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Synthetic tools for carbohydrate-protein interaction studies

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Synthetic tools for carbohydrate-protein interaction studies

A thesis submitted to
Bangor University in candidature
for the degree of

Doctor of Philosophy

In the

School of Chemistry

by

Viviane Fournière

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Elsevier for kind reprint permission.

Pensée spéciale à tous ceux qui n'en auront jamais vu la fin.

Abstract

Carbohydrate-protein interactions are important for the tuning of many biological processes. Tools are required to investigate the carbohydrate recognition domains. Several examples are presented in this work.

The first project consists in the synthesis of neoglycoconjugates for binding studies. Here a simple and efficient route to functionalise chitobiose, chitotriose and chitotetraose with a suitable linker avoiding protecting group chemistry is described. Unprotected chitooligomeres were used as starting material. After amination of the anomeric position of the saccharides, the linker was attached by amide formation. An amide bond was chosen to mimic the peptide bond.

Two projects discuss the synthesis of a pentasaccharide and a heptasaccharide. Using as many identical building and reactions as possible in both the synthesis, the oligosaccharides was assembled following a linear pathway. For the pentasaccharide synthesis, a convergent pathway was attempted first. Starting respectively with a monosaccharide or a disaccharide, each sugar was added after the other, usually followed by a protecting group manipulation. Experience gained during the pentasaccharide synthesis for protecting group manipulation, strategic group pattern and glycosylation was used for the heptasaccharide synthesis. 1,2-*cis* glycosylation was improved via a new

one-pot procedure. At the end, a hydrogenolysis provided the fully deprotected target molecule.

The last project deals with selenoglycosides. They are intended to improve structure elucidation of lectins by X-Ray crystallography. Since both α and β selenoglycosides are of interest, a route to the two anomers using a common precursor was investigated. The β-selenoglycosides of common mono- and disaccharides were prepared. The respective glycosyl halides were added to the "in situ" reduced dimethyldiselenide. The anomerisation of the β anomers were performed using $BF_3 \cdot OEt_2$. This route was applied on a H1/Lewis^b determinant as well. The major problem in this part of the project was to obtain the β orientation of the selenomethyl group without using a participating group. A solution was eventually found by employing a large excess of the reducing agent during the introduction of the seleno group as a smaller excess led to an α/β mixture.

Contents

List of paper and contribution report

This thesis is partially based on the following papers I and II. Chapter 3 refers to paper I and its supplementary material. Chapter 4 refers to paper II.

I. **Synthesis of the Lewis b pentasaccharide and a HSA-conjugate thereof**. Fournière, Viviane; Skantz, Linnéa; Sajtos, Ferenc; Oscarson, Stefan; Lahmann, Martina. *Tetrahedron*, **2010**, 66(39), 7850-7855.

II. **Synthesis of the B-Lewis b heptasaccharide using a versatile lacto-N-tetraose intermediate**. Fournière,Viviane; Skantz, Linnéa; Oscarson, Stefan; Lahmann, Martina. *Manuscript*.

The author wishes to clarify her contributions to the paper I and II.

I. Performed all synthesis work, except HSA conjugation. Characterised all new compounds. Contributed partly to the writing of the manuscript.

II. Performed all synthesis work except compound 3. Characterised all new compounds. Contributed partly to the writing of the manuscript.

Abbreviations

Ac	acetyl
AFM	atomic force microscopy
AgOTf	silver trifluoromethyl sulfonate
BabA	blood group antigen bindin adhesin
BDA	2,3-butane diacetal
Bn	benzyl
BSA	bovine serum albumin
Bu	butyl
CSA	camphorsulfonic acid
CRD	carbohydrate recognition domains
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DMTST	dimethylsulfonium trifluoromethyl sulfonate
DSS	disuccinimidyl suberate
EDA	ethylene diamine
Et	ethyl
Fuc	fucose
Gal	galactose
Glc	glucose
HOBt	hydroxybenzotriazole
Lac	lactose

LNT	lacto-*N*-tetraose
MAD	multiple-wavelength anomalous diffraction
MALDI-TOF	matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry
Man	mannose
Me	methyl
MS	mass spectrometry
NHS	*N*-hydroxy succinimide
NIS	*N*-iodo succinimide
NMR	nuclear magnetic resonance
Ph	phenyl
pTSA	*p*-toluenesulfonic acid
R_f	retention factor
RT	room temperature
SAD	single-wavelength anomalous diffraction
SAM	self-assembled monolayer
SPR	surface plasmon resonance
TBAB	*tert* butyl ammonium bromide
tEG	tetraethylene glycol
TFA	trifluoroacetic acid
THAP	trihydroxy-acetophenone monohydrate
THF	tetrahydrofuran
TLC	thin layer chromatography

1 Introduction

1.1 Carbohydrates in General

Together with proteins, lipids and nucleic acids, carbohydrates are biomolecules and are the most abundant class of organic compounds found in any living system. Carbohydrates are the major source of metabolic energy, both for plants and animals, and they form the supporting material for plants and crustaceans.[1]

Carbohydrate comes from "hydrated carbon" describing a family of compound with $C_n(H_2O)_n$ as empirical formula. Carbohydrates are simple organic compounds. They are aldehydes or ketones with many hydroxyl groups added to the residual chain (Fig 1 **1**, **2**, **3**). Other functional groups like the amino group (Fig 1 **4**), are common. A monosaccharide is assigned to the D or the L series according to the configuration at the highest-numbered centre of chirality (Fig 1 **6**, **7**). Carbohydrates are compounds with several stereocentres, and thus this class of substances consists of a large number of stereoisomers. The monosaccharides prefer to cyclise into 5- or 6-membered rings (Fig 1 **2**, **3**), using the carbonyl function to form hemiacetals. The resulting new stereogenic center, the anomeric center, has a special reactivity and can be involved in reaction without affecting the other hydroxyl groups. The two anomers are called α and β. For hexopyranose systems, the highest asymmetrically substituted

carbon atom is called the 'configurational atom'. In the α anomer, the exocyclic oxygen atom at the anomeric centre is formally cis, in the Fischer projection (Fig 1 **1**), to the oxygen attached to the anomeric reference atom, the first atom out of the ring; in the β anomer these oxygen atoms are formally trans (Fig 1 **5**, **6**).[2]

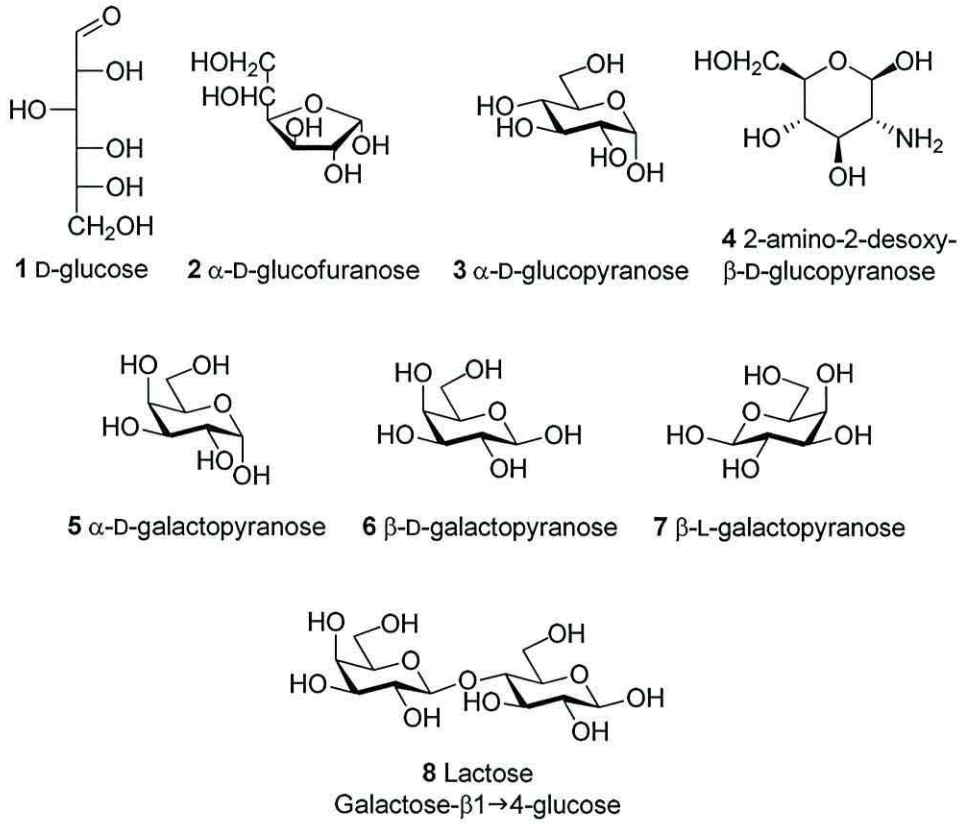


Figure 1 Typical carbohydrate structures.

Carbohydrates are found as mono-, oligo- or polysaccharides (Fig 1, **8**) and are often associated with proteins and lipids to form glycoconjugates as glycoproteins or glycolipids (Fig 2). Those structures are frequently

displayed on cell surface for interaction with the surrounding environment. They are involved in biological processes and are target for the development of pharmaceuticals like vaccines[3] and inhibitors[4, 5].

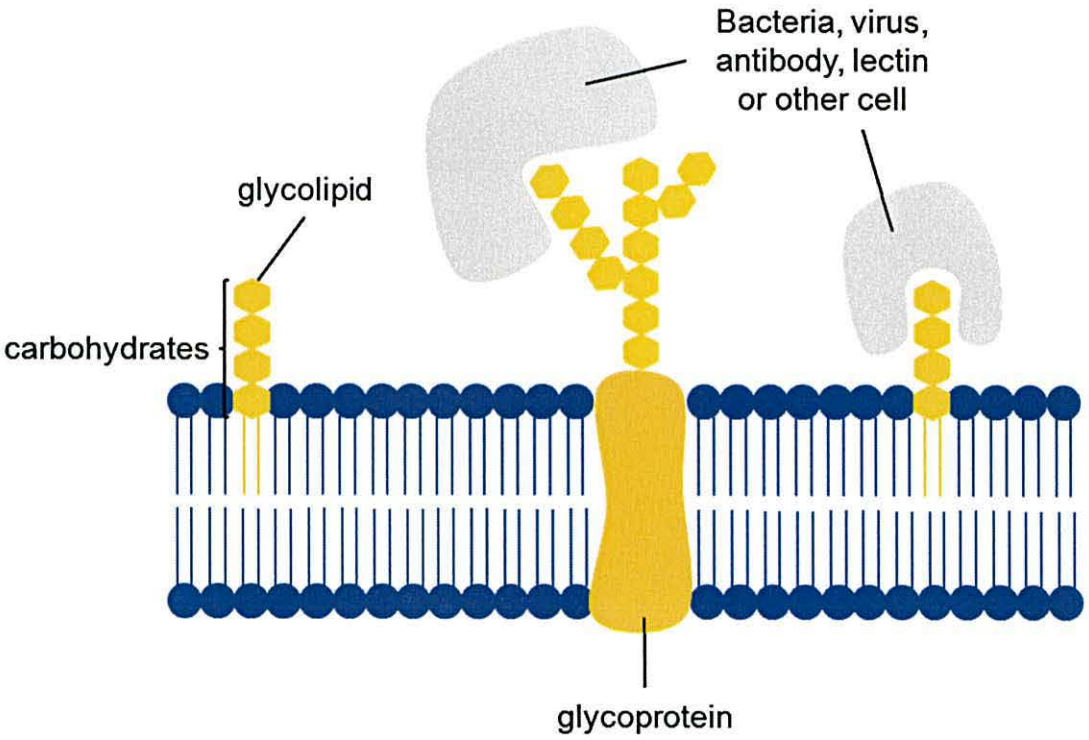
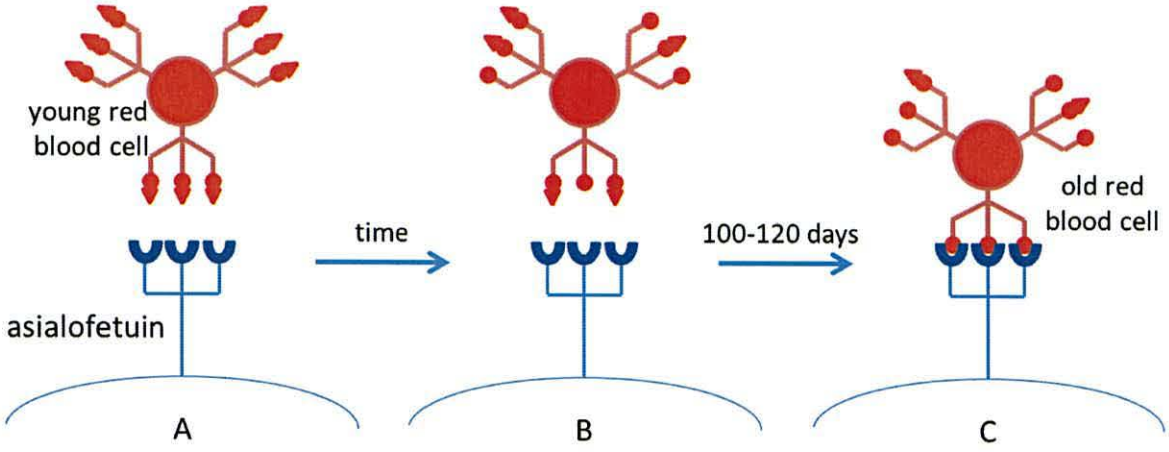


Figure 2 Carbohydrate recognition, adapted from 6.

1.2 Carbohydrate-Protein Interactions

All the target molecules in this thesis are synthesised to be used in interaction with proteins. There are two major types of interactions between proteins and carbohydrates. The first one involves enzymes. This type of interaction results in the modification of the sugar moiety. The other

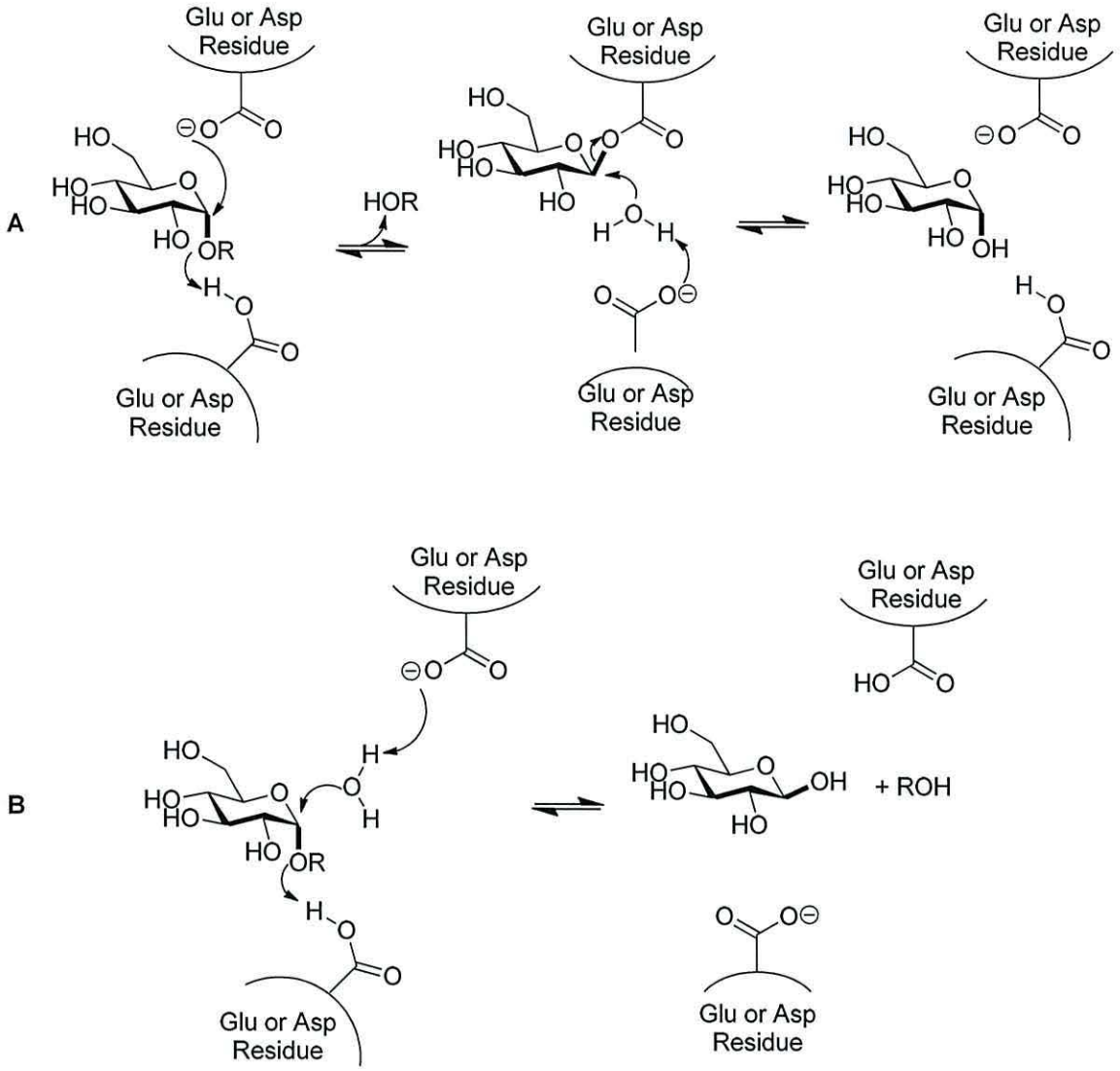
interaction can be described as recognition action, often for the purpose of fine tuning a biological process by also employing multivalent effects (Scheme 1). In this case, the carbohydrate molecule binds to a protein, a so called lectin. The binding process can initiate a number of changes but the carbohydrate moiety will be not chemically changed.



Scheme 1 The multivalent effect on erythrocyte ageing: A. Asialofetuin does not recognise a young red blood cell; B. With time, erythrocytes lose terminal sialic acids revealing galactoses to be presented to the receptor. Asialofetuin can recognise those galactoses selectively but the single binding event is too weak, thus the cell is considered not old enough and released back into the blood stream. C. An old red blood cell offers 3 galactoses with the correct geometry to occupy all binding sites in the receptor. This multivalent recognition is much stronger, retaining the cell to be removed from the blood stream, internalised and degraded. Adapted from 7.

1.2.1 Enzymes

There are three different classes of enzymes using carbohydrate substrates: the glycoside hydrolases (or glycosidases), the glycoside kinases and glycoside transferases. Glycoside hydrolases are for instance found in the intestinal tract and in saliva where they degrade bulk carbohydrates such as starches, lactose, and other food derived saccharides. They are also involved in the biosynthesis and degradation of glycogen[8] in the body. After recognition of a glycoside substrate, glycosidases catalyse the hydrolysis of the glycosidic linkage. Common substrates for glycoside hydrolases are polysaccharides. The sugar chain can be degraded either from the terminal unit, furthest apart from the reducing end by *exo* glycosidases or within the saccharide chain by *endo* glycoside hydrolases.[9] The glycoside bond can be cleaved either with inversion or retention of the anomeric configuration (Scheme 2). After hydrolysis of the glycoside bonds, the two products are released.



Scheme 2 Mechanism of retaining (**A**) and inverting (**B**) glycosidases, redrawn from 10.

Glycoside kinases are also known as phosphoryltransferases. A kinase is a type of enzyme that transfers phosphate groups from high-energy donor molecules, such as ATP, to specific substrate molecules.[11] In the case of glycoside kinases, the substrate is a carbohydrate molecule. For instance, glucokinase is an enzyme that facilitates phosphorylation of glucose to

glucose-6-phosphate.[12] Glucokinases can be found in cells in the liver, pancreas, gut and brain of humans and most other vertebrates.[13]

Glycosyltransferases are enzymes that act as catalysts for the transfer of a monosaccharide unit from an activated sugar phosphate (known as the "glycosyl donor") to an acceptor molecule, e.g. an alcohol. The result of glycosyl transfer can be a monosaccharide glycoside, an oligosaccharide, or a polysaccharide. Glycosyl transfer can also occur to protein residues, usually to tyrosine, serine or threonine to give *O*-linked glycoproteins, or to asparagine to give *N*-linked glycoproteins. Glycosyltransferases, by analogy with glycoside hydrolases, can catalyse the transfer of a glycosyl moiety with either retention or inversion of configuration. A few glycosyltransferases are used in chemoenzymatic synthesis.[14] They are relatively expensive but their selectivity makes them valuable tools, for example to introduce sugar units at specified positions on an unprotected oligosaccharide.

1.2.2 Lectins

Lectins are small non-enzymatic proteins. They are carbohydrate-recognition proteins and glycoproteins which interact with sugars through specific sites called “carbohydrate recognition domains” or CRD, which are highly specific for their sugar moieties. Carbohydrates contain biological information in their 3-dimensional structures, and this is of importance for

cell-cell adhesion and cell communication. In these processes, the carbohydrate portion of the glycoconjugates functions as ligands for those specialised proteins. In this ligand-receptor interaction, non-covalent carbohydrate-protein complexes are formed. In contrast to enzymes, the chemical structure of a sugar moiety is not changed by a lectin. For example, calreticulin and calnexin are homologous lectins. They ensure that the proteins are correctly folded.[15]

The term lectin was first proposed in 1954[16] and derived from the Latin verb "legere" which means "to pick out", "to choose" or "to select". It refers to the high selectivity and specificity of the recognition. The most widely accepted definition of the term lectin was proposed in 1980[17] and adopted by the nomenclature committee of the International Union of Biochemistry. This definition states that a lectin is "a carbohydrate binding protein of non-immune origin that agglutinates cells and/or precipitates polysaccharides or glycoconjugates".

Lectins are either attached to membranes or form soluble entities. Whereas the calreticuline is a soluble protein, the calnexin is bond to a membrane.[18] The protein structure and the details of recognition sites can be studied using the data obtained from X-ray crystallography, after protein crystallisation in presence of the substrate. It can be very tedious to find suitable crystallisation conditions for a soluble lectin; however, crystallisation of membrane bond lectins is rarely successful. Thus, other means for the investigation of membrane bond lectins have to be used. Lectins can bind to a soluble carbohydrate or to a carbohydrate moiety as

part of a glycoprotein or glycolipid. They are found in most of the organisms and are involved in various biological processes, like cell adhesion, inflammation, protein immunomodulation, folding.

Certain carbohydrates can also be antigens recognised by antibodies.[19] Antibodies are proteins used by the immune system to identify and neutralise foreign objects, such as bacteria and viruses. Carbohydrate structures are targets for vaccination because unique cells surface carbohydrates act as biological markers. These antigenic carbohydrates may be used to induce a specific immune response that prevents infection. Vaccines are already approved for human use.[3]

1.3 Oligosaccharide synthesis

Assembling monosaccharides in order to build larger carbohydrate structures with control of the sterochemistry is of importance. Well-defined structures are necessary for carbohydrate-protein interaction studies as the recognition can be highly selective. Those structures are formed by creating new *O*-glycosidic linkages. Due to the significance of this linkage for the overall biological properties and the difficulties in making this bond, numerous synthetic approaches have been investigated since the first reactions were performed, more than a century ago, by König and Knorr.[20, 21]

To successfully form a glycosidic linkage with high stereoselectivity between two monosaccharides, two major aspects have to be investigated. The first is the choice of the glycosyl donor, more precisely its possible leaving group and the corresponding promoter system.[20] The second is the selection of the protecting group pattern for both the donor and the acceptor.[22] Other reaction conditions such as temperature, pH, and the solvent also influence the outcome of the glycosylation reaction.[23, 24]

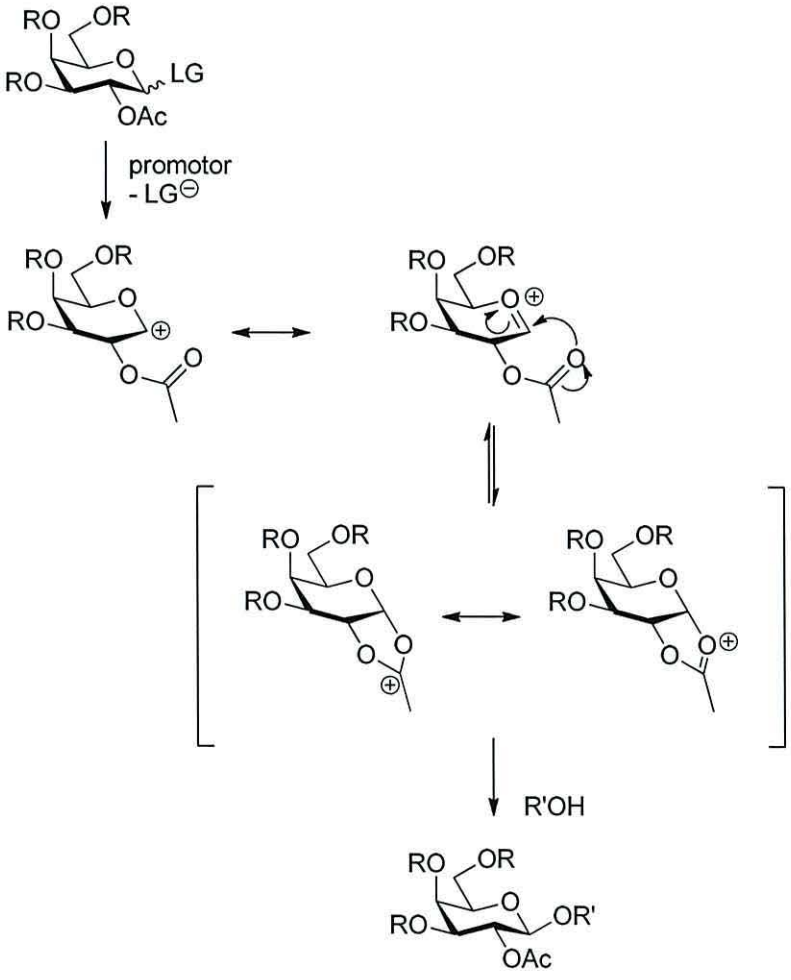
1.3.1 Protecting groups and neighbouring-group participation

Regioselectivity is a major issue in carbohydrate chemistry because the differentiation of the hydroxyl groups can be difficult, and has to be considered both when synthesising building blocks and forming glycosidic linkages.

