. S. Hodges, *Infect. Immunol.*, **57** (1989) 3720-3726.

137. K. K. Lee, H. B. Sheth, W. Y. Wong, R. Sherburne, W. Parancych, R. S. Hodges, C. A. Lingwood, H. Krivan and R. T. Irvin, *Mol. Microbiol.*, **11** (1994) 705-713.

138. C. McInnes, C. M. Kay, R. S. Hodges and B. D. Sykes, *Biochemistry*, **32** (1993) 13432-13440.

139. A. P. Campbell, C. McInnes, R. S. Hodges and B. D. Sykes, *Biochemistry*, **34** (1995) 16255-16268.

140. A. P. Campbell, H. B. Sheth, R. S. Hodges and B. D. Sykes, *Int. J. Pept. Protein Res.*, **48** (1996) 539-552.

141. N. Baker, G. C. Hansson, H. Leffler, G. Riise and C. Svanborg-Eden, *Infect. Immun.*, **58** (1990) 2361-2366.

142. P. Doig, W. Paranchych, P. A. Sastry and R. T. Irvin, *Can . J. Microbiol.*, **35** (1989) 1141-1145.

143. L. Saiman and A. Prince, *J. Clin. Invest.*, **92** (1993) 1875-1880.

144. H. C. Krivan, D. D. Roberts and V. Ginsburg, *Proc. Natl. Acad. Sci. USA*, **85** (1988) 6157-6161.

145. J. Kihlberg, S. J. Hultgren, S. Normark and G. Magnusson, *J. Am. Chem. Soc.*, **111** (1989) 6364-6368.

146. T. L. Lowary and O. Hindsgaul, *Carbohydr. Res.*, **249** (1993) 163-195.

147. D. M. Percival and S. G. Withers, *Biochemistry*, **31** (1992) 505-512.

148. M. C. Chervenak and E. J. Toone, *J. Am. Chem. Soc.*, **116** (1994) 10533-10539.

149. M. M. Palcic, L. D. Heerze, M. Pierce and O. Hindsgaul, *Glycoconjugate J.*, **5** (1988) 49-63.

150. R. Dehmlow, *Angew. Chem. Int. Ed. Engl.*, **16** (1977) 493-505.

151. C. K. De Bruyne and G, van der Groen, *Carbohydr. Res.*, **25** (1972) 59-65.

152. H. M. Zuzumond, P. A. M. van der Klein, G. A. van der Marel and J. H. van Boom, *Tetrahedron Lett.*, **33** (1992) 2063.

153. G. Zemplén, *Ber.*, **60** (1927) 1555-1564.

154. O. Kanie, S. Crawley, M. M. Palcic and O. Hindsgaul, *Carbohydr. Res.*, **243** (1993) 139-164.

155. W. M. Pearlman, *Tetrahedron Lett.*, **17** (1967) 1663-1664.

156. K. M. Nicholas, *J. Am. Chem. Soc.*, **107** (1985) 4999-5001.

157. M. Meldal and K. J. Jessen, *Chem. Commun.*, 1990 483-485.

158. T. H. Watts and C. M. Kay, Can. J. Biochem., **60** (1982) 867-872.

159. A. R. Ferscht, J. P. Shi, J. Knill-Jones, D. M. Lowe, A. J. Wilkinson, D. M. Blow, P. Brick, P. Carter, M. M. Waye and G. Winter, *Nature*, **314** (1985) 235-238.

160. I. J. Goldstein and R.D. Poretz In *The Lectins*; I. E. Liener and N. Sharon. I. J. Goldstein Eds.; Academic Press: London, 1986; pp. 35-244.