The different reactivity of the hydroxyl groups allows the introduction of orthogonal groups. Protecting groups influence to various extends the reactivity by electronic and steric effects.[22] Some protecting groups control the regioselectivity via anchimeric assistance (participating effect).

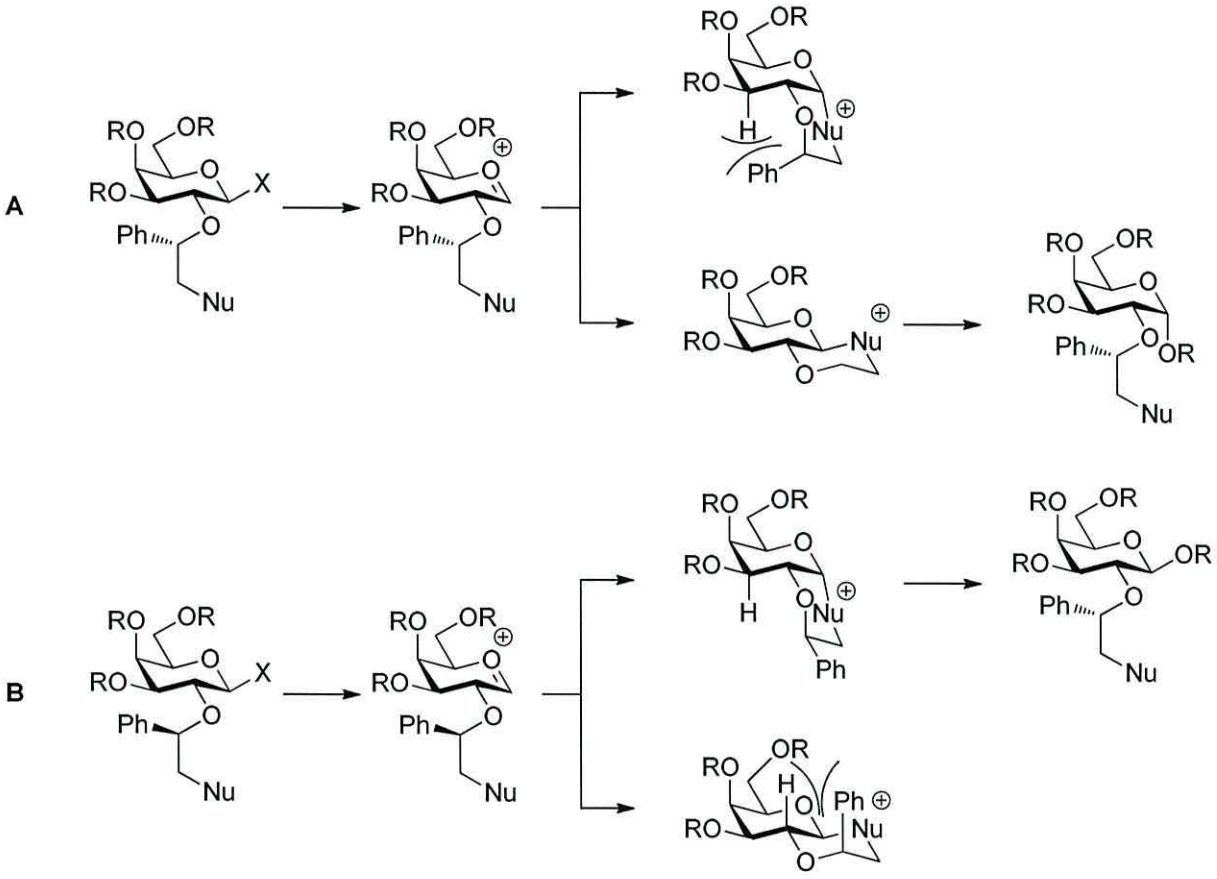
Participating groups are classically esters on the C-2 position, giving the neighbouring-group participation. During glycosylation processes (Scheme 3), activation of a given glycosyl donor initiates departure of the leaving group, leading to a stabilized oxocarbenium ion. The neighbouring acyl

group of the donor assists this departure and an acyloxonium is formed. Consequently, the dioxalane ring can only suffer a nucleophilic attack from the other side to form a 1,2-*trans* glycosidic linkage. Sometimes this nucleophilic attacks leads to an orthoester but this reaction can be avoided by varying the ester group or tuning the pH of the reaction conditions. In addition, orthesters can be made deliberately and serve as glycosyl donors.



Scheme 3 Neighbouring participation. Adapted from 22.

Participation during glycosylation can also originate from directing groups like dialkyl phosphates, chiral auxiliary groups like a substituted ethyl moiety containing a nucleophilic group (Scheme 4), and remote participating groups.



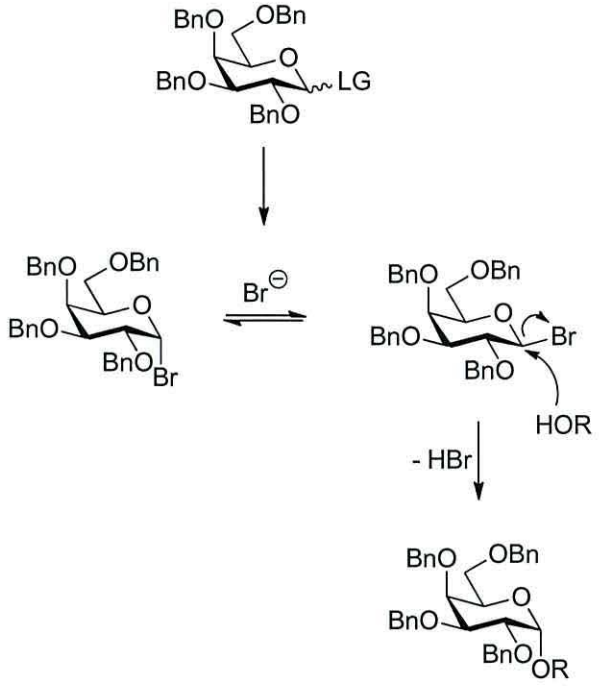
Scheme 4 Mechanism of the chiral auxiliary group participation (**A:** 1,2-*cis* glycosylation; **B**:1,2-*trans* glycosylation). Adapted from 22.

1.3.2 1,2-cis glycosylation

In contrast to the synthesis of 1,2-*trans* glycosidic linkages, 1,2-*cis* glycosylation is not as straightforward. Efficient synthesis requires a non-participating group at C-2 or a conformation constraining protecting group. Despite those efforts, the formation of the undesired anomer is often not completely suppressed, resulting in anomeric mixtures with α/β-ratios depending on the reaction conditions.

However, efficient methods exist to prepare *cis*-linkages but they are carbohydrate-dependent. The 1,2-*cis* glycosylation using a β-glycosyl bromide as intermediate, for example, is especially effective for α-galactosides and α-fucosides but less selective for α-glucosides, and cannot be used for β-mannosides. This method is based on the "in situ" anomerisation of the glycosyl bromide in the presence of tetraalkylammonium halide.[25]

Under those reaction conditions, the glycosyl donor is first converted into a glycosyl bromide (Scheme 5). The more reactive β-anomer reacts immediately with the nucleophile in a S_N2-type reaction giving an α-linkage, while the relatively more stable α-anomer is converted "in situ" into the β-anomer in order to preserve the equilibrium before reacting. The regioselectivity of the reaction is kinetically controlled.



Scheme 5 1,2-*cis* glycosylation reaction. Adapted from 10.

Other common techniques to prepare 1,2-*cis* glycosidic linkages include intramolecular aglycon delivery (for mannosides and glucosides)[26] or the use of a triflate as leaving group (for mannosides).[27]

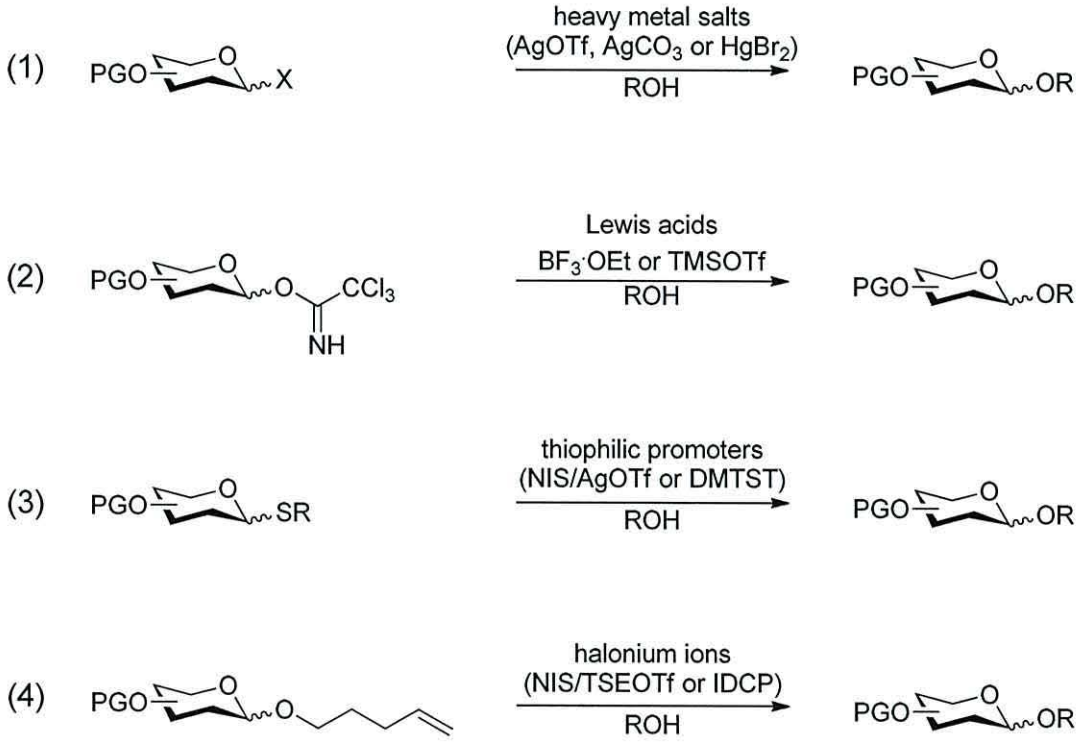
1.3.3 Glycosyl donors

The synthesis of oligosaccharides requires glycosyl donors with a feasible leaving group at their anomeric position. The majority of anomeric groups used in glycosyl donors need to be converted into a good leaving group prior or during the glycosylation reaction. This activation step is done by a suitable promoter system. Once activated by a promoter, the donor reacts with a nucleophilic group, usually a hydroxyl group, from a glycoside

acceptor to create a glycosidic bond. Methods to form this linkage have been extensively investigated.[20]

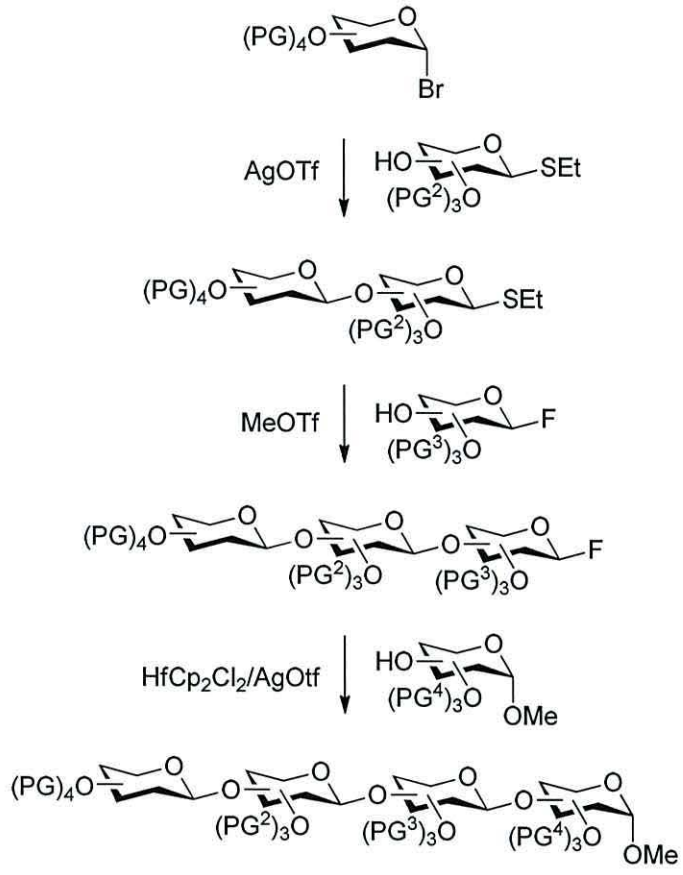
Some widely used leaving groups are (Scheme 6):

1. Glycosyl halides, promoted by heavy metal salt such as Ag or Hg[28]
2. Trichloroacetimidates, promoted by a catalytic amount of a Lewis acid[20]
3. Thioglycoside, promoted by thiophilic reagents[28, 29]
4. n-pentenyl glycoside, promoted by an electrophilic halonium ion[30]



Scheme 6 Common glycosyl donors and examples of their promoter systems.

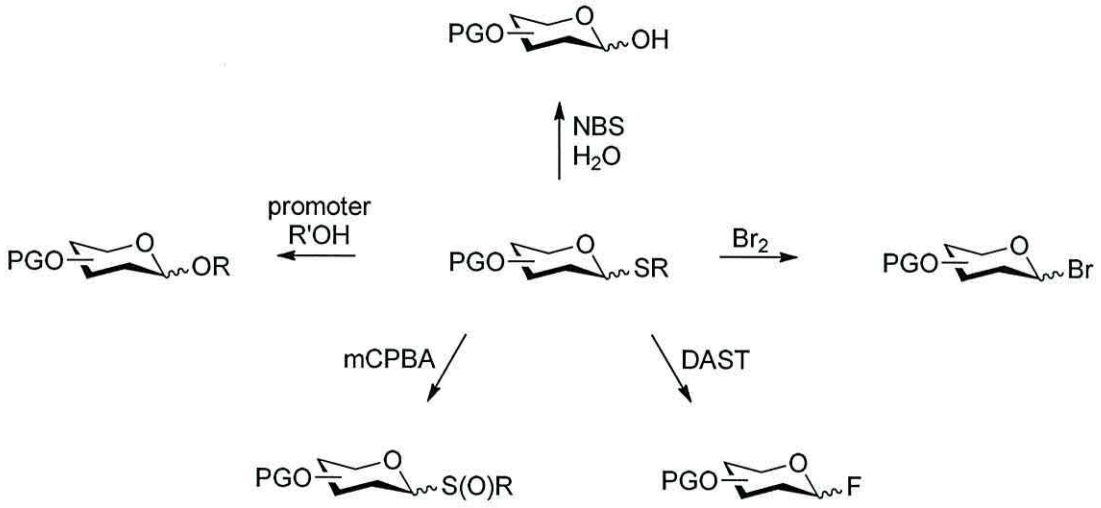
Orthogonal couplings can be carried out by carefully choosing orthogonal promoter system/leaving group couples of fully or partially protected glycosyl donors and acceptors (Scheme 7). The orthogonality can be enhanced by employing various principles in carbohydrate synthesis, as match-mismatch[31], armed-disarmed[32], and solvent effects[33].



Scheme 7 Example of orthognal glycosylation adapted from 34.

Along with trichloroimidates, thioglycosides are possibly currently the most used donor leaving groups. The interest in thioglycosides is easily understood as they are easily synthesised and highly stable under various reaction conditions, allowing protecting group manipulation or

glycosylation by chemoselective activation. They can as well be starting material for other glycosyl donors like glycosyl bromides, obtained in a one-step reaction, or tricholoroacetimidates, obtained via a hydrolysis step (Scheme 8). Thioglycosides are also activated via a large number of promoter systems with a broad spectrum of properties.[20] Thus they are also very suitable for orthogonal couplings, either by combining two different thio residues (e.g. SEt and SPh) [35] or by converting one of the thio groups into a suitable orthogonal leaving group.



Scheme 8 Examples of thioglycoside conversions into various glycosyl donors.

1.4 *Helicobacter pylori*

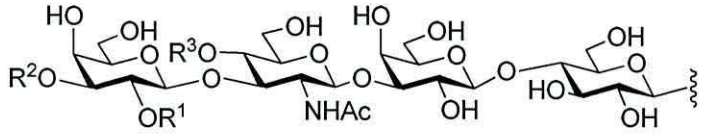
1.4.1 Helicobacter pylori

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium, previously known as *campylobacter pyloridis.*[36] It was isolated and identified in 1982 by Barry Marshal and Robin Warren.[37] The spiral-shaped pathogenic bacterium is found on the human gastric mucosa[38] and has been recognised as a causative agent for gastric ulcers and associated with gastric cancers.[39] The infection is usually asymptomatic and approximately 50% of the world's population has been estimated to be infected.[40] The infection can be chronic if not treated. *H. pylori* is well adapted and produces urease to survive in the stomach by creating an alkaline environment. The prevalence of *H. pylori* infections varies widely by geographic area, age, race, ethnicity, and socioeconomic status.[41]

H. pylori adheres to epithelial cells of the stomach. The attachment is mediated by adhesins recognising specific receptors. The blood group binding adhesin (BabA) is considered as one of the most important and binds to the fucosylated Lewis^b antigen displayed on the surface of stomach epithelial cells.[42] However, other adhesins and receptors interact together to adhere bacteria on the epithelial cells, e.g. sialic acid-binding adhesin (SabA) binding Lewis^x antigens.[43]

1.4.2 Lewis^b blood group antigens

H. pylori binding to epithelial cells by the BabA proceeds via recognition of specific carbohydrate structures belonging to the Lewis blood group antigen family. All known, recognised Lewis blood group structures contain a common sub-structure, a lacto-*N*-tetraose (LNT) (Fig.3).[44] These structures are closely related to the ABO blood group determinants.



Position	LNT	H-1	A	B	Le^a	Le^b	A-Le^b	B-Le^b
R^1	H	α-L-Fuc	α-L-Fuc	α-L-Fuc	H	α-L-Fuc	α-L-Fuc	α-L-Fuc
R^2	H	H	α-D-GalNAc	α-D-Gal	H	H	α-D-GalNAc	α-D-Gal
R^3	H	H	H	H	α-L-Fuc	α-L-Fuc	α-L-Fuc	α-L-Fuc

Figure 3 Blood group antigens.

1.5 The aims

The aim of the research conducted focussed on the development and synthesis of various tools based on biologically active carbohydrates. These tools are designed for studying the interaction between lectins, engineered chitinases and their substrates, and for detailed *H. pylori* binding studies.

In Chapter 2, chitin oligosaccharide glycoconjugates were desired to study engineered chitinases using AFM. Due to the enzyme's active site characteristics, a linker had to be introduced. This linker had to carry a functional group allowing self-assembled monolayer chip preparation.

Various oligosaccharides were of interest to further investigate the BabA of *H. pylori* (chapter 3 and 4). A pentasaccharide had to be synthesised to find the minimal epitope recognised by the protein. Regarding previous analyse results, a short non-functionalised liker was introduced. A route to prepare a heptasaccharide was designed, using as many building blocks as possible from the earlier elaborated pentasaccharide.

In chapter 5, a library of selenomethyl glycosides was constructed for structure analysis of lectins. Both anomeric configurations and a simple route to those were needed.

2 Chito-oligosaccharide derivatives for AFM studies

2.1 Background

Chitin is a linear *N*-acetylglucosamine polysaccharide (Fig 4) and the principal component of the exoskeleton of invertebrate. This polymer is also present in the cell walls of most fungi and many algae[45]. Chitin has a highly ordered, crystalline structure. In the chitin crystal, the chains form hydrogen-bonded sheets linked by C=O and H-N-groups. In addition, each chain has intramolecular hydrogen bonds between the neighbouring sugar rings. On the monosaccharide level, there is a hydrogen bond between the OH-group on C-3 and the endocyclic oxygen. The accumulation and geometry of those hydrogen bonds explains the stiffness and the poor solubility of chitin[46].

Chitin is an omnipresent but valuable polymer. Extensive research is still carried out on chitin and chitosan, a partially deacetylated chitin derivative. Water-soluble chitosan is obtained by controlled deacetylation and is used e.g. in the health care sector in wound-healing products due to its antibacterial properties[47]. Chemical derivatisation is difficult to achieve. Bioengineering of available chitinases might allow the exploitation of chitin and chitosan for a number of applications, e.g. pharmaceuticals, skin care, food, agriculture, waste degradation or water treatments[48, 49]. Before this can be done, the enzymes need to be studied.

The degradation of chitin into poly- and oligosaccharides with defined dp-ranges is a challenging task. The depolymerisation of chitin can be obtained by using chitinases[50] or by acidic degradation[51]. Chitinases are glycosidases that selectively recognise chitin as substrate to cleave the glycosidic linkage of chitose units. Degradation of chitin occurs via cleavage of the glycosidic bonds. Chitin oligosaccharides can be obtained via those processes. Controlling the degree of polymerisation is an issue as the acid catalysis degradation is reported to be non-random and monomers preferentially formed[52, 53].

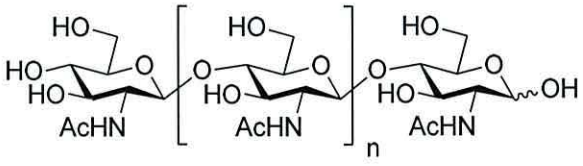


Figure 4 Chitin polymer.

Chit42 is a chitinase extracted from the filamentous fungus *Trichoderma harzianum*[54]. Preliminary studies suggest that the active site is a deeper binding groove to ensure a stronger binding with the substrate but also allowing some flexibility at more distance subsites. The enzyme cleaves preferentially between the second and the third sugar unit from the reducing end. AFM experiments are used to understand and characterise the protein-carbohydrate binding of the wild-type enzyme, and different engineered chitinase variants of Chit42, expressing amino acid mutations

along the binding site[50]. As the monosaccharide binding is known to be too weak, longer oligosaccharides have to be used as substrates. Chit42 studies are of interest because of its potential biocontrol against crop pathogen infections[55].

Measuring binding forces using AFM was realised for the first time in 1994[56]. The strength of the interaction between biotin and streptavidin was studied in this example The AFM works by scanning a sample in a raster fashion with a very small tip mounted at the end of a flexible microcantilever while maintaining gentle contact with the sample surface. This relative motion is performed with sub-Ångström accuracy by a piezoelectric actuator (usually a tube, sometimes a tripod). Interacting with the sample, the cantilever deflects a laser beam. The tip–sample interaction can be monitored with high resolution exploiting a laser beam impinging on the back of the cantilever. The beam is reflected towards a split photodetector configuring an optical lever which amplifies cantilever deflections[57] (Fig 5).

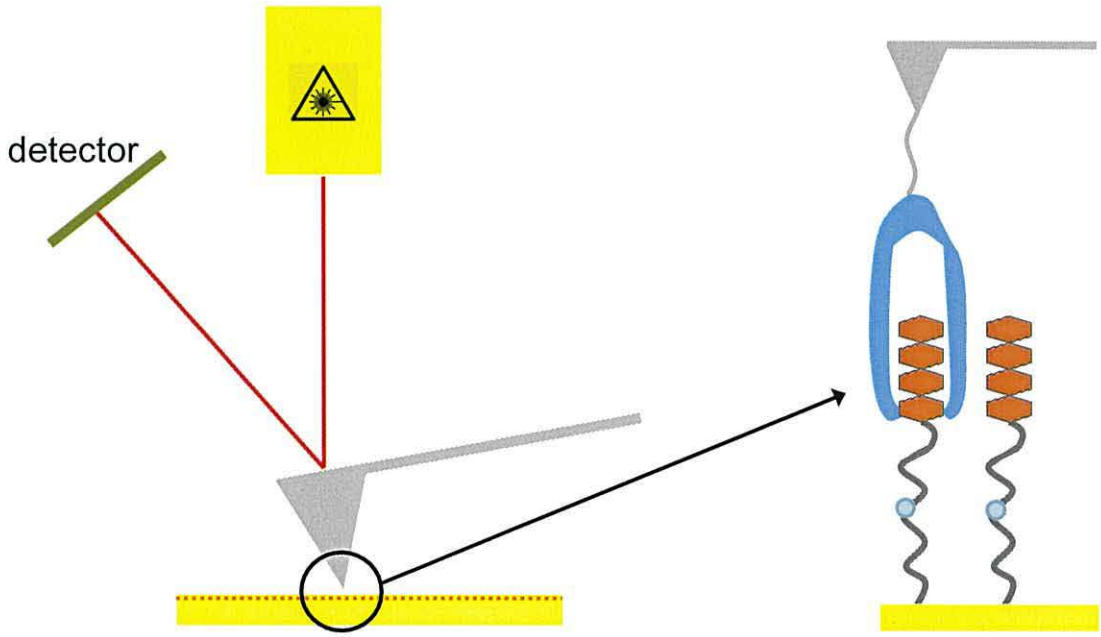


Figure 5 Principle of AFM measurements. The enlargement shows the oligosaccharide substrate assembled via a long spacer on the Au-surface while the enzyme has been immobilised on the cantilever via a linker. Adapted from 57.

The data of interest for the binding is collected as the force curve. The force curve consists in an approach–retract cycle between the tip and the sample during which the cantilever deflection is measured as a function of the relative motion.

In the force recording mode, the sample is probed at a fixed spot on the surface. The area of the probed surface is basically dependent on the sharpness of the used tip. Each measurement consists of an approach-retraction cycle where the probe tip returns to its starting point at the end of the measurement.

A measurement begins at a state where the probe, in this case the immobilised enzyme, is lifted some 100 nm above the sample, the carbohydrate surface. Then the probe-sample distance is decreased until

contact is achieved by raising the sample using a Z-piezo scanner. The total force exhibited at the tip during the process is monitored using the laser reflection from the sensing element - the cantilever of the probe. When the cantilever is deflected upwards by a predefined value at which contact is expected to be established, the sample is moved to the opposite direction until the starting point is reached. Binding events are hereby seen when the tip withdraws from the sample.

The illustration (Fig 6) shows a force curve measurement where weak attractive forces are present at the approach leading to a jump-into-contact event, which can be seen as decrease of the sensed force to a negative value. Here, the enzyme equipped tip is close enough to produce an attraction. This is followed by area period where sample and probe are in direct contact, characterised by a linear increase and decrease when moving the sample further to the direction of the probe and force the contact, and moving it away, respectively. When the sample is moved away beyond the point where no direct contact force is present, the interaction specific force takes effect (here ca. 1.5 nN). The binding force can be deduced from this plot as the maximum force sensed before the cantilever returns to its resting position characterised by a zero force. The magnitude of the adhesion force can be used to compare interaction strengths between different molecules in a variety of environments[58-60].

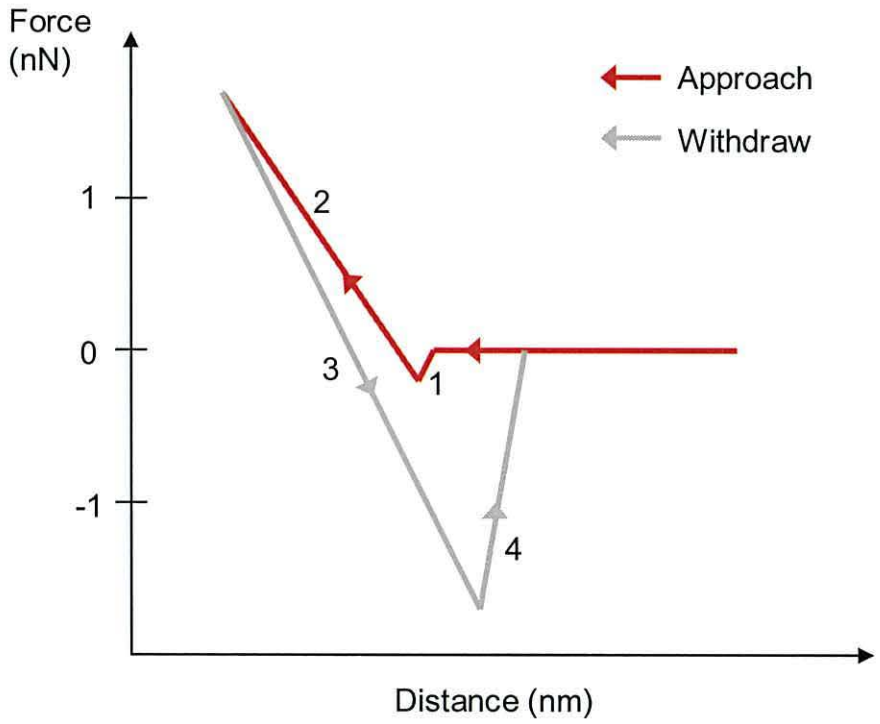


Figure 6 Force curves. Adapted from 57

In order to be able to carry out the AFM experiments, the enzyme substrates have to be immobilised on a surface via a functionalised linker. It was anticipated that carbohydrate SAMs would offer a convenient platform for high throughput characterisation of carbohydrate–protein interactions, including multivalent interactions. SAMs are formed spontaneously by adsorption of alkanethiols under reductive conditions from their solutions onto clean gold surfaces[61, 62]. The characteristics of these monolayers are well described as highly ordered monolayers with linkers tilted at an angle of 20-30° from normal to the metal surface. Carbohydrate SAMs offer extensive control over the presentation pattern, the density, and orientation of the carbohydrate ligands[63]. Gold is most

frequently used as SAM base because it does not form stable oxides under ambient conditions, and there is no interaction between the gold and the protein[64]. The AFM measurements are usually performed in aqueous solutions in order to prevent interfering capillary forces[65].

There are two principal methods to form functionalised SAMs (Fig 7). Either, the linker reacts first with the gold surface to form the SAM, and then the substrate is attached to the linker via another small spacer. The other way is to functionalise the linker first, and then let it react with the gold surface to form the functionalised SAM directly. For the present study, the first method, **A**, was used.

This method introduces sugar moieties onto the preformed functionalized SAMs using selected chemical reactions[66].

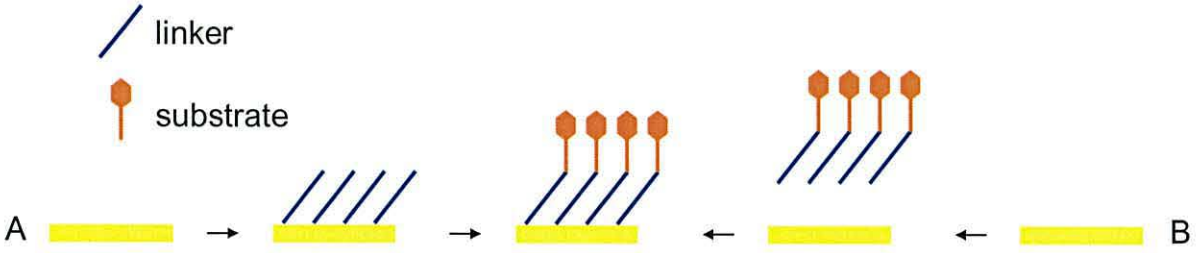


Figure 7 Two principal routes to produce functionalised SAM adapted from 61

Binding forces can also be measured with a surface plasmon resonance (SPR) device[67]. The underlying physical principles of SPR are based on surface electromagnetic waves, created by a polarised light (laser) under total internal reflexion. When those waves propagate on the conductive

gold layer, covering a glass surface, they are sensitive to any changes on the surface like molecule adsorption. When the waves interact with surface irregularity, part of the light is absorbed and the reflective light intensity is reduced. Analyse of this reflective light reduction allows a real-time measure of surface interaction (Fig 8) [68].

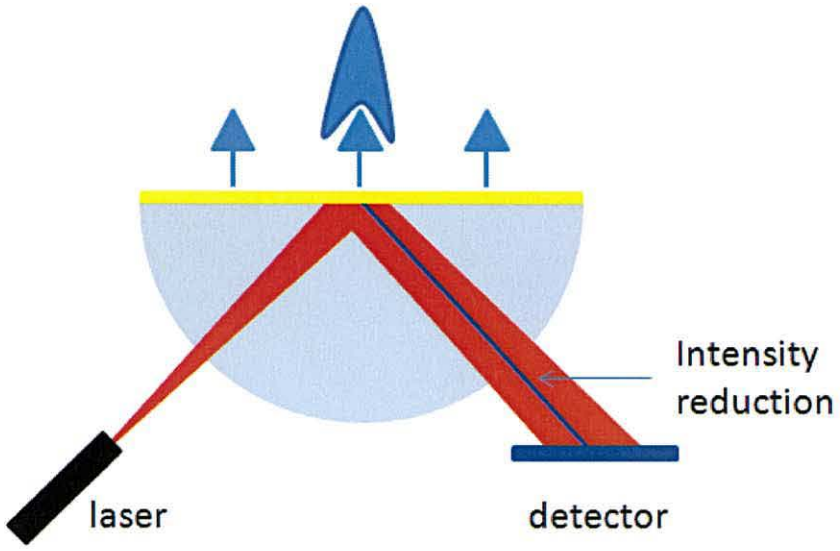


Figure 8 Principle of SPR measurement. Adapted from68

When the affinity of two ligands has to be determined, the binding constant must be determined. SPR devices are well suited for binding constant determination. While one of the interactants is immobilised to the sensor surface, the other are free in solution and passed over the surface. Association and dissociation is measured in arbitrary units and displayed in a graph called the sensorgram. [69]

For the binding analysis of engineered proteins, the chito derivative ligand is attached to the SPR sensor chip, thus immobilised. A solution containing the to be analysed protein is injected in a small flow cell over the sensor chip, under continuous flow. As the analyte binds to the ligand, the accumulation of mass in form of the interacting protein on the surface results in a decrease of emitted light. The change in emitted light is registered and plotted (Fig 9)

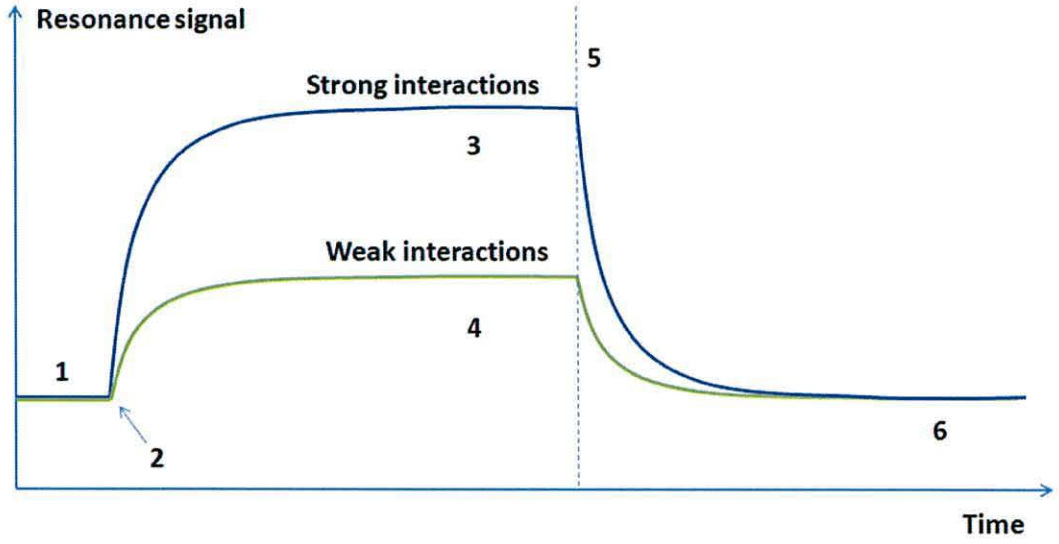


Figure 9 SPR curves. 1: Background noise; 2. Analyte injection; 3. Strong interaction equilibrium; 4. Weak interaction equilibrium; 5. Regeneration solution injection; 6. Complete dissociation. Adapted from 68.

2.2 Synthetic Strategy

Macromolecular chitin and larger chitooligomers are not or scarcely water soluble, similarly protected chitoderivatives can sometimes be very difficult

to dissolve in a suitable solvent. However, shorter unprotected chitooligomers are fairly well soluble in aqueous systems. Unfortunately, pure chitooligomers are rather expensive compounds. For the AFM investigation of engineered chitinases, linker equipped oligomeric chitin derivatives were required. For the preparation of the SAMs, the functionalised derivatives were intented to be attached to a carboxylic acid. With respect to the above mentioned issues, the reaction pathway should be efficient to keep loss of material to a minimum with multi-step synthesis, and avoid extensive use of protecting group.

Three chitooligomers **9**, **10** and **11** were chosen as substrate for the enzyme study (Fig 10). The synthesis of the glycoconjugates starting from the monosaccharide level would have been possible but not economically viable. The chitooligomers available in the laboratory were obtained by enzymatic digestion followed by size exclusion chromatography and recrystallisation[70] or by controlled hydrolysis[71].

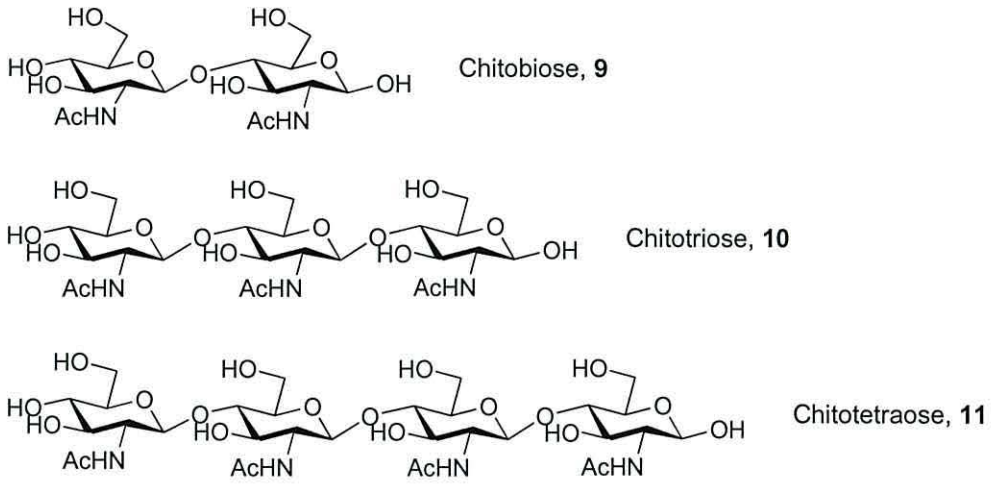


Figure 10 The different chitooligomers.

There are many options to introduce a suitable linker to oligosaccharides required for both later attachment to the SAMs but also to possibly mimic carbohydrate units. *O*-glycosylation is the most common one, and this technique is especially useful when the oligosaccharide is prepared by synthesis, but other connections such as *N*-glycosylation are possible (Fig 11).

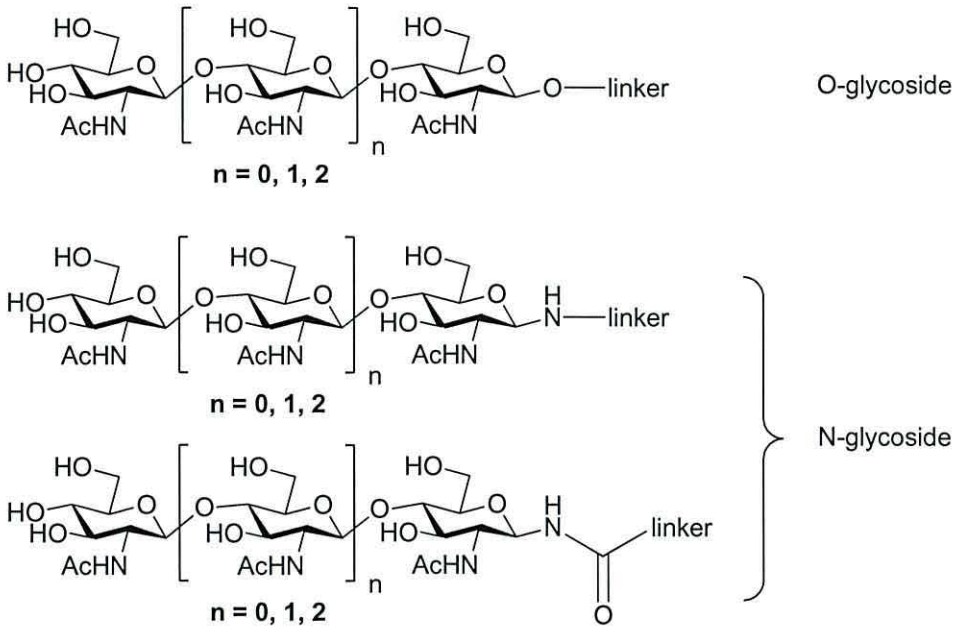
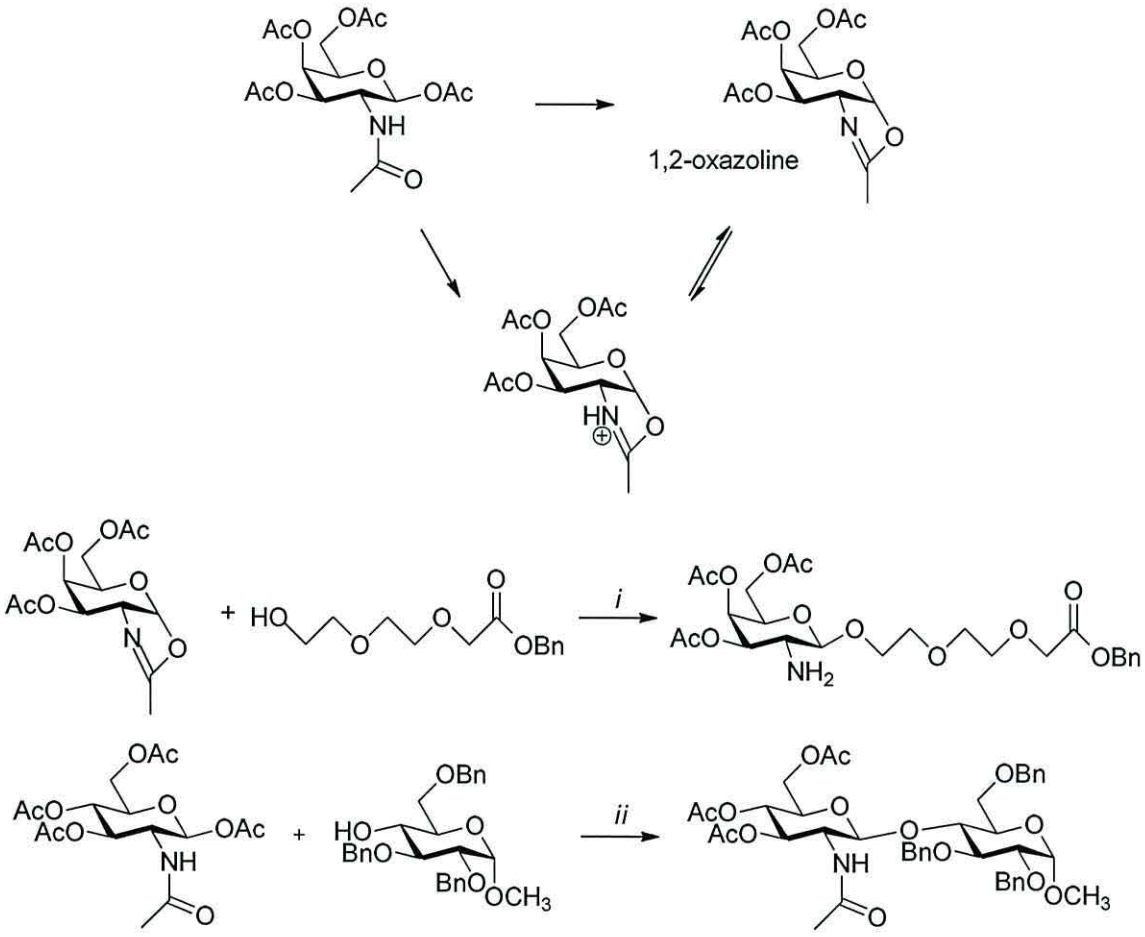


Figure 11 Different possible anomeric linkages.

The use of an *O*-glycosidic linkage was excluded, because of the requirement of multi-step synthesis which would proportionally decrease the yield and increase the risk of solubility issues. Oxazolines can be used as donors for glycosylations. They have been used mainly to introduce non-carbohydrate linkers[72] (Scheme 9). Recently, rare earth metal triflates have been found to be suitable to use oxazolines also for glycosylation with carbohydrate acceptors in good yields[73]. However, due to the number of *N*-acetyl groups in the starting chitooligomers, regioselectivity might be an issue. Oxazoline formation may not only occur on the reducing end but also at other glycosidic linkages along the oligosaccharide chain leading to degradation of the oligomers. In addition, it was decided to avoid protection group chemistry in the sugar moiety.



Scheme 9 Oxazolineformation and use. *i.* TSMOTf, DCM; ii. $Sc(OTf)_3$, DCM.

Another option to functionalise the anomeric position is to introduce a nitrogen atom. Using nitrogen gives the possibility to use an amino or amide function to attach the linker. The formation of an anomeric amine as final linkage group function was excluded because of its tendency to hydrolyse[74, 75]. Therefore, the formation of a glycosyl amide was selected to be the best candidate. In addition the amide linkage mimics the natural *N*-linked glycosides[76].

The enzyme presents deep groove so it may partly interact with the linker closest to the sugar moiety. Mimicking a natural function, e.g. an amide bond, for the sugar spacer linkage may help the enzyme to accept the glycoconjugate as substrate. The length and properties of an ideal linker were not known. However, it was anticipated that a long linker will increase the chance of substrate accessibility.

As the chosen functionality is an amide, the linker has to contain the carboxylic group. This carboxylic group has to be activated to react with the amino derivative. The linker should be long enough to allow the glycone moiety to enter in the groove correctly. DSS is an amine-reactive, homobifunctional reagent that produces an 8-atom linkage. Many of the reported applications of DSS involved receptor binding on cell surfaces[77, 78]. As NHS-ester, DSS is highly reactive towards amine nucleophiles[79]. The linker is known to have a poor solubility in water, and can be easily removed by precipitation in water and filtration, while the final product remains in the water phase. Although this linker is relatively stable and can be stored recrystallised at low temperature, the activated carboxylates have half-lives on the order of hours at physiological pH[80].

Additional aspects had to be taken into consideration when designing the target compounds. They need to be attachable through a suitable functional group at the terminus of the linker to a second, longer linker forming the SAM of a gold chip (Fig 12). Since the other end of the linker will be attached to a carboxyl group exhibited by the SAM surface to form an amide, the end of the linker should be an amine. To avoid

polymerisation of the linker, the amino function should not be carried by the linker carrying also the activated carboxyl group. A practical solution is to use a diacid linker and adding finally a small diamine to the glycoconjugates.

Taking account of all the above, compounds **12**, **13** and **14** were designed (Fig 12) as target molecules.