161 H. Dietrich and R. R. Schmidt, *Carbohyr. Res.*, **250** (1993) 161-164.

162. R. B. Moffett, *Org. Synth.*, **4** (1963) 652-653.

163. J. H. Clark, J. M. Miller and K. -H. So, *J. Chem. Soc.*, *Perkin Trans.*, **1**, (1978), 941-946.

164. P. M. Collins and R. J. Ferrier In *Monosaccharides*; John Wiley&Sons: Chichester, 1995; 119-120.

165. J. C. Swoden, *Adv. Carbohydr. Chem.*, **6** (1951) 291-318.

166. R. Gigg and C. D. Warren, *J. Chem. Soc. C*, **15** (1968) 1903-1911.

167. X. Wang and P. H. Gross Gross, *Liebigs Ann.*, **1995** 1367-1370.

168. T. F. Tam and B. Fraser-Reid, *J. Org. Chem.*, **45** (1980) 1344-1346.

169. J. Dupuis, B. Giese, J. Hartung and M. Leising, *J. Am. Chem. Soc.*, **107** (1985) 4332-4333.

170. M. P. Dillon, H. Maag and D. M. Muszynski, *Tetrahedron Lett.*, **36** (1995) 5469-5470.

171. H. Streicher, A. Geyer and R. Schmidt, *Chem. Eur. J.*, **2** (1996) 502-510.

172. A. Dondoni, M. -C. Scherrmann, A. Marra and J. -L. Delephine, *J. Org. Chem.*, **59**, 7517-7520.

173. K. Tamooka, H. Yammamoto and T. Nakai, *J. Am. Chem. Soc.*, **118** (1996) 3317-3318.

174. R. R. Schmidt, M. Behrendt and A. Teopfer, *Synlett*, (**1990**) 694-696.

175. I. Tavorska and F. R. Taravel, *Adv. Carbohydr. Chem. Biochem.*, **51** (1995) 15-61.

176. K. C. Nicolaou, R. E. Dolle, D. P. Papahatjis and J. L. Randall, *J. Am. Chem. Soc.*, **106** (1984) 4189-4192.

177. S. Hanessian, C. Bacquet and N. Lehong, *Carbohydr. Res.*, **80** (1980) C17-C22.

178. C. Modena In *The Chemistry of the Thiol Group*; S. Patai Ed.; Wiley&Sons: New York, 1974; pp. 785-839.

179. H. Paulsen, *Angew. Chem. Int. Ed. Engl.*, **21** (1982) 155-173.

180. J. P. Horwiz, A. J. Tomson, J. A. Urbanski and J. Chua, *J. Org. Chem.*, **27** (1962) 3045-3048.

181. D. R. Williams, P. L. Brown and J. W. Benlow, *J. Am. Chem. Soc.*, **111** (1989) 1923-1925.

182. K. Luthman, M. Orbe, T. Waglund and A. Claesson, *J. Org. Chem.*, **52** (1987) 3777-3784.

183. E. Atherton and R. C. Sheppard In *The Fluorenylmethoxycarbonyl Amino Protecting Group in the Peptides*; S. Udenfried and J. Meienhofer Eds.; Academic Press: Orlando Fl, Vol. 9, 1987; pp. 1-38.

184. I. Schön and L. Kisfaludy, *Synthesis*, **1986** 327-333.

185. C. C. Lenzof, *Acc. Chem. Res.*, **11** (1978) 327-333.

186. J. J. Ritter and P. P. Minieri, *J. Am. Chem. Soc.*, **70** (1948) 4045-4048.

187. A. J. Ratcliffe, P. Konradsson and B. Fraser Reid, *J. Am. Chem. Soc.*, **112** (1990) 5665-5667.

188. J. Podlech and D. Seebach, *Angew. Chem. Int. Ed. Engl.*, **34** (1995) 471-472.

189. C. N. C. Drey and E. Mietwa, *J. Chem. Soc. Perkin* Trans., **1** (1982) 1587-1592.

190. A. A. Bothner-By, R. L. Stephens, J. Lee, C. D.Warren and R.W. Jeanloz, *J. Am. Chem. Soc.*, **106** (1984) 811-813.

191. T. -L. Hwang and A. J. Shaka, *J. Magn. Reson. Ser. B*, **102** (1993) 155-165.

192. H. Greeen and R. Freeman, *J. Magn. Reson.*, **93** (1991) 93-141.

193. U. Koert, *Angew. Chem. Int. Ed. Engl.*, **36** (1997) 1836-1837.

194. P. Perlmutter in *Conjugate Addition Reactions in Organic Synthesis*, Tetrahedron Organic Chemistry Series Vol. 9, (1992) pp. 1-373, Oxford, England: Pergamon Press.

195. J. M. Lancelin, P. H. A. Zollo and P. Sinay, *Tetrahedron Lett.*, **24** (1983) 4833-4836.

196. C. S. Wilcox and M. D. Covart, *Carbohydr. Res.*, **171** (1987) 141-160.

197. A. Hasegawa, T. Nagahara, H. Ohki and M. Kiso, *J. Carbohydrate Chem.*, **11**, (1992) 699-714.

198. R. U. Lemieux, Z. Ablas, H. Burzynska and R. M. Ratcliffe, *Can. J. Chem.*, **60** (1982) 63-68.

199. Compounds 36, 37 and 38 were synthesized by Mr. H. Jiao at the Department of Chemistry, University of Alberta, Edmonton.

200. A. Dondoni and M. -C. Scherrmann, *J. Org. Chem.*, **59** (1994) 6404-6412.

201. A. J. Duggan, M. A. Adams and J. Meinwald, *Tetrahedron Lett.*, **19** (1978) 4327-4330.

202. J. C. Chabala and J. F. Vincent, *Tetrahedron Lett.*, **19** (1978) 937-940.

203. Z. J. Witczak, J. Sun and R. Mielguj, *Bioorg. Med. Chem. Letters* , **5** (1995) 2169-2174.

204. W. Paranchych, P. A. Sastry, L. S. Frost, M. Carpenter, G. D. Armstrong and T. H. Watts, *Can. J. Microbiol.*, **25** (1979) 1175-1181.