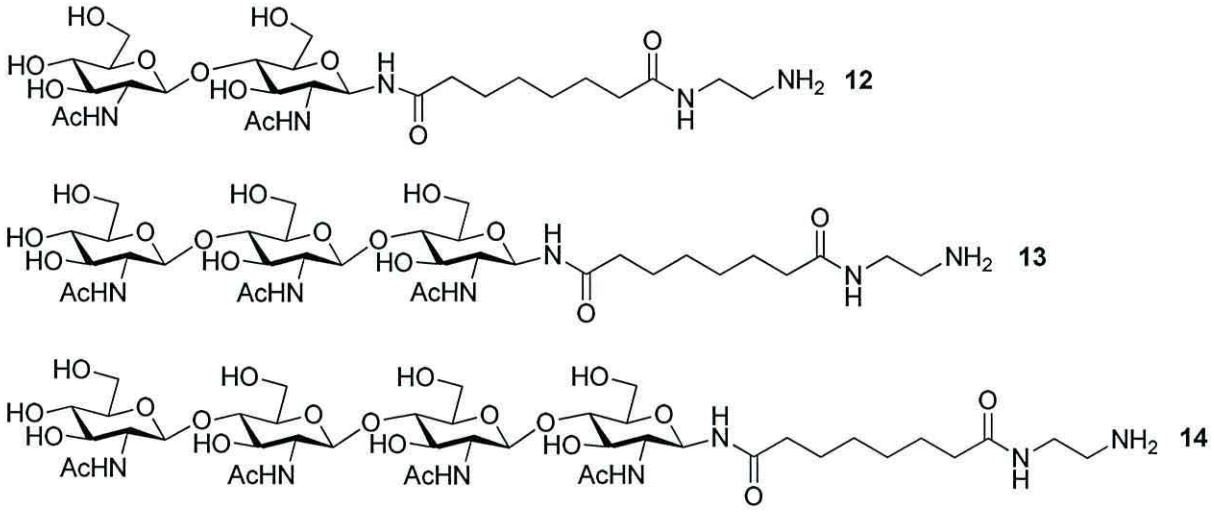
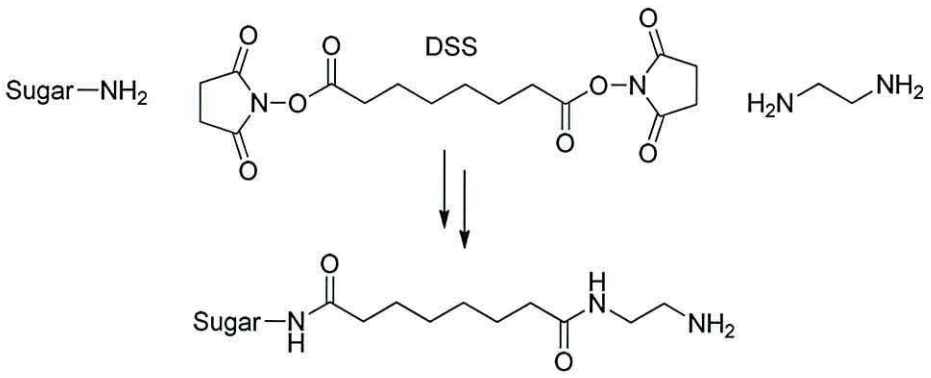


Figure 12 target molecules.

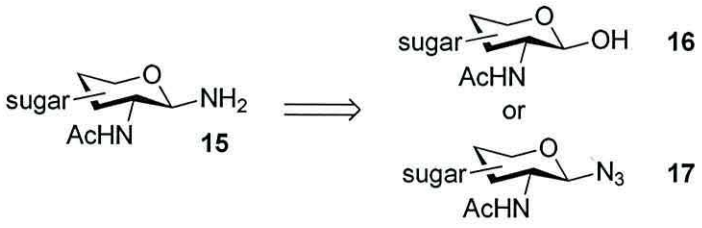
2.3 Results and discussion

The synthesis of the target derivatives required chito oligomers with an amine at the anomeric position, DSS, and EDA (Scheme 10).



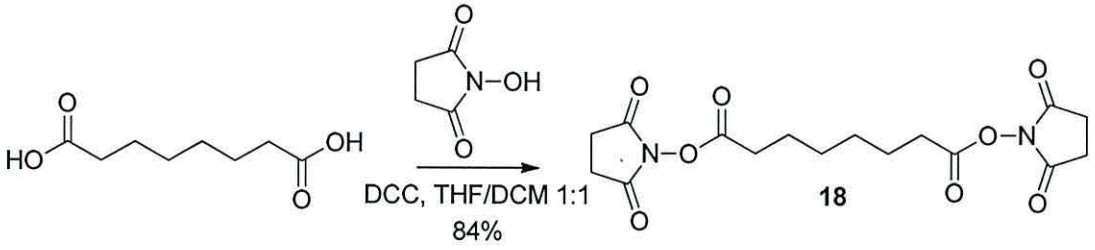
Scheme 10 Synthesis overview.

Two routes were possible to get the required sugar amine derivatives **15**: reduction of azide **17** or direct substitution of the hemiacetal **16** (Scheme 11). Since azide reduction requires an additional step, the substitution was tried first[81, 82].



Scheme 11 Two routes to the amine derivative.

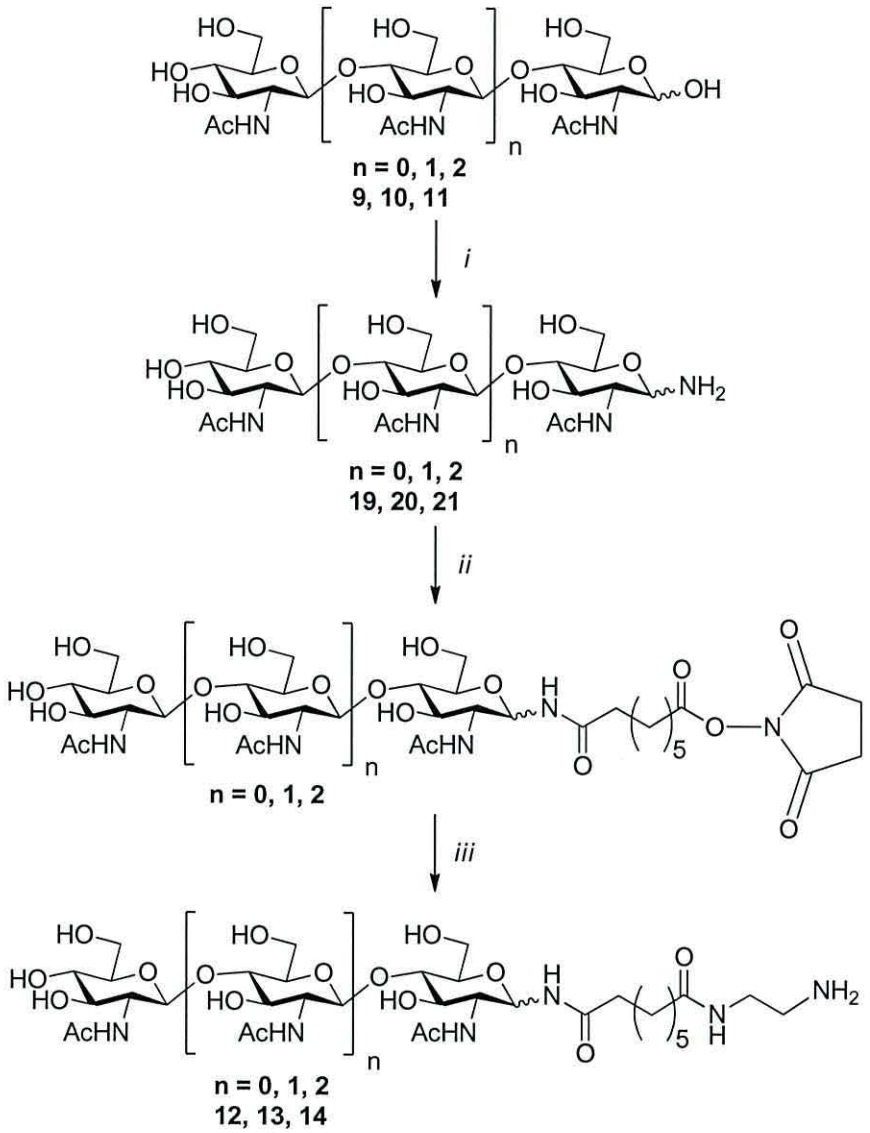
The linker (DSS) **18** was synthesised using NHS and suberic acid according to known procedures (Scheme 12)[83].



Scheme 12 DSS **18** synthesis.

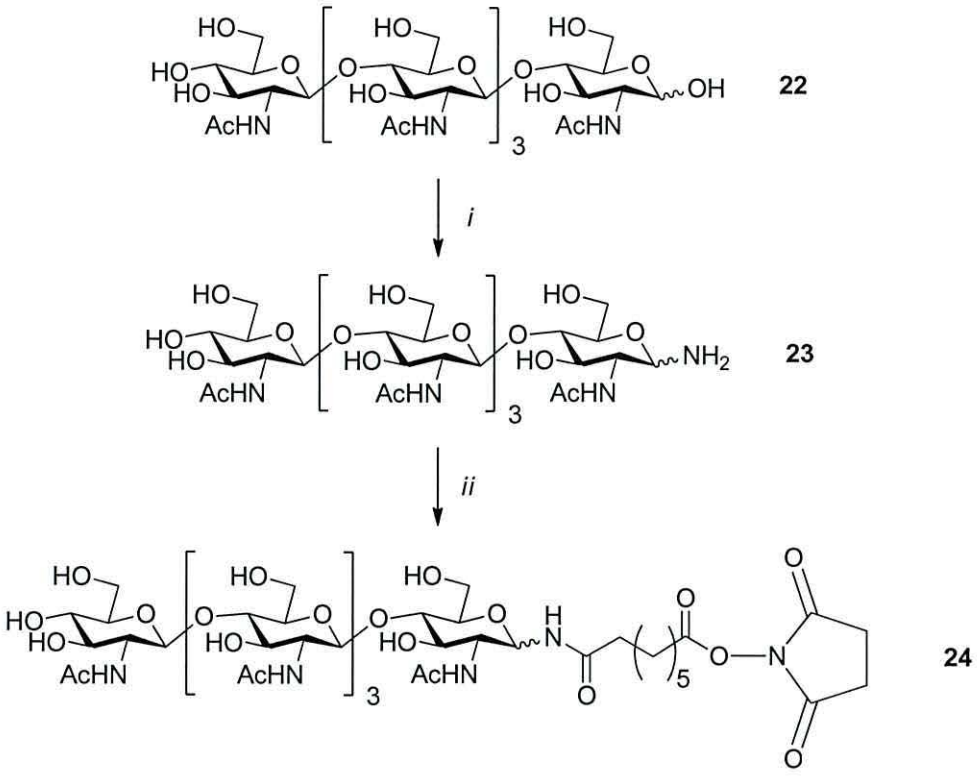
The chitooligosaccharides **9**, **10** and **11** were used as starting materials (Scheme 13). Several procedures for the introduction of the anomeric amino group are known, e.g. using pure ammonia[74] or saturated aquous ammonia[84] together with the unprotected sugar. Here, NH_4HCO_3 was chosen for the chitin derivatives instead of the $(NH_4)_2CO_3$ because the reaction was expected to form a pure β anomer[70]. Unprotected 1-amino sugars are prone to hydrolyse very rapidly, thus reaction conditions have to be elaborated for each case[85]. Reaction conditions requiring extended reaction times (several days) and open-vessel[86] procedures were excluded due to expected hydrolysis. Eventually reaction conditions were adapted from a procedure reported to be done in a microwave[87]. Instead, reactions were carried out using NH_4HCO_3 in DMSO at 40° overnight. Higher temperatures were tried but degradation of the sugars was observed. The crude reaction mixture was then freeze-dried to remove DMSO and the excess of ammonium carbonate. The residue obtained (**19**, **20**, **21**) was used without further purification due to the instability of the amine yielding mainly hydrolysis product, i.e. the starting material, and the

coupling reaction had to be accomplished as soon as possible. The reaction was done again in DMSO, and HOBt was used as activating agent[86]. 10 Equivalents of linker were used to avoid undesired disubstituted linker. It was possible to monitor the reaction by TLC and complete conversion was obtained after few hours. The excess of linker and part of the activator were precipitated with water and both removed by filtration. Since the stability of the activated ester in water is temperature dependent, ice-cold water was used, and the next reaction was started directly after filtration. For the last coupling reaction, 20 equivalents of ethylene diamine were added to the water. The final product was obtained after few hours. Purification was performed by ion exchange chromatography and reverse phase chromatography yielding the three desired compounds (**12**, **13**, **14**) with a yield between 44% and 57%. All compounds gave satisfying MS data.



Scheme 13 Chitooligosaccharide derivatives' synthesis. i $(NH_4)_2CO_3$, DMSO, 30 °C; ii DSS, 1-hydroxybenzotriazole, DMSO; iii $H_2NCH_2CH_2NH_2$, H_2O, 44%-57%.

The same procedure was applied to synthesise the conjugate from the chitopentaose **22** (Scheme 14) but already the derivative containing the activated linker **24** was not soluble in water. Since the target compounds were intended for analysis in aqueous systems, the synthesis of the chitopentaose derivative was abandoned.



Scheme 14 Chitopentaose derivative synthesis. i $(NH_4)_2CO_3$, DMSO, 30 °C; ii DSS, 1-hydroxybenzotriazole, DMSO.

2.4 Conclusion

Glycoconjugates mimicking chit42 natural substrates were synthesised. The SAMs were formed in SPR device using a carboxyl-terminated tEG alkanethiol-linker and the chitoglycoconjugates. Preliminary studies suggested a slow association-dissociation process with an engineered chit42 mutant without effect coming from the length of the chitoligomer (Appendix). The SAMs were also used for studying WGA with SPR device[88].

3 Synthesis of the Lewis^b pentasaccharide (paper I)

3.1 Background

The most common therapy to treat bacterial infections is by applying antibiotics. They are natural or synthetic compounds killing or slowing down the growth of bacteria. The emergence of antibiotic resistant strains of bacteria is the downside of the evolution pressure coming from the use of antibiotics. Using antiadhesives, compounds that inhibit the binding of the intruding bacteria to the host rather than killing it may be a viable alternative as it may reduce the emergence of strains resistant against antiadhesives [89].

In 1996, Åberg, Norberg and collaborators [90] presented a large scale synthesis of the Lewis^b tetrasaccharide (Fig 13, A). Acrylamide copolymers of this structure and copolymers of the di-, tri-, and hexasaccharide were prepared, and tested for their ability to inhibit bacterial binding to the gastric tissue. These results showed that the tetrasaccharide is a poor ligand for the adhesin. On the other hand, the Lewis^b hexasaccharide (Fig 13, B) has been found the best ligand so far for the BabA. Another study compared Lewis^b oligosaccharides conjugated to different carriers. The conjugates all resulted from reductive aminations, thus the reducing end ring was open. This study concluded that the carrier has a significant effect

on the adhesion, and that the native hexasaccharide conjugate (Fig 13, C) is well recognised even if the glucose on the reducing end is open.

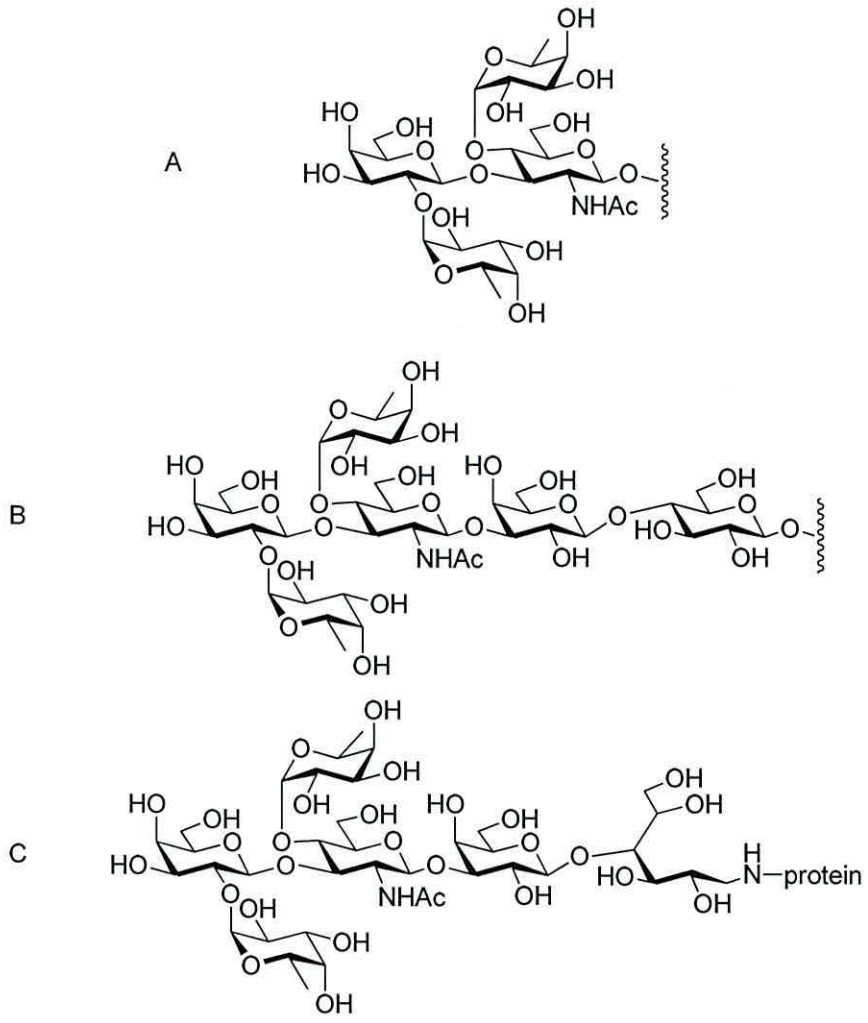


Figure 13 Lewis[b] structures.

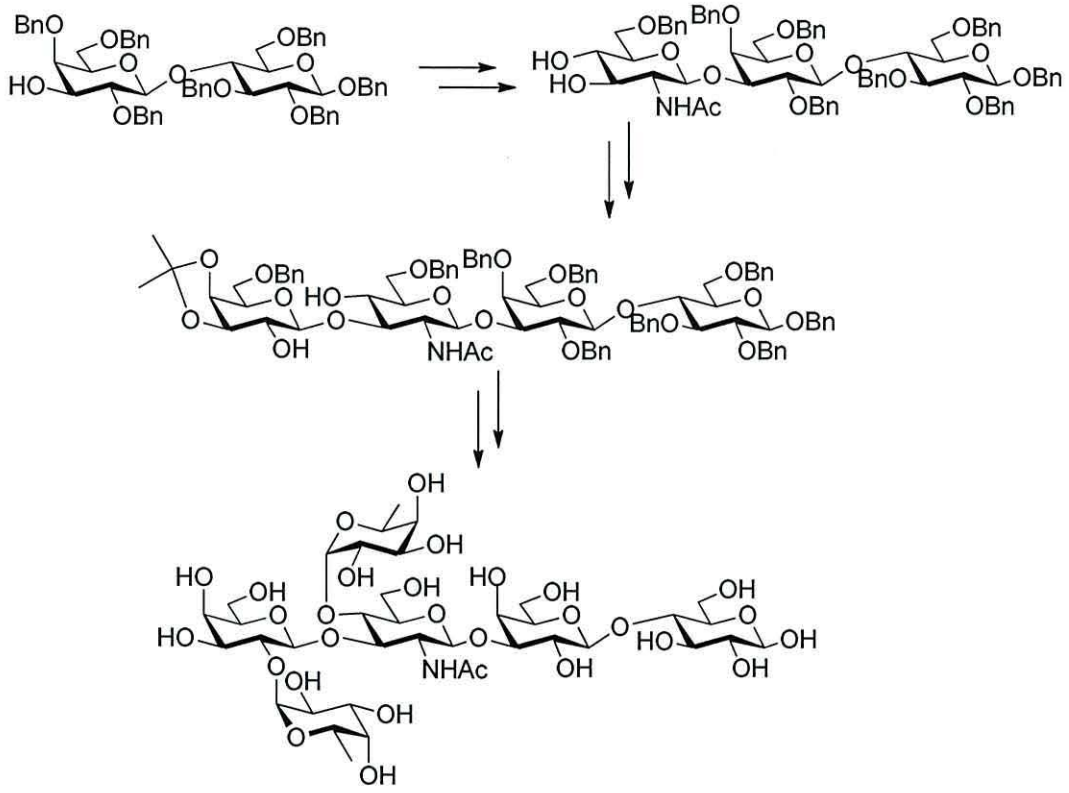
With these results in mind we were interested to investigate the minimal epitope required for a good binding to BabA. Thus, it was decided to synthesise the Lewis[b] pentasaccharide and compare its affinity to BabA with the analogues hexa- and tetra-saccharide. The pentasaccharide was

designed to have an additional linker to mimic the open glucose present when the hexasaccharide underwent the reductive amination.

3.2 Retrosynthetic pathways

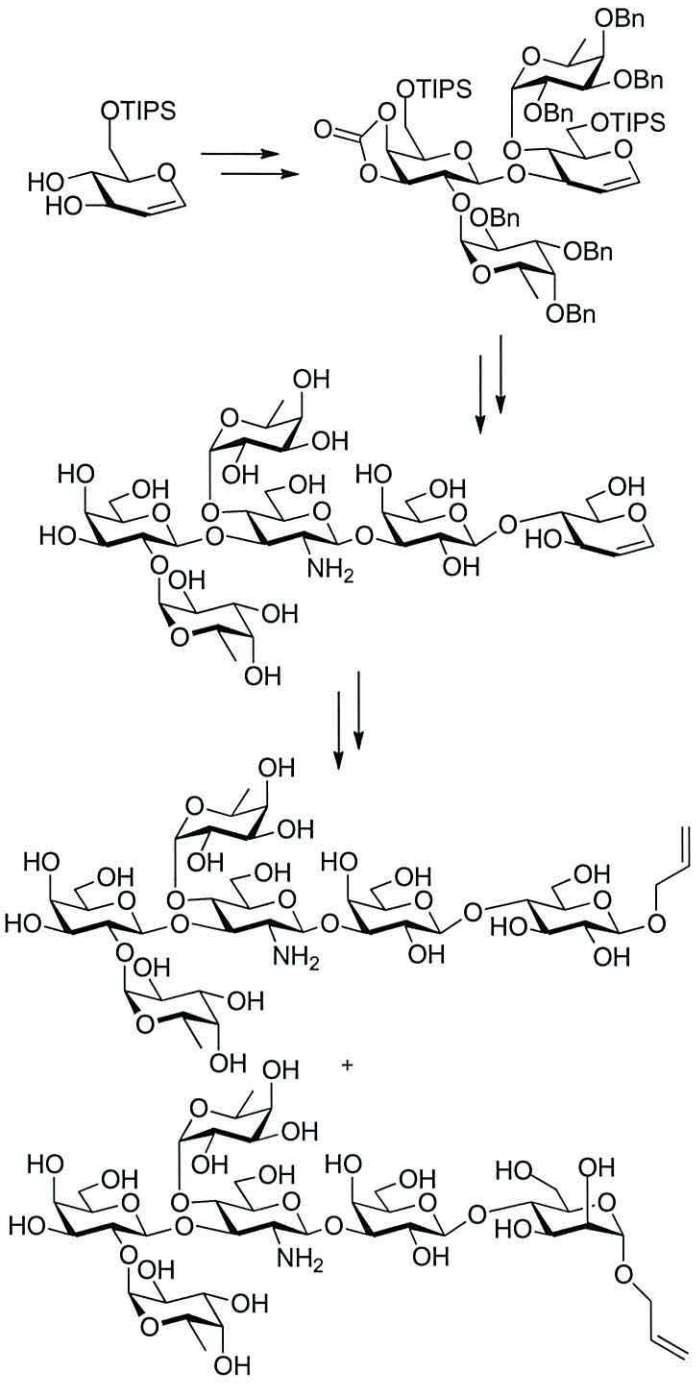
The synthesis of oligosaccharides can be achieved in various ways. Several aspects need to be considered, including choosing between a linear vs. convergent approach, the overall economy of the synthesis, the orthogonality both in the glycosylation and the protecting group patterns, the stereochemistry of the anomeric centres, the acceptor nucleophilicity and the donor electrophilicity. While the synthesis of the Lewis^b hexasaccharide has been published previously, there was no synthesis of a deprotected Lewis^b pentasaccharide.

Sato *et al.* [92] reported a linear pathway starting from the lactoside and using thioglycosides for the hexasaccharide. They prepared the linear tetrasaccharide but since the protecting group pattern was not suitable for the introduction of the fucose on the terminal galactose, several steps had to be carried out to modify the pattern. After complete deprotection followed by peracetylation, the linker was introduced using highly toxic mercury salts. A major drawback of this publication is that the data is not well documented (Sheme 15).



Scheme 15 Sato *et al.* approach. Adapted from [92].

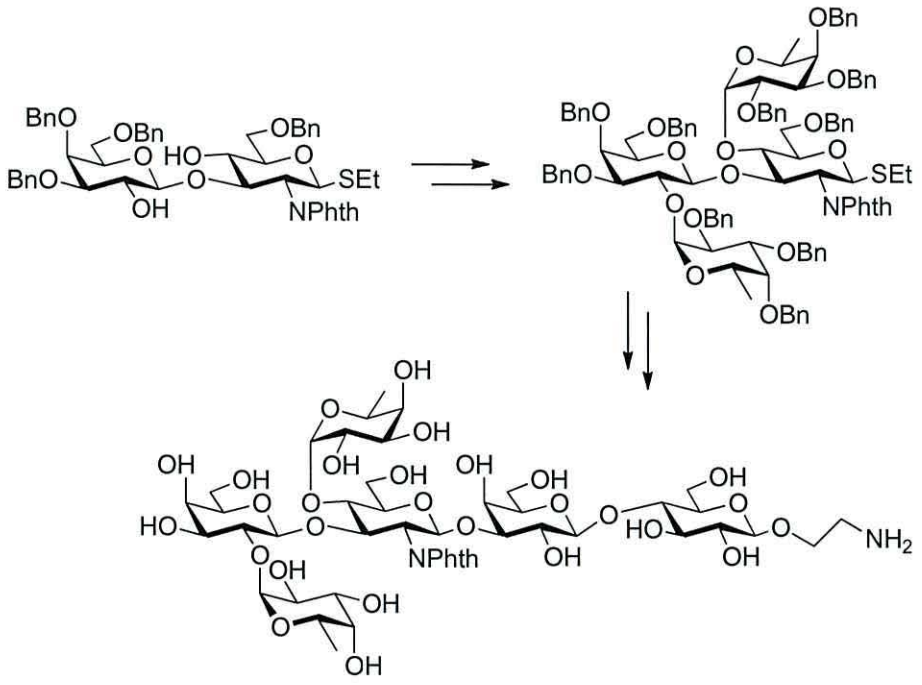
Danishefsky *et al.* [93] presented a pathway using glycals as donors. After building up the oligosaccharide using this glycal approach, the reducing end glycal is converted into Brigl's anhydride, i.e. the 1,2-epoxide, to introduce stereoselectively a β-anomeric allyl group. This is subsequently transformed by ozonolysis into an aldehyde to be used for further conjugation (Sheme 16).



Scheme 16 Danishefsky *et al.* approach. Adapted from [93].

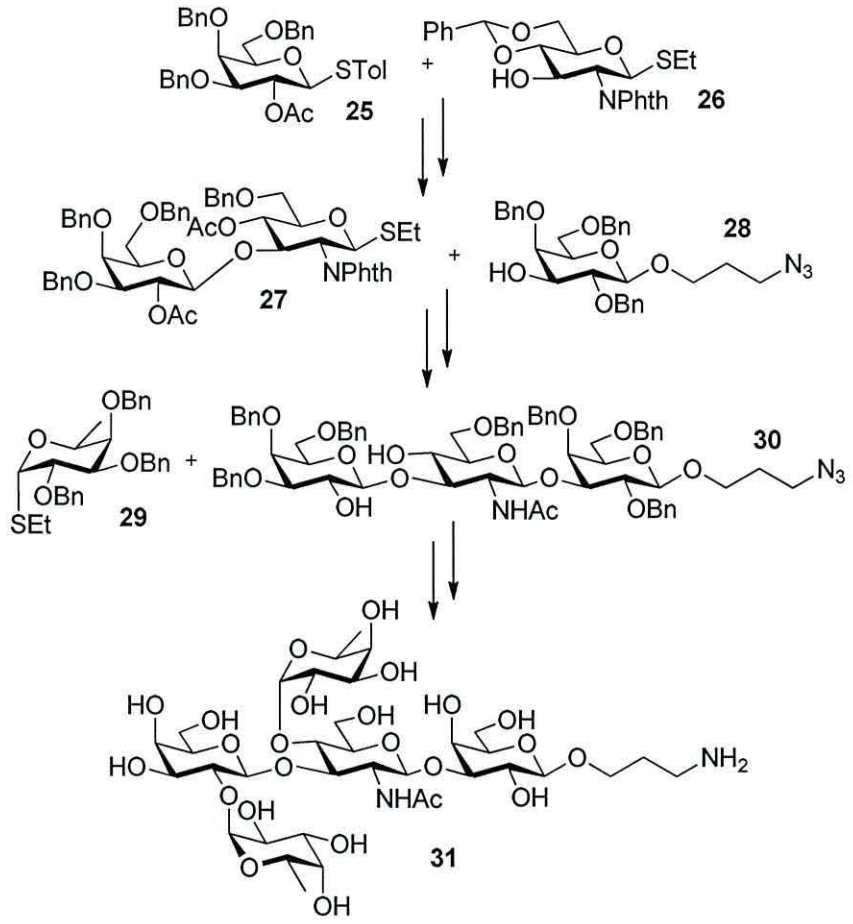
Cherniak *et al.*[94] synthesised the hexasaccharide following a [4+2] approach using a tetrasaccharide donor similar to Norberg's but coupling it to the lactoside could only be achieved with a large excess of the promoter

DMTST (30 equiv.). In addition, the removal of the phthalimido group on the hexasaccharide level was difficult (Scheme 17).



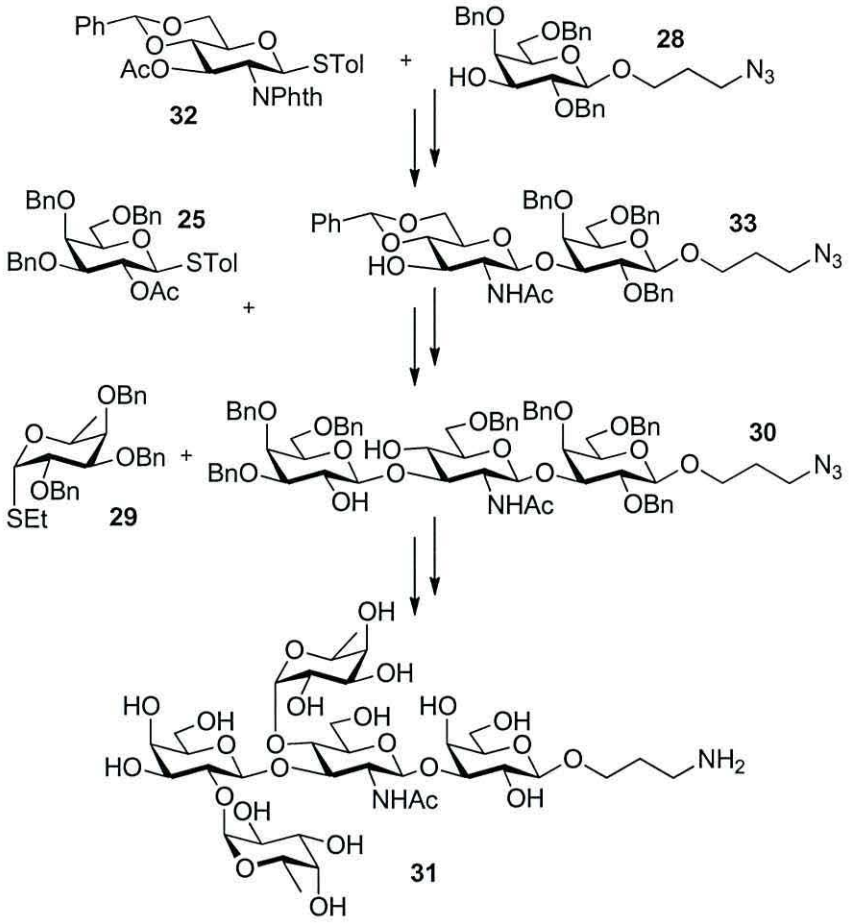
Scheme 17 Cherniak *et al.* approach. Adapted from [94].

For the synthesis of the pentasaccharide **31**, two pathways were attempted. The first one was an adaptation of a pathway published in our group for the synthesis of the hexasaccharide, consisting in a convergent approach giving the linear trisaccharide backbone **30** before adding the fucoses **29** (Scheme 18) [95].



Scheme 18 First projected pentasaccharide synthesis – convergent pathway.

The other pathway followed a linear approach assembling trisaccharide backbone **30** using the spacer equipped galactoside **28** as first glycosyl acceptor, before finally introducing the two fucosyl residues (Scheme 19).

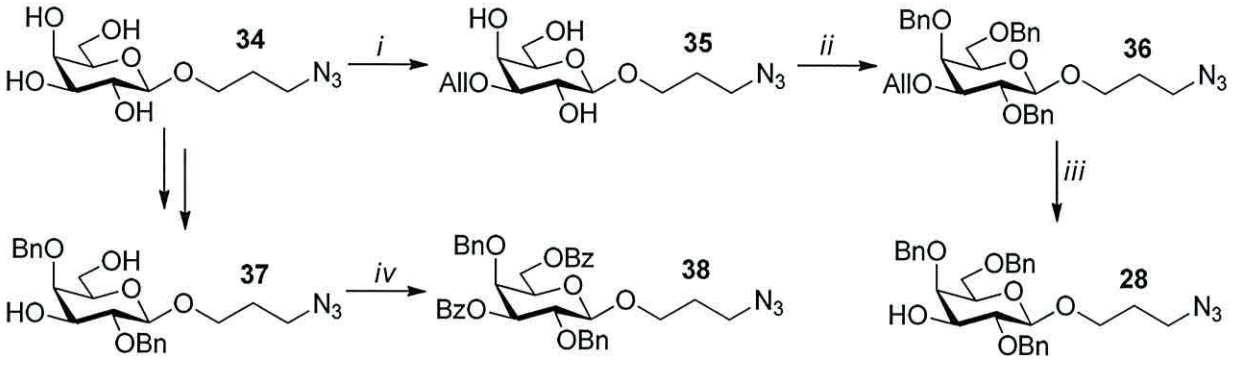


Scheme 19 Second projected pentasaccharide synthesis – linear pathway.

3.3 Synthesis of the spacer equipped galactoside acceptor **28**

Starting from the azidopropyl galactoside **34**[96], the 3-OH was regioselectively protected with a temporary allyl group by using allyl bromide and CsF after stannylene activation in MeOH to give galactoside **35**. The remaining free hydroxyl groups were then benzylated with NaH/BnBr yielding **36** (42% yield over two steps). Removal of the allyl

group using $PdCl_2$ in boiling ethanol afforded acceptor **28** (45% yield, Scheme 20).

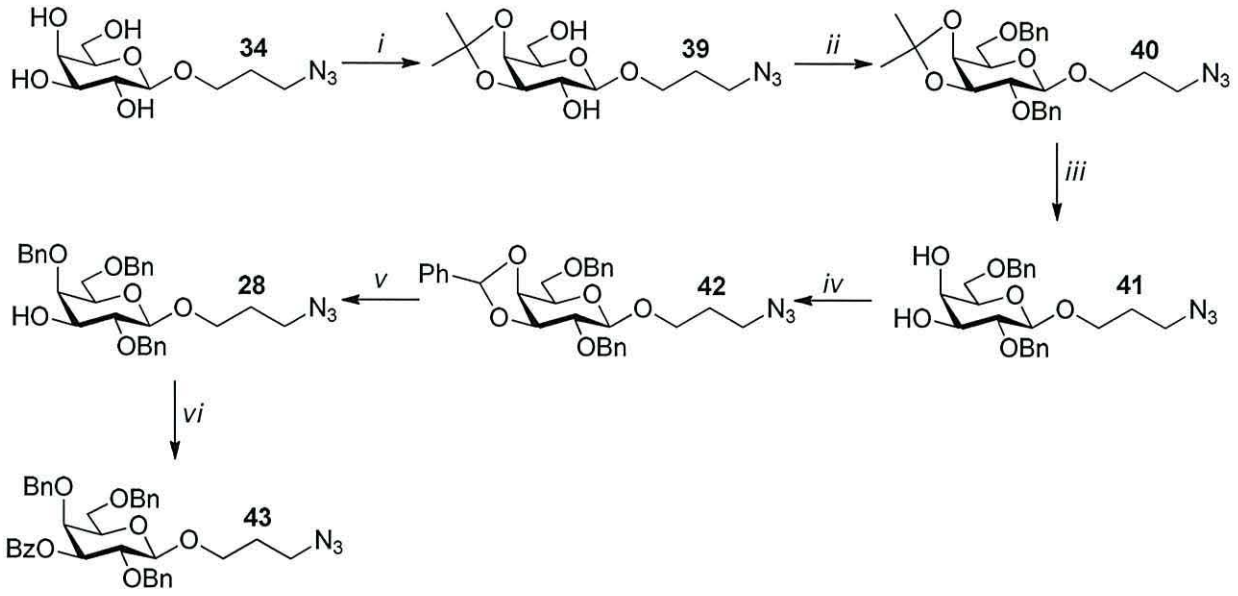


Scheme 20 Attempt to prepare acceptor **28**. *i.* 1. Bu_2SnO, MeOH 2. AllBr, CsF, DMF; *ii.* NaH, BnBr, DMF, 42% (over 2 steps); *iii.* $PdCl_2$, MeOH/EtOH 1:1, 45%.

Up-scaling this pathway gave unexpectedly dihydroxy galactoside **37** instead of the desired galactoside **28**. To confirm the structure, part of the product was benzoylated with benzoyl chloride in pyridine with DMAP giving **38** (^{1}H NMR, $CDCl_3$, δ 4.27 ppm (dd, $J_{5,6}$=7.0 Hz, $J_{6,6'}$=11.0 Hz, H-6), 5.18 ppm (dd, $J_{2,3}$=10.2 Hz, $J_{3,4'}$=3.1 Hz, H-3)). The reaction was tried again using dry toluene with methanol (1:1) and dry toluene instead of methanol but the resulting compound was again the dihydroxy galactoside **37**.

Thus, galactoside **34** was treated with 2,2-dimethoxypropane and CSA in acetone. The expected 3,4-isopropyl protected galactoside **39** was purified from the mixture in 56% yield. Benzyl bromide and sodium hydride in DMF

were used again for benzylation of the remaining free hydroxyl groups (**40**, 66% yield) before cleaving off the isopropylidene acetal group using 10% aqueous trifluoroacetic acid giving **41** in 95% yield [97]. Then the 3,4 benzylidene acetal derivative **42** was introduced using benzaldehyde dimethyl acetal and pTSA in dry THF. This reaction yielded the *exo* isomer with 95% yield. Solvent, concentrations and equivalents for the reagents were of importance to prevent *endo*/*exo* mixtures. Although close on TLC, a slight difference in the R_f value allowed removal of impurities of the undesired stereoisomer by chromatography. Acceptor **28** was prepared by regioselective reductive opening of the benzylidene acetal with $NaBH_3CN$ in dry THF. The product had to be carefully purified because remainders from the reducing agent reduced the reactivity of acceptor **28** during the glycosylation reaction significantly. The regioselective opening of the *endo* isomer would have given the benzyl ether on C-3 and the hydroxyl group on C-4 [98]. To establish the position of the free hydroxyl group, a sample of **28** was benzoylated giving **43** (Scheme 21). A clear downfield shift was observed (^{1}H NMR, $CDCl_3$, δ 5.05 ppm (dd, $J_{2,3}$=10.2 Hz, $J_{3,4}$=3.1 Hz, H-3)).

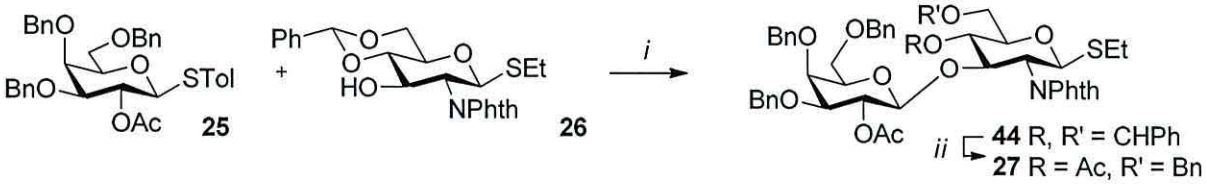


Scheme 21 New route to prepare glycoside **28.** *i.* 2,2-Dimethoxypropane, CSA, 56%; *ii.* BnBr, NaH, DMF, 66%; *iii.* TFA (90% aq), DCM, 95%; *iv.* $Bn(OMe)_2$, pTSA, THF, 95%; v. $NaBH_3CN$, HCl/Et_2O, THF, 90%.

3.4 Synthesis of the trisaccharide backbone - Convergent pathway

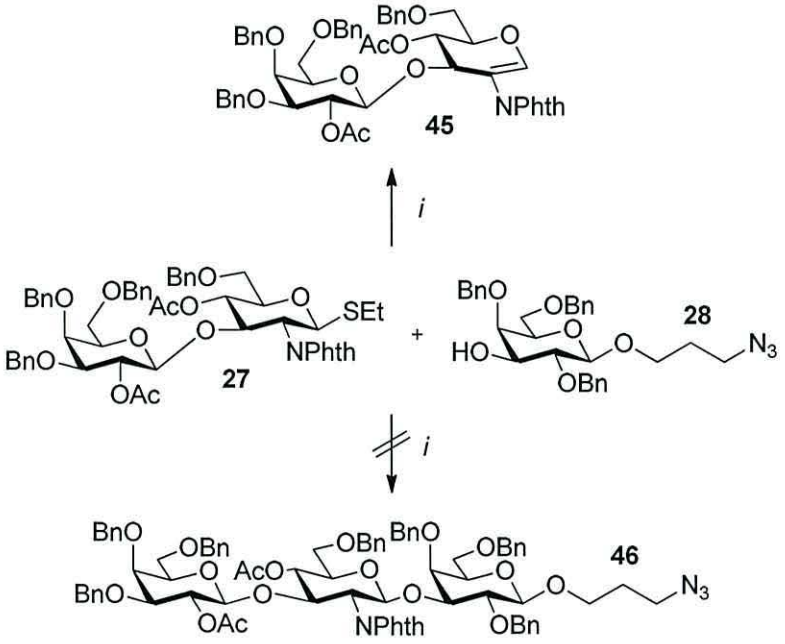
The first coupling between 2-*O*-acetyl galactoside donor **25**[99,100] and acceptor **26** employed the concept of chemoselective glycosylations[101]. The thiotolyl donor **25** was first converted into the corresponding α-bromogalactoside using bromine in DCM. Any excess of bromine was removed by co-evaporation with toluene prior to addition to thioethyl acceptor **26** to avoid reaction with its thio group. The coupling reaction was performed using AgOTf as promoter providing disaccharide **44** (60% yield). β-Selectivity was ensured by the participating acetyl group and

confirmed by NMR data ($J_{1'\text{-}2'}$=10.4 Hz). Also no orthoester, sometimes an issue when using acetyl groups as participating groups under Koenigs-Knorr conditions, was observed. It is generally accepted that the 4,6–benzylidene group has an influence both on the reactivity and the stereochemistry during coupling reactions[102]. From previous experience, it was found that the benzylidene acetal had to be removed when employing disaccharide **44** as glycosyl donor to achieve both regioselective selectivity and suppress the formation of elimination product **45**. Thus, the benzylidene acetal was first regioselectively reduced to give the benzyl ether on the C-6 position. The remaining free hydroxyl group was then acetylated giving donor **27** in 65 % yield over two steps (Scheme 22). After final acetylation, a significant downfield shift was observed (^{1}H NMR, $CDCl_3$, δ 4.97 ppm (dd, $J_{3,4}$=9.2 Hz, $J_{4,5}$=10. Hz, H-4)) confirming the regioselectivity of the reductive opening.



Scheme 22 Coupling to the disaccharide building block **27**. *i.* 1. Br_2, dry DCM; 2. 2,6-Di-*tert*-butyl-4-methylpyridine, AgOTf, 60%. *ii.* 1 $NaBH_3CN$, HCl in Et_2O, dry THF; 2. Ac_2O, pyridine, 65%;

The coupling reaction to the galactoside acceptor **28** was tried using NIS/AgOTf as promoter. According to TLC and a mass spectroscopic analysis of the crude mixture supported the presence of the expected trisaccharide **46**. However, NMR analysis of the isolated major product revealed that the elimination product from the donor **45** (Scheme 23) had been formed predominantly. Several unsuccessful attempts were made to improve the formation of the desired trisaccharide **46**, among others by changing the reaction temperature before this path was abandoned.

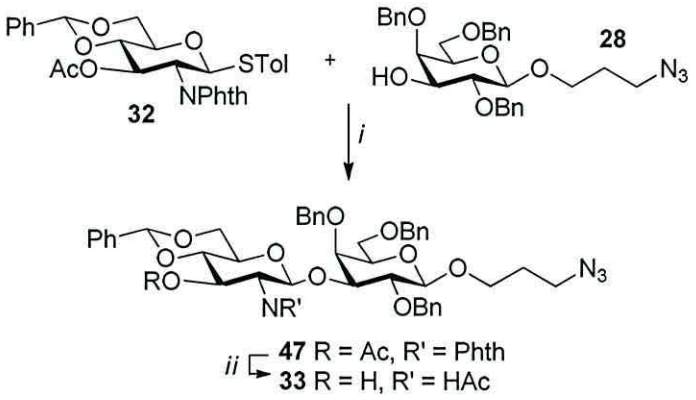


Scheme 23 Unsuccessful attempt to obtain the trisaccharide **46** by an [2+1] approach.

i. NIS, AgOTf, DCM.

3.5 Synthesis of the trisaccharide backbone - Linear pathway

For this approach, azidopropyl acceptor **28** was coupled with the 2-phthalimido protected donor **32**[103] giving disaccharide **47** (93 %). This coupling was performed with NIS and AgOTf as promoter system. To allow the coupling to the trisaccharide **48**, the acetate had to be cleaved off, and the phthalimido protecting group had to be converted into an acetate. The removal of the phthalimido group was achieved using ethylene diamine in ethanol (99 %). Cleavage of the phthalimido is often an issue and hydrazine, a highly hazardous reagent, is commonly used [104]. In this sequence, less dangerous ethylene diamine was used instead. The use of pure ethanol but not necessarily anhydrous is important for this reaction as the presence of impurities lead to unwanted side reactions. Still, the presence of too much water induces half opening of the phthalimido ring only. As partial *O*-acetate migration to the nitrogen occurred, remaining *O*-acetates were removed with NaOMe before the mixture of disaccharides was then *N*-acetylated in the presence of Ac_2O. The reaction slowed down as the pH decreased when forming AcOH. Buffering the reaction mixture by the addition of Et_3N prevented both the acid-base reaction between the free amine and AcOH and the cleavage of the acetal. The sequence afforded disaccharide **33** in 83 % yield (Scheme 24). The NMR shift of the amide CH_3 was significantly upfield shifted (^{1}H NMR, $CDCl_3$, δ 1.54 ppm (s, 3H, $NCOCH_3$)).

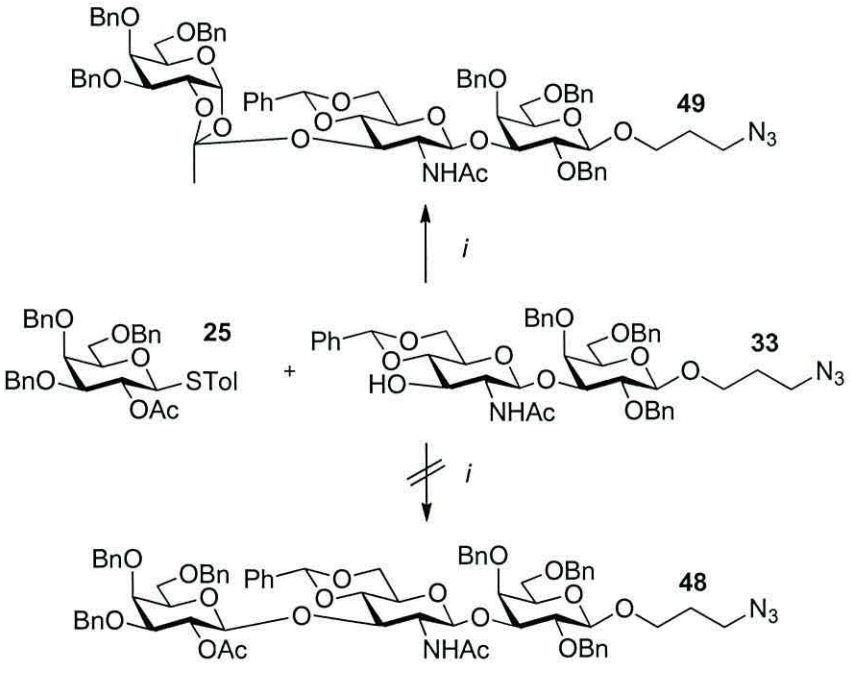


Scheme 24 Assembling the disaccharide **33**. *i.* NIS, AgOTf, CH_2Cl_2, 93%; *ii.* 1. EtOH/EDA (10:1), reflux; 2. NaOMe/MeOH; 3. Ac_2O, Et_3N, MeOH, 83%.

After one success allowing characterisations, several unsuccessful attempts were made to couple disaccharide acceptor **33** with the 2-*O*-acetylated galactosyl donor **25**. The temperature, the promoter system, and the solvent were varied. So, DMTST in Et_2O or DCM, and NIS/AgOTf in Et_2O or DCM were used at different temperatures (Table 1). Instead of getting the expected trisaccharide **48**, the orthoester **49** was obtained as major product in the best case (Scheme 25) (^{13}C NMR, $CDCl_3$, δ 57.2 ppm (orthoester-CH_3), 121.4 ppm (orthoester-CO_3)).

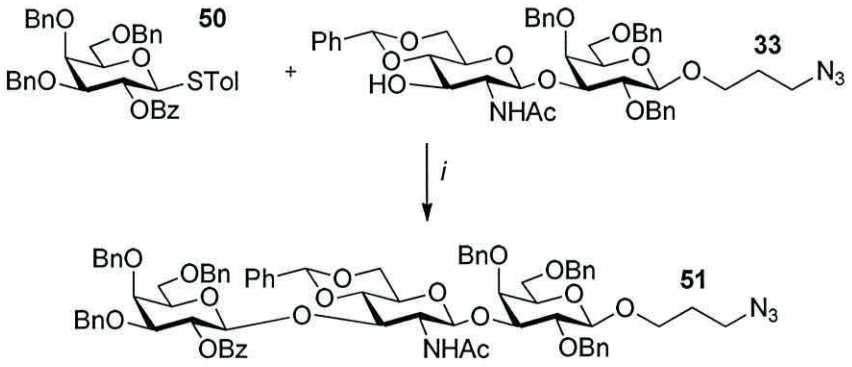
Table 1 Overview of the reaction conditions used for the couplings between galactoside donor **25** and disaccharide acceptor **33**.

promoter	Solvent	Temperature	Observation
NIS/AgOTf	DCM	RT	Degradation
NIS/AgOTf	DCM	0°C	Orthoester-degradation
NIS/AgOTf	DCM	-20°C	Orthoester-degradation
NIS/AgOTf	Et_2O	RT	Orthoester
NIS/AgOTf	Et_2O	0°C	Orthoester
NIS/AgOTf	Et_2O	-20°C	Degradation
DMTST	DCM	RT	Orthoester
DMTST	DCM	0°C	Degradation
DMTST	Et_2O	RT	Orthoester
DMTST	Et_2O	0°C	No reaction



Scheme 25 Attempts to produce trisaccharide **48**. *i*. NIS/AgOTf, DCM.

Since 2-*O*-acetates are prone to produce orthoesthers under glycosylation conditions[105], this group was changed to a benzoate giving donor **50**. With this new galactoside donor **50**[99], the reaction was achieved using the NIS/AgOTf promoter system at room temperature with 4Å molecular sieves, and the expected tetrasaccharide **51** was isolated as major compound in 54 % yield (Scheme 26).



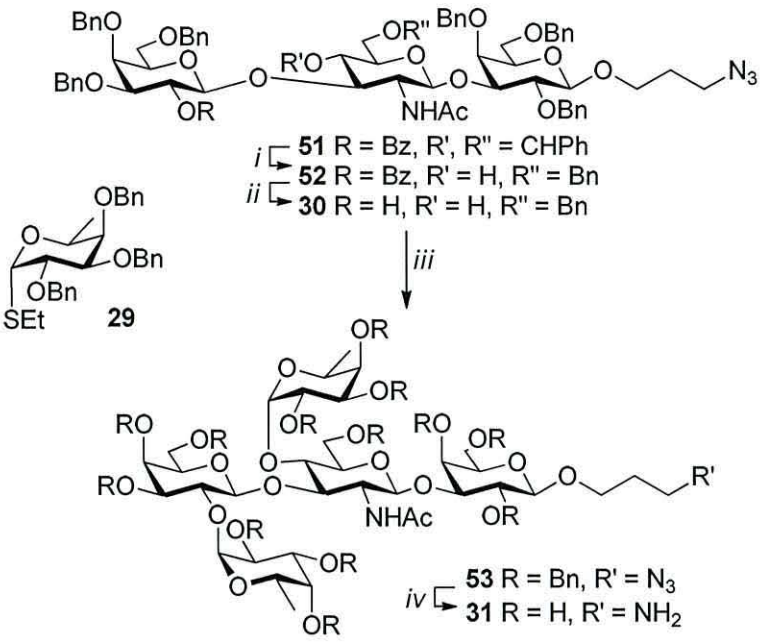
Scheme 26 Synthesis of the trisaccharide **48** with the new galactoside donor **47**.

i. NIS/AgOTf, DCM, 4Å, 54 %.

To prepare the trisaccharide backbone **30**, the protective groups had to be removed. Firstly, the benzylidene acetal was reduced with $NaBH_3CN$ in THF to give **52** before cleaving the benzoate with NaOMe affording the desired trisaccharide **30** (47 % yields over the sequence). As the transesterification was slow, heating was used to accelerate the reaction.

The α-fucosylation was then performed under halide-assisted reaction conditions using a one-pot procedure to avoid manipulation of the

bromofucose intermediate. Previously published works[94, 95] prepared the bromofucose separately which is the option when an orthogonal glycosylation step is required. Still, co-evaporating the excess of bromine before dissolving and adding the donor to the reaction mixture increases the risk of hydrolysis. A possibility is to quench the excess of bromine with an alkene like cyclohexane but this often slows down the subsequent glycosylation step possibly due to dilution effects. Since orthogonality was not an issue in this fucosylation, donor **29** and acceptor **30** were dissolved in DCM together with tetrabutyl ammonium bromide and activated 4Å molecular sieves before adding bromine, and the reaction mixture was stirred overnight at room temperature. The expected pentasaccharide **53** (67 %) was isolated and then deprotected using Pd/C and H_2 in EtOAc/EtOH (95 %)/H_2O (2:2:1) yielding the deprotected pentasaccharide **31** with 82 % (Scheme 27). Deprotection of the pentasaccharide and the reduction of the amine were concomitant. To avoid poisoning the catalyst, equimolar aqueous HCl was used during hydrogenolysis [106].



Scheme 27 From the trisaccharide **51** to the deprotected pentasaccharide **31**. *i*. $NaBH_3CN$, HCl in Et_2O, THF; *ii*. NaOMe, MeOH, 47 % (2 steps); *iii*. 1. Et_4NBr, CH_2Cl_2/DMF (10:1), 4Å MS, rt; 2. Br_2, 24 h, 67 %; *iv*. H_2 (1 atm), 10% Pd/C, HCl, EtOAc/EtOH/H_2O, rt, 24 h, 82 %.

3.6 BSA conjugation

The Lewis[b] pentasaccharide is intended to be used in affinity studies. Depending on the technique, different conjugates need to be prepared. To allow the conjugation to BSA, a linker has to be introduced between the protein and the oligosaccharide. Different linkers, for example squaric acid [107], can be used but the linker influences the binding affinity between the carbohydrate and the protein. Different aspects have to be considered. The major issues are the distance to the carrier to improve availability of the substrate, the polarity of the linker to avoid electrostatic interactions

with the carrier surface, the density of substrate on the surface of the carrier, and the sterical accessibility of the substrate for the receptor (Fig. 14) [108]. In this study DSS was chosen as it has been previously used in our group with success[95].

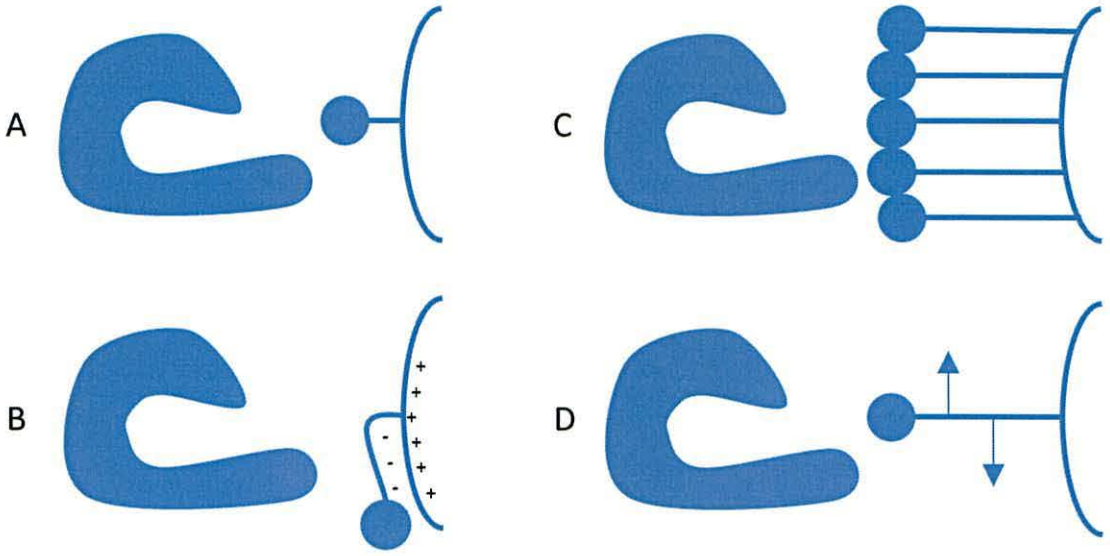
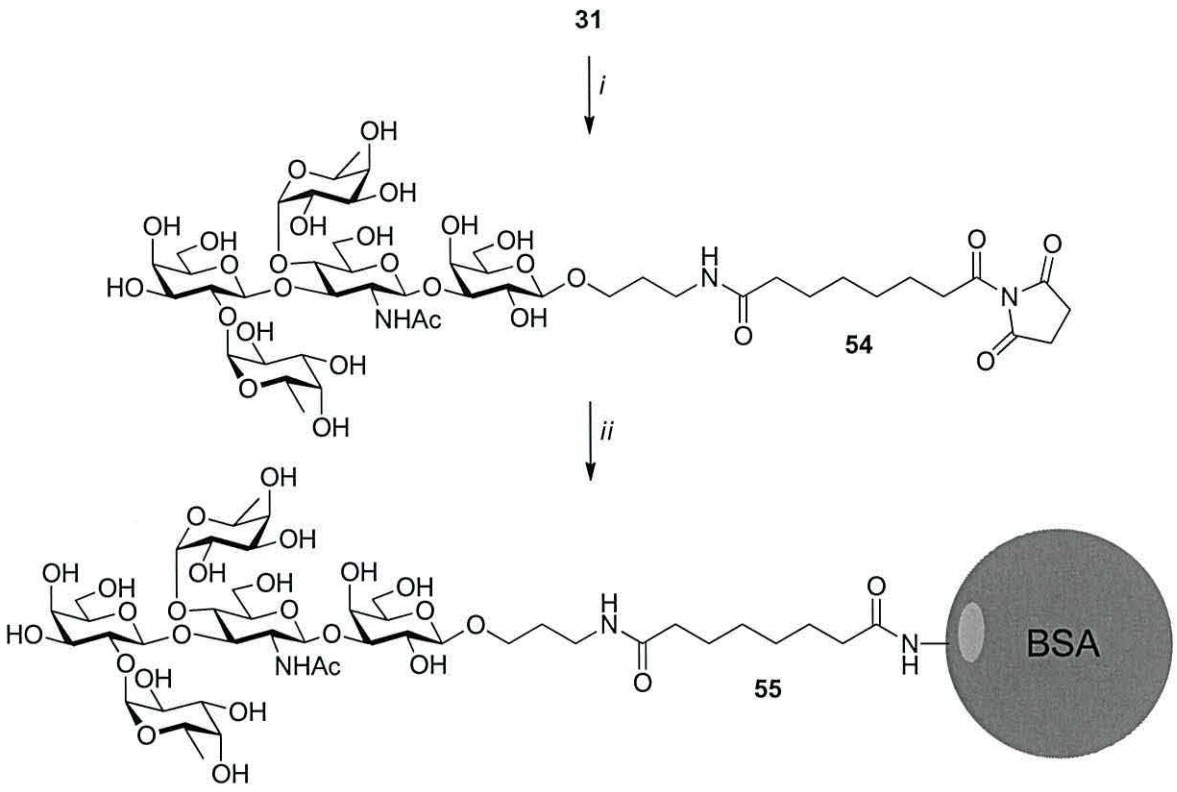


Figure 14 Some aspects to be considered when designing linkers; A: length. B: polarity. C: density on surface. D: steric hindrance.

Firstly, the pentasaccharide **31** was reacted with DSS in DMSO in the presence of Et_3N to obtain **54**. The reaction was monitored with MALDI-TOF. The pentasaccharide **54** with the activated linker was then mixed with BSA to afford **55** with an average of 15 incorporations (Scheme 28 and appendix for calculation)



Scheme 28 Preparation of the BSA conjugate. *i.* DSS, Et_3N, DMSO; *ii.* BSA, pH 10 buffer, H_2O.

3.7 Conclusion

Attempts were made to adapt the convergent approach that had been used earlier for the synthesis of the hexasaccharide. The linear pathway was then followed to prepare the linear trisaccharide backbone. As scaling-up the preparation of the starting galactoside **28** was unsuccessful, a new pathway to prepare it was investigated. Considering that the coupling with galactoside **25** carrying a 2-*O*-acetate led to the formation of an orthoester trisaccharide, this donor had to be changed for galactoside **50** carrying a 2-*O*-benzoate. In summary, the pentasaccharide of the Lewis^b family was successfully prepared introducing the two fucosyl units in one stereoselective coupling into the trisaccharide backbone prior to deprotection. The final BSA conjugate will be sent to collaborators to study BabA affinity.

4 Synthesis of the B-Lewis^b heptasaccharide (paper II)

4.1 Background

In the ABO blood group system, A and B blood groups consist of a combination of the O antigen with the A and B determinant respectively (Fig. 15).

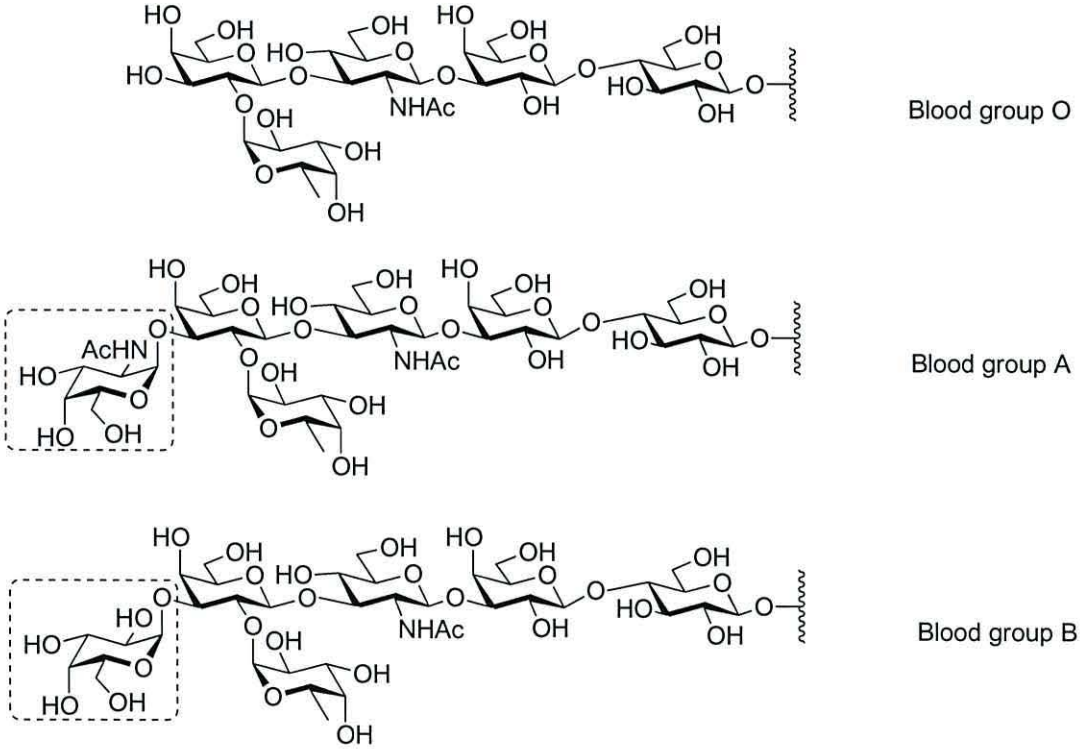


Figure 15 Blood group antigens

In the Lewis system, it is the same and the A-Lewis^b and B-Lewis^b antigens are combinations of the A or B determinant with the Lewis^b antigen (Fig. 16).

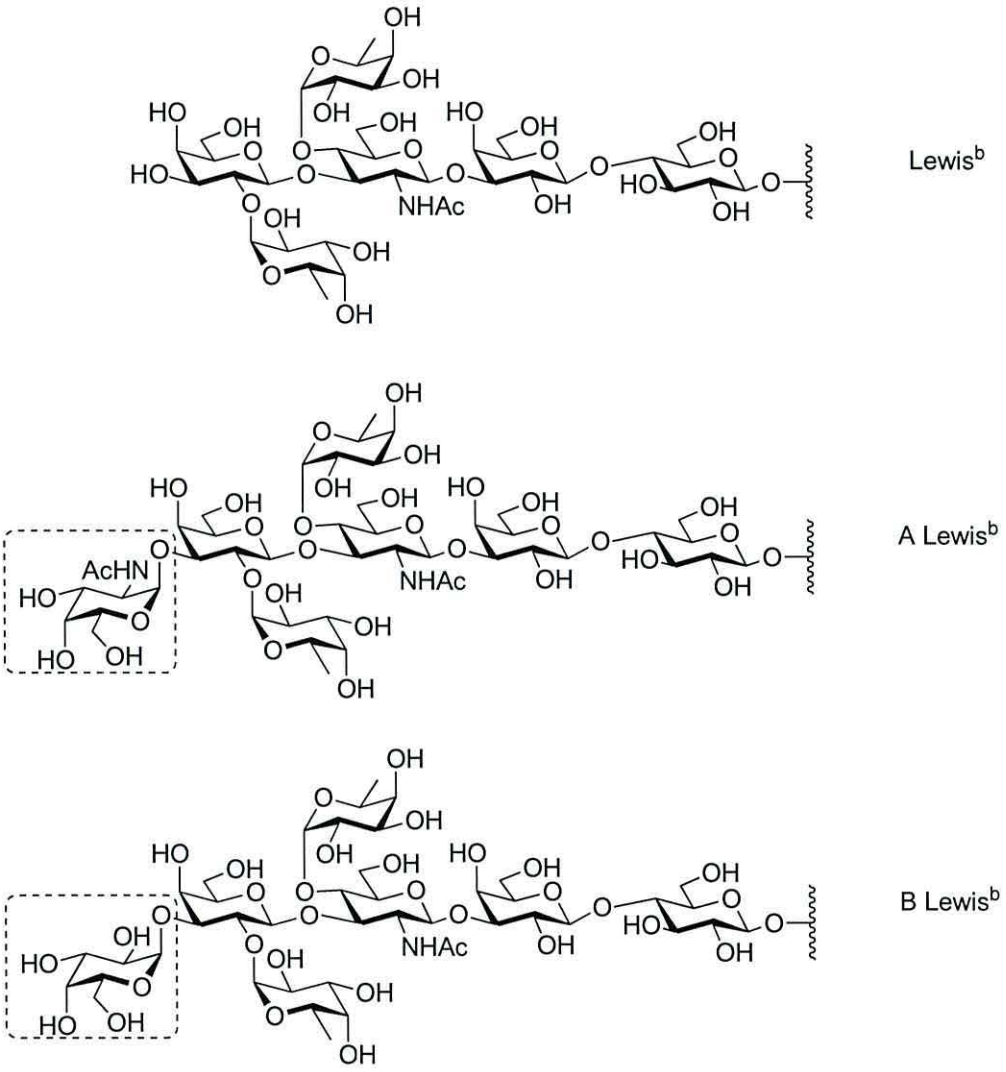


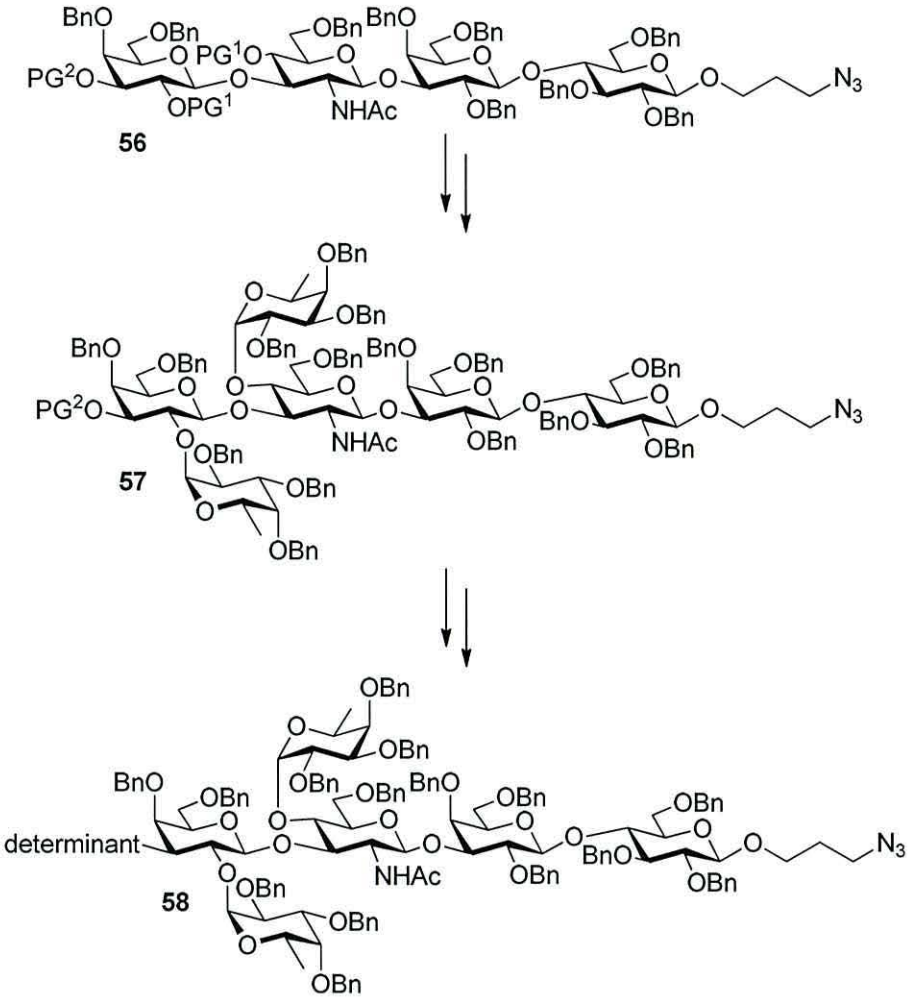
Figure 16 Blood group antigens

4.2 Retrosynthesis

Since the synthesis of building blocks is relatively time consuming and the target oligosaccharides are quite similar, it was decided to reuse as many

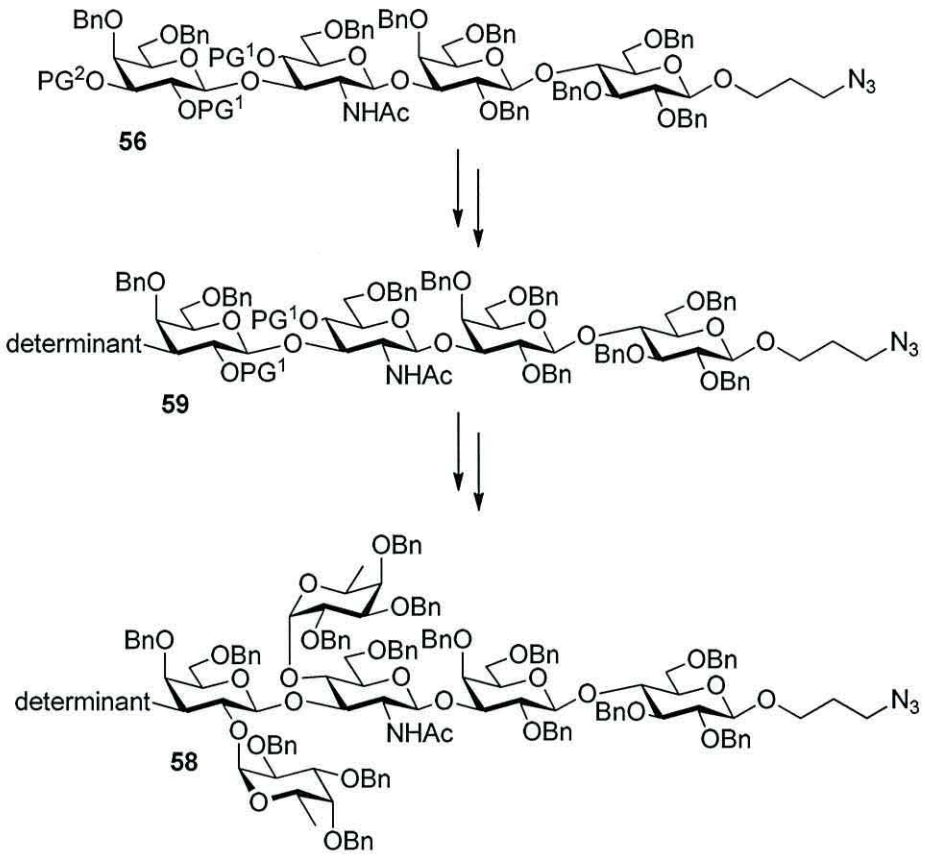
building blocks as possible from the earlier elaborated pentasaccharide synthesis [109]. However, the synthetic pathway should allow access to both the A-Lewis^b and the B-Lewis^b heptasaccharides.

The first idea was to prepare a suitable protected Lewis^b hexasaccharide **57** (Scheme 29) followed by introduction of the respective determinant (**58**). This pathway required a new building block for the non-reducing end galactose, equipped with a 3-*O*-protecting group that is inert against treatment with bromine and orthogonal to the other protecting groups used. A possible risk with this pathway is that the fucosyl residues have to survive a subsequent glycosylation reaction. Perbenzylated fucosyl residues are known to be less stable and thus easy to cleave off.



Scheme 29 The first pathway: introducing the fucoses before the determinant.

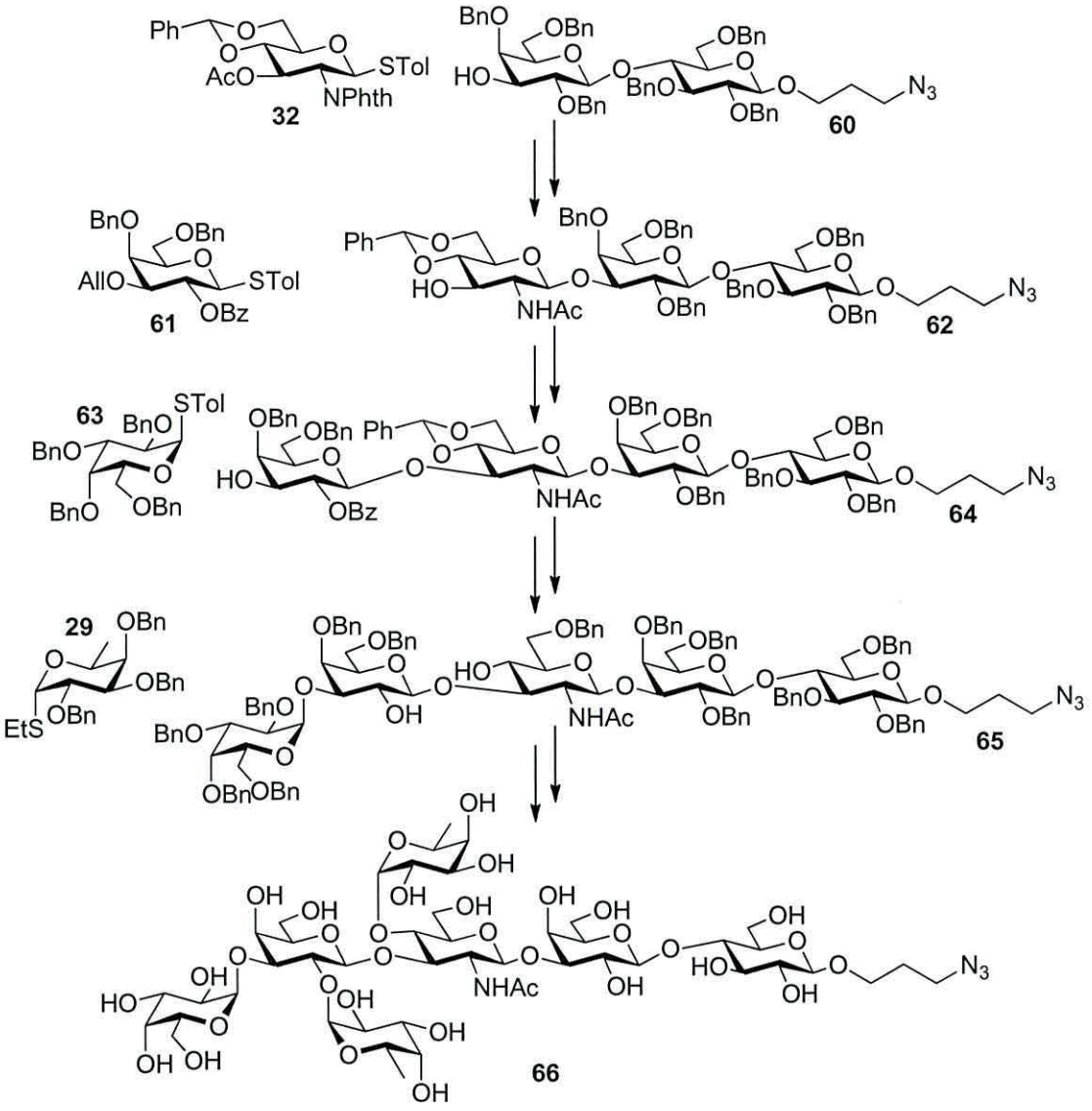
Another option was to synthesise the linear pentasaccharide **59**, including the respective determinant, before introducing the fucosyl residues (Scheme 30). A minor disadvantage of this pathway is that the differentiation between the A-Lewis^b and B-Lewis^b has to be done on the pentasaccharide level. Also in this case a new galactosyl building block is required.



Scheme 30 The second pathway; introducing the determinant before the fucoses.

Both pathways share the key tetrasaccharide **56** (Scheme 29 and 30). While it was expected that subsequent glycosylations and protecting group chemistry would be similar to those in the Lewis^b pentasaccharide synthesis, the efficiency of the synthetic sequence had to be improved. Also, a suitable galactosyl donor had to be found.

It was decided to go for the second pathway as it permits more flexibility in the choice of the 3-*O*-galactosyl protecting group required for the attachment of the determinant and less chemical transformations with intermediates carrying the labile fucosyl residues (Scheme 31).



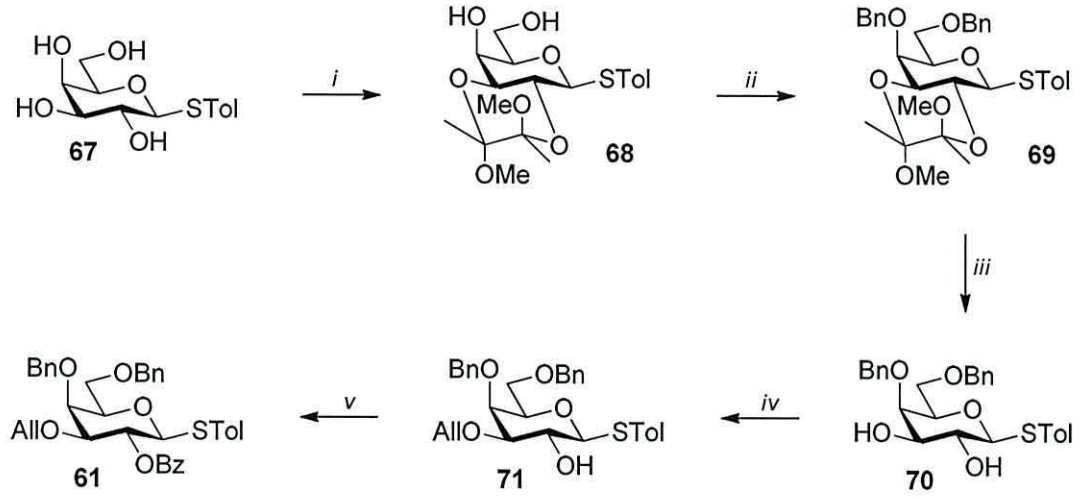
Scheme 31 Projected synthetic scheme for the B-Lewis^b heptasaccharides.

4.3 Synthesis of the B-Lewis^b heptasaccharide

*4.3.1 Galactoside **61***

As orthogonal protecting group, the versatile allyl ether protecting group was selected for the key galactosyl building block. The reliable synthesis

of a similar building block had been developed earlier in our group [110]. Except for the change to the thiotolyl group instead of the previously used thioethyl group, only slight improvements in the yields were made for the preparation of this galactoside. The advantages in using thiocresol instead of ethane thiol are not only a matter of less odour but also that a smaller excess of reagent is required and that the products are more prone to be crystalline reducing the need of chromatographic purification. Starting from deprotected galactose **67**[111] with a thiotolyl residue as leaving group at the anomeric position, a temporary BDA protecting group was introduced regioselectively at the 2-*O* and 3-*O* position to obtain the desired compound **68**[112]. The two remaining unprotected hydroxyl groups were then protected with benzyl ether groups giving compound **69** before the temporary BDA was cleaved off in aqueous TFA to yield the expected diol **70** in 70% yield over the 3 steps. The last two hydroxyl groups had to be discriminated for selective protection. Several options were possible but in the 2-*O* position a participating group was required to ensure β-selectivity in the glycosylation step, while the 3-*O* protection group had to be stable under basic conditions. The 3-OH was selectively activated using a stannylene acetal before introducing an allyl ether giving compound **71**. The remaining hydroxyl group was protected with a benzoate ester, yielding the galactoside **61** in 64% over the 2 steps (Scheme 32). A benzoate ester was chosen to avoid orthoester formation as seen earlier during the pentasaccharide synthesis when using an acetate protecting group.



Scheme 32 Synthesis of the key galactoside **61**. *i.* 1,2-butanedione, trimethylorthoformate, CSA, MeOH; *ii.* NaH, BnBr, DMF; *iii.* TFA/H_2O 10:1, DCM (70 %, 3 steps); *iv.* 1. Bu_2SnO, MeOH; 2. AllBr, CsF, MeCN; *v.* BzCl, DMAP, pyridine (64 %, 2 steps).

4.3.2 Assembling the heptasaccharide

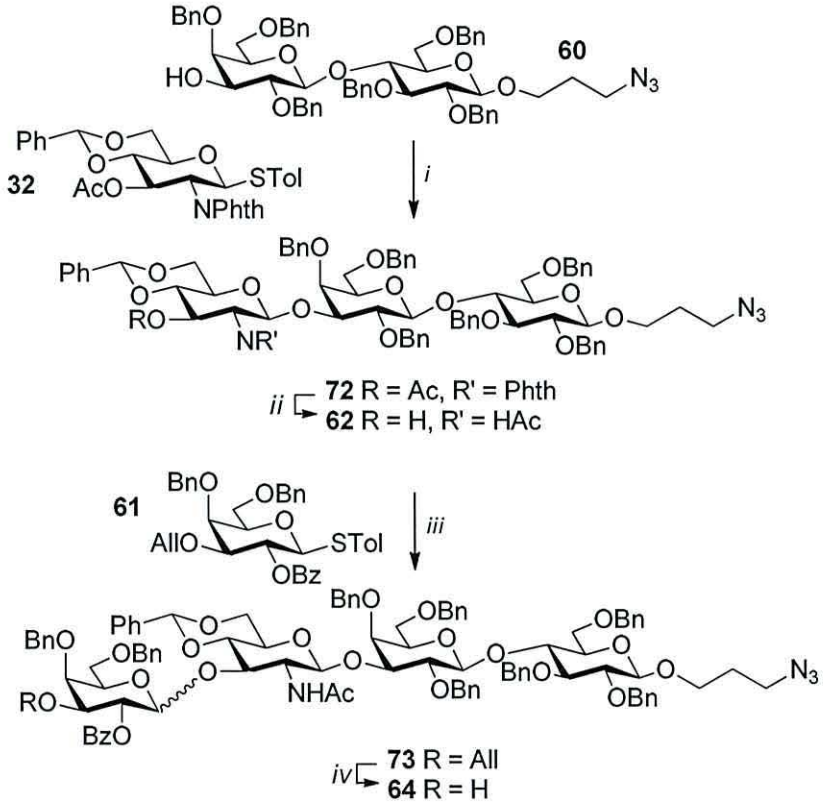
The heptasaccharide was assembled using the experience gained during the syntheses of the pentasaccharide discussed in a previous chapter and the hexasaccharide obtained earlier in our group[95].

The first coupling involved lactoside **60** as acceptor and the phthalimido protected glycosylamine donor **32**. The coupling was carried out using NIS and AgOTf as promoter system giving the expected *N*-phthalimido protected trisaccharide **72** in 78% yield. The protecting group pattern was then modified, removing the phthalimido group first, followed by deacetylation in C-3 and selective acetylation of the free amine in C-2. Due to the experienced difficulties to reproduce the acetylation of the resulting free amine during the pentasaccharide synthesis, another

pathway was investigated. After removing the phthalimido protecting group, the amine was first acetylated using acetic anhydride in pyridine. During the process, the deprotected hydroxyl group obtained via migration of the acetyl group to the free amine was acetylated as well. The last step to change the pattern was the deprotection of the 3-hydroxyl group to allow the next coupling. Using NaOMe, the acetyl ester was cleaved off via transesterification to yield compound **62** with 88% over the sequence. This pathway was more reproducible than the previous protecting group manipulation despite the detour thus reacetylating the free hydroxyl group in C-3 before deacylation.

The next coupling was achieved by reaction with the trisaccharide **62** and the new donor **61** using NIS and AgOTf as promoter system giving the expected tetrasaccharide **73** as α/β mixture (20%/80%). Due to large signal overlap, several signals were used to establish the obtained α/β ratio [significant signals used for the calculation: ^{1}H NMR, $CDCl_3$, δ 5.5 ppm (dd, *J*=9.2 Hz and 10.0 Hz, H(β)-2''''; 5.59 ppm (dd, *J*=4.0 Hz and 10.5 Hz, H(α)-2''''); 5.64 ppm (m, (β)OCH_2CHCH_2); 5.77 ppm (m, (α)OCH_2CHCH_2)]. Several experiments changing both the ratio of NIS and AgOTf as well as the temperature to promote the formation of the β linkage were attempted without success. The unwanted α diastereoisomer was removed by flash column chromatography affording the desired β diastereoisomer in 72% yield. Before the next coupling, the allyl ether was isomerised using a catalytic amount of 1,5-cyclooctadienebi(methyldiphenylphosphine) iridium(I) catalyst followed by

hydrolysis of the vinyl ether intermediate with NIS giving the expected tetrasaccharide acceptor **64** in 68% yield. Palladium(II) chloride was tested as well to cleave the allyl protecting group but the yield was lower because of hydrolysis of the benzylidene acetal, possibly due to small amounts of HCl originating from impurities in the catalyst (Scheme 33).



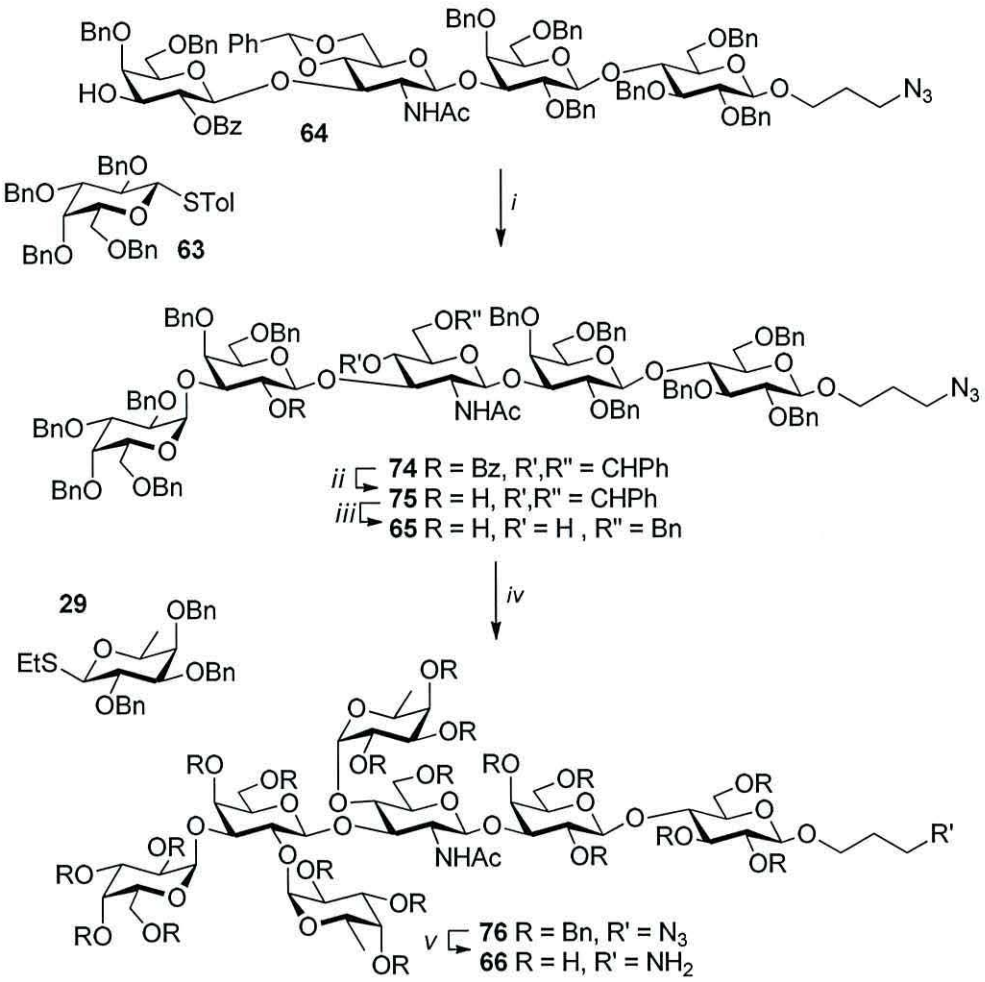
Scheme 33 From lactoside **60** to tetrasaccharide **64**. *i.* NIS, AgOTf, DCM, 78%; *ii.* 1. EDA, abs. EtOH; 2. pyridine Ac_2O; 3. MeOH, NaOMe, 88 %; *iii.* NIS, AgOTf, DCM, 72 %; *iv.* 1,5-cyclooctadienebi(methyldiphenylphospie) iridium(I) PF_6, THF, NIS, H2O, 68%.

At this stage, the determinant could be added. The coupling was done with the B determinant as it is cheaper to synthesise. To get the required α linkage, a halide assisted coupling was carried out again as one-pot

reaction in DCM as described in a previous chapter. Usually the halide assisted couplings are done in a two steps sequence, this is, the glycosyl bromide is produced in a different flask and then added to the reaction mixture. In the one-pot procedure, the tetrasaccharide acceptor **64** was dissolved in DCM together with donor **63**. Then, TBAB and 4Å molecular sieves were added, and after one hour stirring at room temperature, bromine was dropped into the reaction mixture to form the bromogalactoside "in situ". The mixture was left stirring overnight for complete conversion to give the target pentasaccharide **74** in 79% yield after chromatography ^{1}H NMR, $CDCl_3$, δ 5.25 ppm (d, *J*=5.1 Hz , (α)H-1''''').

A sequence of protecting group manipulations was necessary to prepare the pentasaccharide for the last coupling. Benzoyl esters are routinely removed under basic conditions or via catalytic hydrogenation. The latter was not an option due to the presence of benzyl ethers as permanent protecting groups, but sometimes a variation in the base can produce better result. The benzoyl ester was removed via transesterification using NaOMe but the reaction was slow. Increase of the temperature from room temperature to 40 °C afforded the expected pentasaccharide **75** in 52**%** yield. Also, NaOH was tried but the reaction was as expected even slower and more degradation products were formed. Subsequently, the benzylidene acetal was opened towards the 6-*O* position under reductive conditions. The reaction was carried out in the presence of $NaBH_3CN$ and HCl in THF at room temperature giving the diol pentasaccharide **65** in 62%

ready for coupling. The coupling to the heptasaccharide was done following the halide assisted protocol as described previously. The bromo donor was prepared "in situ" to provide the coupling between pentasaccharide **65** acceptor and fucoside **29** The expected heptasaccharide **76** was isolated in 83% yield (^{1}H NMR, $CDCl_3$, δ 5.25 ppm (d, *J*=2.9 Hz, H-1^{fuc-1}); 5.57 ppm (d, *J*=3.0 Hz, H-1^{fuc-2})). The final deprotection of the benzyl ether and concomitant reduction of the azide was carried out with Pd/C and H_2 in EtOH. Again, one equivalent of HCl was added to avoid catalyst poisoning. The reaction was followed by mass spectroscopy and took 4 days at atmospheric pressure. Aliquots of water were added after 2 and 3 days to allow the partially and fully deprotected heptasaccharides to be soluble in the reaction medium. After P2 size exclusion column, the final deprotected heptasaccharide **66** was obtained in 85% yield, ready for conjugation to a carrier (HR-ESI calcd for $C_{47}H_{82}N_2O_{34}Na$ $[M+Na]^+$ 1218.4749. Found 1218,3956) (Scheme 34).



Scheme 34 from tetrasaccharide **64** to deprotected heptasaccharide **66**. *i*. TBAB, Br_2, DCM, 79%; *ii*. NaOMe, EtOH, 52%; *iii*. $NaBH_3CN$, HCl in ET_2O, THF, 62%; *iv*. TBAB, Br_2, DCM, 83%; *v*. Pd/C, H_2, $EtOH/H_2O$, 85%.

4.4 Conclusion

The targeted B-Lewis^b heptasaccharide **66** was successfully synthesised, following a linear approach. Our experience for the synthesis of the Lewis^b pentasaccharide was well applied both for the numerous protecting group manipulations and the strategic choices for the protecting group patterns. A BSA heptasaccharide conjugate will be prepared for further BabA affinity studies.

5 Selenoglycosides

5.1 Background

To understand cellular processes, knowledge of the three-dimensional structure of lectins and other macromolecules is important. Two techniques are widely used for the structural determination of macromolecules at atomic resolution: X-ray diffraction of crystals and NMR. While NMR does not require crystals and provides more detailed information on the dynamics of the molecule in question, it can generally be used only for compounds with a molecular weight of less than 30,000. X-ray crystallography can be applied to compounds with molecular weight up to at least 10^6. For many proteins, the difference is decisive in favour of X-ray diffraction[113].

Five different techniques exist for analysing X-ray diffraction spectra. The direct determination method is used for small molecules. The position of the different atoms is determined directly by spectra analysis. High resolution data are necessary. For the molecular replacement technique, a known structure is required, which serves as a model for the unknown structure. Homology in the amino acid sequence is an indication of whether a model is suitable. The structure of the model is borrowed and refined. Molecular replacement is the most rapid method but requires a known homologous protein structure.

The isomorphous replacement technique is based on the introduction of heavy atoms at a few specific positions. The X-ray diffraction pattern of the native protein crystal is compared with that of a crystal of the same type but containing, in addition, at least one heavy atom. The intensity differences between the native and the labelled patterns are then exclusively due to the attached heavy atoms (Fig 17). Intensity of heavy atoms in the isomorphous replacement method is proportional to the atomic numbers of the heavy atoms. The position of the heavy metal is then determined.

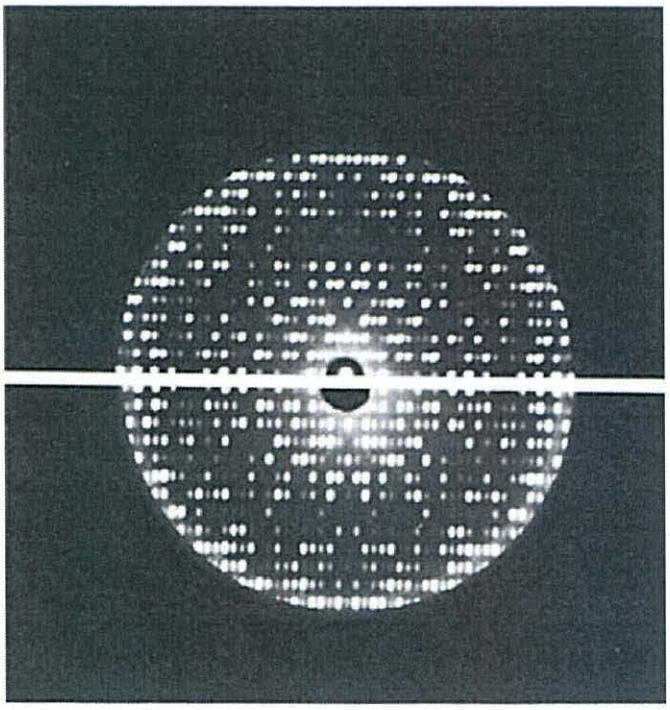


Figure 17 Intensity differences between the native (top) and heavy derivatives (bottom) crystals from [113].

Single- and multiple-wavelength anomalous diffraction (SAD and MAD respectively) are, with the isomorphous replacement, the most generals methods. In those methods, the difference in intensity can profitably be exploited. In general, the atom used for anomalous scattering is selenium. The wavelength of extension of this atom is 0.98 Å, in the used wavelength range. In the MAD, the wavelength dependence of the anomalous scattering is used[114]. Due to anomalous effect small changes of the wavelength around the absorption edge of the anomalous atom produce measurable intensity differences in the diffraction pattern. The principle of this method is rather old, but it was the introduction of the tuneable synchrotron radiation sources that made it a technically feasible method for protein structure determination. Of course, the protein should contain an element that gives a sufficiently strong anomalous signal. Therefore, the elements in the first periods of the periodic system are not suitable. For instance, one selenium is enough for 150 amino acids[115]. One way to introduce Se into a protein is by growing a micro-organism on a selenomethionine-containing substrate instead of a methionine-containing substrate. Condition for application of the method is that the wavelengths are carefully chosen to optimize the difference in intensity; usually, diffraction data are collected at three different wavelengths.

The disadvantages of MAD technique are the collection of data at three different wavelengths, the inherently long exposure time, and the danger of radiation damage to the crystal. These disadvantages are much less serious if the crystal structure could be solved by data collection on a

single crystal with one wavelength only. A single wavelength is also sufficient in isomorphous replacement, but native and derivative crystals are required. For a structure determination with SAD, the crystal must contain an anomalous scatterer that provides a sufficiently strong anomalous signal[116]. The anomalous atom position is determined and the structure analyse starts from that atom.

Selenium is a useful atom for X-ray structural study of proteins[117] and has been shown to be good tool for the structure elucidation of several lectins [118]. Different seleno derivatives from common mono- and disaccharides as well as the selenated Lewis^b determinant were synthesised to be used as ligands for structure analysis of lectins.

5.2 Common mono- and disaccharides

5.2.1. Synthetic pathway

To provide ligands that can be recognised by the studied lectins, the selenomethyl group was chosen as it is the smallest and the least prone to unfavourably interact with the protein.

The synthesis of β-methylselenoglucoside was already described by Stick *et al.* This group synthesised both peracetylated and perbenzylated derivatives after reducing dimethyldiselenide with sodium borohydride in ethanol. No α-anomer was produced.

Stick's general approach was already an adaptation of a work published earlier by Benhaddou *et al*. Alkyl/aryl α- and β-selenoglucosides were prepared either from reduction of dialkyl or diaryl diselenide or from reduction of respective α- and β-diglucosyl diselenides via reduction using potassium borohydride in ethanol and acetonitrile. First potassium phenyl selenolate was generated "in situ" using potassium borohydride in ethanol and 2,3,4,6-tetra-*O*-benzyl-α-glucopyranolsyl chloride was added to form phenyl-β-glucosyl selenide. Generalisation of this approach was made with dimethyl and dimethyl diselenides. Treating 2,3,4,6-tetra-*O*-benzyl-α-glucopyranolsyl chloride with selenourea lead to α-diglucosyl diselenide. The anomeric configuration was retained. Further reduction with potassium borohydrate and the desired alkyl halide afforded target alkyl α-selenoglucoside. 2,3,4,6-Tetra-*O*-acetyl-α-glucopyranolsyl bromide was used to prepare β-diglucosyl diselenide leading to alkyl β-selenoglucoside following the same treatment as above. The synthesis of phenyl selenoglucosides were first reported by Bonner and Robinson [121] using selenophenol and potassium hydroxide in ethanol yielding phenyl tetraacetyl-β-D-selenoglucoside. Witczak and Whistler [122] proposed the synthesis of selenoglycoside from "in situ" reduction of diphenyl diselenide with hypophosphorus acid. The resulting phenyl selenol reacted with per acetylated glycosides and BF_3 to give α- or β-seleno derivatives.

Selenoglycosides could also be prepared from glycals. Frenzel *et al*[123] described the use of Brigl's anhydride with selenophenol followed by acetylation to obtain α anomer. Santoyo-Gonzales *et al*[124] demonstrated

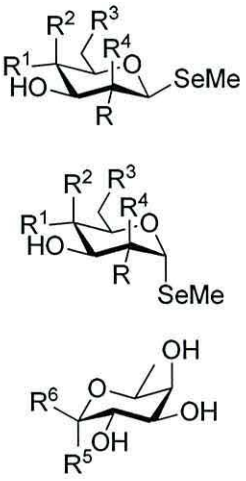
that glycal could lead to 2-amino-2-deoxy-selenoglycosides via azidophenylselenation using (diacetoxyiodo)benzene, diphenyl diselenide and sodium azide in DCM but α/β mixtures were obtained.

Sakakibara *et al*[125] published the synthesis of phenyl selenoglucosides using phenyl trimethylsilyl selenide in the presence of a catalytic amount of trimethylsilyl triflate. Sato *et al*[126] reported selenoglycosidation using $Me_2Sn(SePh)_2$ and $Bu_2Sn(OTf)_2$ in DCM but yielding a α/β mixture. More recently other selenosylcosidation techniques were published. Mukherjee *et al* [127] described zinc-mediated cleavage of deselenide bond. Tiwari and Misra[128] published indium iodide mediated cleavage of diselenides. They both investigated the reaction with glycosyl halides and obtained selenoglycosides with retention of configuration.

Other selenoglycosides derivatives were described. Knap and Darout [129] first described the use of selenocarboxylates to prepare 1-seleno-glycoside derivative. From 1-bromo-α-glucoside as starting material, only the β product was observed. From glycal, only the 2-deoxy-1-seleno-β-D-glucopyranose derivative was obtained. Kaway *et al*[130] published the synthesis of β-*p*-methylselenoglycosides starting from potassium *p*-methylselenobenzoate and α-glycosyl bromide. After activation of the selenoester function with a secondary amine and caesium carbonate, selenolate anions were produced and reacted "in situ" with eletrophiles to give selenoglycosides. Retention of configuration was observed. Nanami *et al*[131] described the same procedure with β-glycosyl chloride, yielding α-

p-methylselenoglycosides. After activation, α-selenoglycosides were synthesised with retention of configuration.

Direct anomerisation is difficult for *O*-glycosides but generally easier for thioglycosides [132], thus it is expected to be easy for selenoglycosides as well. Both α and β isomers are of interest. None of the already published methodologies described an easy way to reach both anomers. A simple route to the two anomers using a common precursor is investigated. The pathway should be efficient in term of yield and reproducibility for different common mono- and disaccharides (Fig 18).



77 βGlcSeMe R=R^1=R^3=OH, R^2=R^4=H
78 αGlcSeMe R=R^1=R^3=OH, R^2=R^4=H

79 βGalSeMe R=R^2=R^3=OH, R^1=R^4=H
80 αGalSeMe R=R^2=R^3=OH, R^1=R^4=H

81 βLacSeMe R=R^3=OH, R^1=OGal, R^2=R^4=H

82 αManSeMe R^1=R^3=R^4=OH, R^1=R^2=H

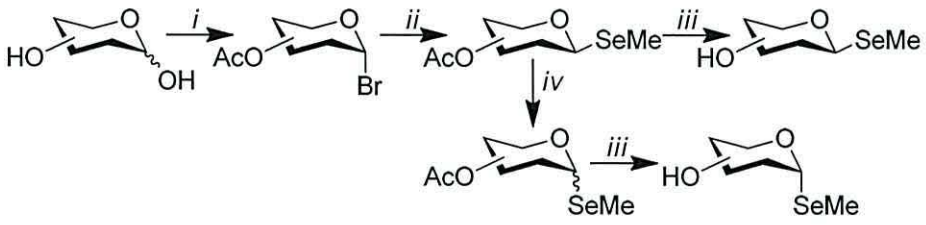
83 βFucSeMe R^5=H, R^6=SeMe
84 αFucSeMe R^5=SeMe, R^6=H

Figure 18 Different common mono and disaccharides of interest.

All glycosides were produced using a modified version of the published pathway (Scheme 35). They were first fully protected using acetyl groups. This protection was chosen because acetyl groups are easily introduced, cleaved and give the preferred β or α anomer as major product due to

participation effect. To functionalise the anomeric centre for the substitution reaction, glycosyl bromides were prepared. The selenium group was then introduced before final deprotection.

For the anomerisation reaction a Lewis acid were used.



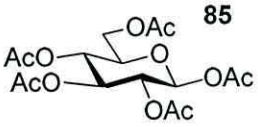
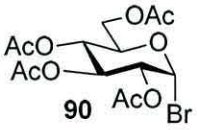
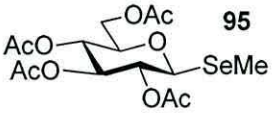
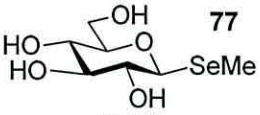
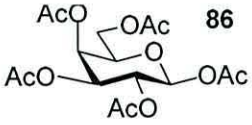
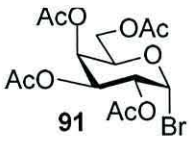
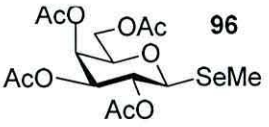
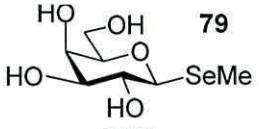
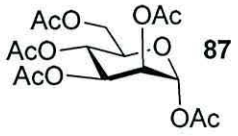
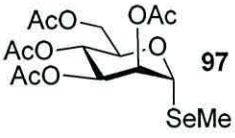
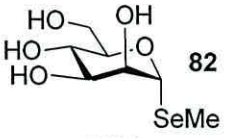
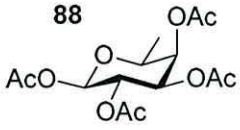
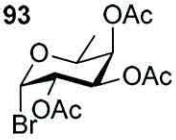
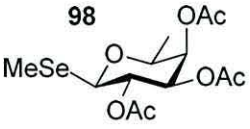
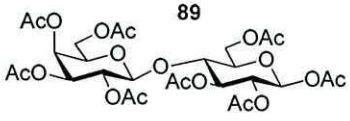
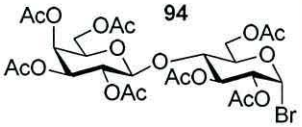
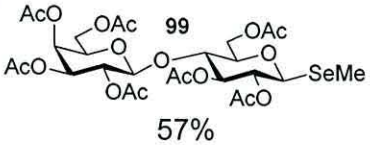
Scheme 35 The common reaction scheme for the synthesis of 2-hydroxyl seleno derivatives. *i.* 1. Ac_2O, NaOAc; 2. HBr/HOAc; *ii.* 1. Se_2Me_2, $NaBH_4$, MeCN; 2. glycosyl bromide, MeCN; *iii.* NaOMe, MeOH; *iv.* $BF_3{\cdot}Et_2O$, DCM.

5.2.2 Synthesis

The unprotected sugars were acetylated in hot acetic anhydride, containing sodium acetate as buffer[10], except for mannose. The fully protected α-mannose was prepared using acetic anhydride in pyridine[133]. The anomeric position had to be activated towards nucleophilic substitution. Thus, the peracetylated products were converted into the bromoglycosides by stirring in HBr/HOAc at 0°C[10]. For the introduction of the selenium, the methylselenol nucleophile was prepared *in-situ* by reduction of the Se-Se bond of dimethyldiselenide using sodium

borohydrate in acetonitrile under reflux. The products were deprotected under transesterification condition (Zemplén procedure) [134] . The resulting yields are summarised in Table 2.

Table 2 Reaction yields.

Glycoside	Bromination	Selenation	Deprotection
 **85**	 **90** 78%	 **95** 91%	 **77** 93%
 **86**	 **91** 70%	 **96** 96%	 **79** 96%
 **87**	 **92** Not isolated	 **97** 15% (2 steps)	 **82** 78%
 **88**	 **93** Not isolated	 **98** 81% (2 steps)	
 **89**	 **94** 53%	 **99** 57%	

For the anomerisation reaction, different concentrations of $BF_3 \cdot OEt$ in dry DCM were tried, but the reaction yields were poor. The reaction was first carried on methyl 2,3,4,6-*O*-acetyl-1-seleno-β-D-glucopyranoside **95** in the presence of $BF_3 \cdot OEt_2$. In general, the conversion rate was low producing variable amounts of degradation products and a mixture of anomers (Table 3). A similar result was obtained with the analogous galactoside. However, when these reactions conditions were tried with a methyl 2,3,4-*O*-acetyl-1-seleno-β-L-fucopyranoside **98** a more effective though still not complete anomerisation was obtained. The difference in case of the fucoside was that the starting material had been purified by flash column chromatography in a petroleum ether/diethyl ether instead of a toluene/ethyl acetate mixture. One could speculate that the petroleum ether/diethyl ether solvent system might have been better in removing impurities originating from the reducing reagent. Serendipitously it was found later during the Lewis b project, that boron salts were difficult to remove and appeared in several occasion to interfere with subsequent reactions.

Table 3 Anomerisation: reaction conditions.

Glycoside	$BF_3{\cdot}OEt_2$	Dry solvent[a]	T	Time	Results
95	2 eq.	DCM	RT	7 h	α/β mixture: 12%/88%; degradation
95	2 eq.	DCM	30° C	5 h	α/β mixture; degradation
95	4 eq.	DCM[b]	RT	3 h	α/β mixture: 18%/82%; degradation
95	2 eq.	DCM[c]	RT	7 h	α/β mixture: 9%/91%; degradation
98	4 eq.	DCM[d]	30° C	4 h	α/β mixture: 24%/76%; degradation
98	2 eq.	DCM	RT	3 h	α/β mixture: 43%/57%; degradation

[a]: 0.1 mmol glycoside/mL concentration; dried over $CaCl_2$.
[b]: under argon.
[c]: freshly dried over $CaCl_2$.
[d]: with 4Å powder.

5.3 Synthesis of the selenium tagged H/Lewis^b determinant

BabA is a membrane bound protein and therefore difficult to crystallise [118]. However the genome of *H. pylori* is known[135] and a truncated protein can be synthesised expressing the outer part only. Once obtained, it is hoped that crystallisation conditions can be found. Those crystals could in turn be soaked with a methylseleno tagged ligand. To study the outer structure of BabA, it has been decided to prepare the methylseleno derivative **100** from the H/Lewis^b determinant. It is expected to be a good ligand for the modified lectin (Fig 19).

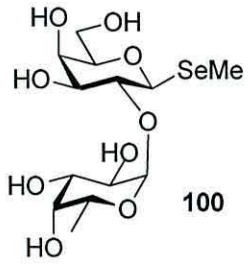


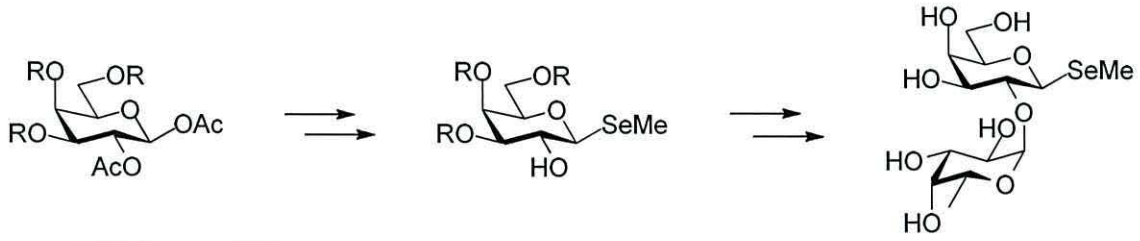
Figure 19 Target disaccharide.

5.3.1 Retrosynthesis

The synthesis of the free disaccharide was already published by Matta [136]. The reaction involved 2,3,4-tri-*O*-acetyl-α-fucopyranosyl bromide with 1,3,4,6-tetra-*O*-acetyl-α-D-galactose in acetonitrile with mercuric bromide and mercuric cyanide as promoter. As expected with a C-2 participating

group on the fucose, both α and β linkages were obtained. Later, Wegmann and Schmidt[137] prepared the disaccharide using 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl trichloroacetimidate with 1 ,6-anhydro-3,4-*O*-isopropylidene-β-D-galactose in Et_2O with catalytic trimethylsilyl triflate giving α/β mixture. Vankayalapati and Singh[138] synthesised it via 2,3,4-tri-*O*-acetyl-α,β-fucopyranosyl phosphate and 1,3,4,6-tetra-*O*-acetyl-α-D-galactose. Using catalytic amount of trimethylsylil triflate, the β likage was formed when 1.5 equivalent of the promoter gave α linkage.

Two general pathways can be followed to prepare the seleno-labelled determinant. The first one is to introduce the selenium before the α-glycosylation. In that case, the galactoside has to carry a participating group on C-2, ascertain the configuration of the selenium group. After removal of the participating group coupling with the fucose can be carried



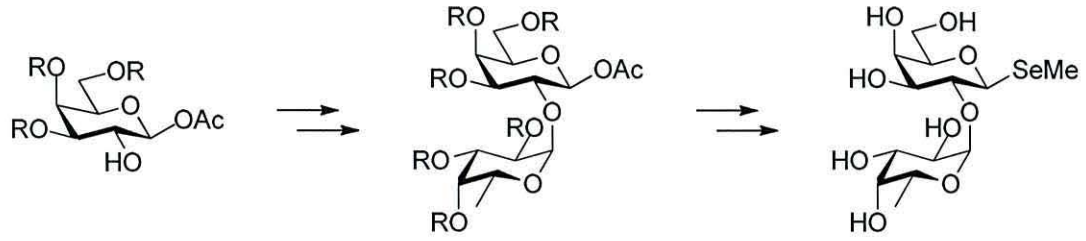
out (Scheme 36).

Scheme 36 First pathway.

The major problem of this pathway is the introduction of the fucose. The methyl seleno group is easily activated by a number of promoter systems

[139,140], including bromine [141] used in halide assisted coupling often employed for α-glycosylations[25], and the problem of undesired activation was not overcome in previous attempts[142] to synthesise the target structure. This, in combination that an α-glycosidic linkage has to be introduced limits this approach. An additional issue is, that selenium as sulphur is a typical poison for Pd-catalysts which are used for convenient removal of e.g. benzyl ether protecting groups. The latter can be surmounted by employing Birch reduction conditions which have been reported suitable in the presence of selenium or sulfur heteroatoms.

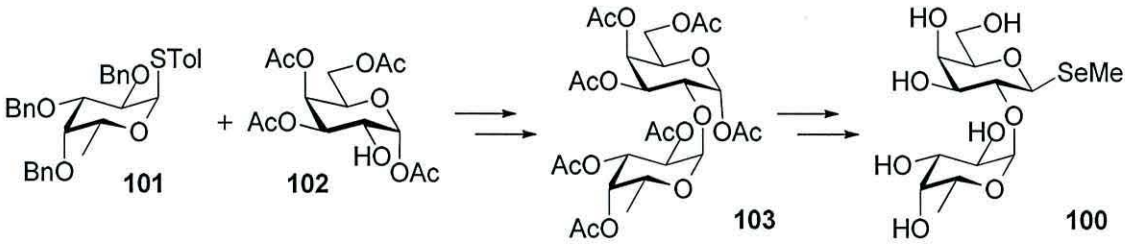
The other alternative is to produce the disaccharide prior to the introduction of the seleno group. (Scheme 37)



Scheme 37 Second pathway.

The major disadvantage of the latter approach is that the participating effect helping for the stereoselective introduction of the selenomethyl group is lost. However, the introduction of the selenomethyl group has been envisaged as an S_N2-type reaction [137]. Thus, starting from an α-bromide, it is expected to obtain the desired anomer.

For the synthesis of the disaccharide, we decided to use our experience in α-glycosylation gained during the synthesis of the previously described pentasaccharide and heptasaccharide: 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide is prepared "in situ". The fucoside donor and the galactoside acceptor were to be synthesised following published pathways[143, 144] (Scheme 38).



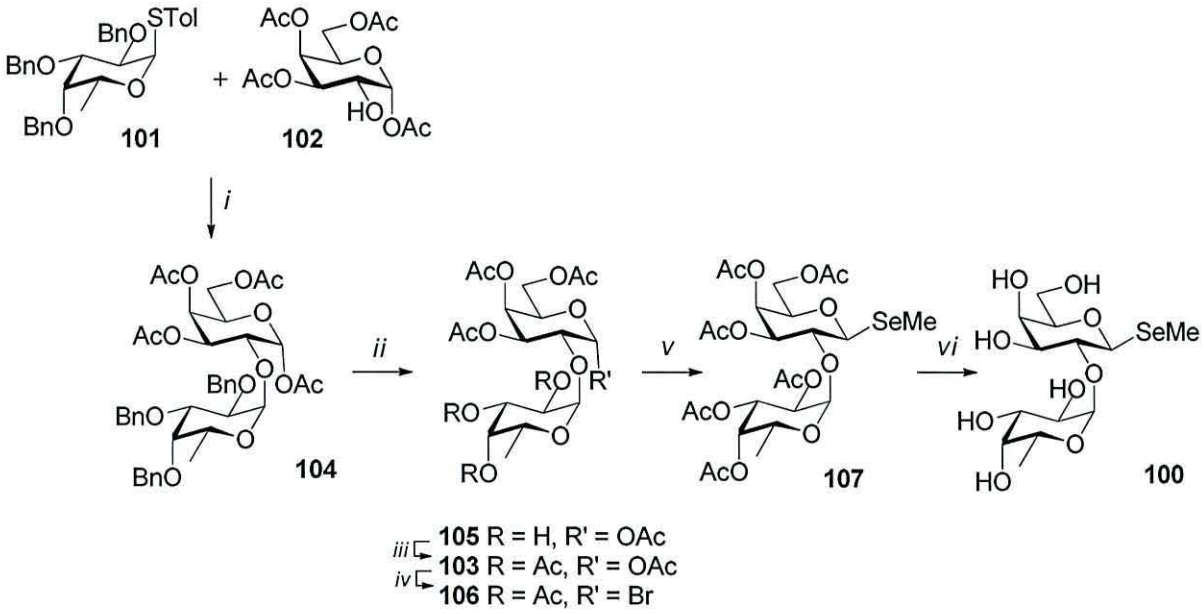
Scheme 38 The projected disaccharide synthesis.

5.3.2 Results and discussion

Galactosyl acceptor **102** was prepared by treating peracetylated galactose with TFA/H_2O in DCM according to the literature[143]. During this reaction only the α acetate **102** was isolated. The fucosyl donor **101** was prepared in 3 steps, starting from peracetylated fucose[144]. After introducing the thiotolyl group, followed by deacetylation under Zemplen[134] conditions, the benzyl ethers were introduced using sodium hydride and benzyl bromide in DMF. For the α-glycosylation, the same methodology as described earlier for the pentasaccharide and the heptasaccharide was employed.

Thus, fucoside **101** and galactoside **102** were mixed together in dry DCM with TEAB and 4Å molecular sieves at room temperature. After one hour stirring, bromine was added to convert the fucoside into the bromofucose donor "in situ". After completion, disaccharide **104** was isolated in 80% yield. The benzyl ethers were then removed by catalytic hydrogenolysis (**105**). This reaction was monitored with MALDI-TOF and completed after a few days. Some detected by-compounds were disaccharides that had lost one or more remaining acetates. This was also noticeable by TLC. However, no attempt on separating this mixture was made, since the next reaction was an acetylation to stabilise the disaccharide. This reaction was performed in pyridine with Ac_2O at room temperature giving the expected peracetylated disaccharide **103** in 60% yield over 2 steps. Then the selenomethyl group had to be introduced. So, disaccharide **103** was converted into the bromide **106**. This substitution reaction is generally fast but produces mainly the β-anomer in the first place. Using HBr-AcOH in DCM and stirring at room temperature for about 2 h ensured that the reaction mixture equilibrated to the thermodynamically favoured α-anomer. The co-evaporated crude bromide was added to a freshly prepared methylselenol containing solution. This reaction mixture was obtained by the reduction of dimethyldiselenide in CH_3CN with an excess of $NaBH_4$. Several attempts were made to elaborate suitable reaction conditions. A large excess of $NaBH_4$ was found to be of importance as a smaller excess led to α/β mixtures of the selenoglycoside. The pure β-anomer of disaccharide **107** was isolated with 63 % yield (^1H NMR, $CDCl_3$, δ 4.71

ppm (d, $J_{1,2}$=9.7 Hz, H-1)). The final deprotection was carried out by transesterification in MeOH with NaOMe yielding the expected selenodisaccharide **100** with 96 % (Scheme 39).



Scheme 39 Synthesis of the seleno disaccharide **100**. *i.* TBAB, Br_2, DCM, 80%; *ii.* H_2, Pd/C, MeOH; *iii.* Ac_2O, pyridine, 60% (2 steps); *iv.* HBr-AcOH 33%, DCM; *v.* 1. Se_2Me_2, $NaBH_4$, MeCN, 2. **106**, MeCN, 63%; *v.* NaOMe, MeOH, 96%.

5.4 Conclusion

A library of selenoglycosides was synthesised, improving the yield of a previously published pathway by changing the reaction solvent. Different purification processes have to be investigated to understand the nature of dragged impurities and their effect on anomerisation and thus to prepare the remaining anomers. A selenium tagged disaccharide was successfully synthesised. Despite the stereocontrol difficulties, both, the desired α-glycosidic linkage to form the disaccharide as well as the β-anomeric selenium tag were installed and the target saccharide obtained in good yields. The final disaccharide **106** will be sent to collaborators for X-ray structural studies of truncated BabA.

6. Conclusion

This thesis described the synthesis of different tools for the investigation of carbohydrate-protein interaction studies. They were prepared as lectin ligands or enzyme substrates depending on the aim of the biological investigation.

Series of new chitin derivatives were synthesised for binding study with chit42, a chitinase. A simple, reproducible and efficient route was used to functionalise the substrate with a bifunctional linker. The derivatives were still soluble, an imperative for the study.

A Lewis^b pentasaccharide and the B-Lewis^b heptasaccharide were synthesised with success following a linear pathway, using the same building blocks whenever possible. Attempts to use a convergent pathway were made. When scaling up led to an undesired glycoside at best, a new route to synthesise the oligosaccharide was investigated. A different building block was prepared to avoid unwanted reaction when occurred. Finally, the BSA-pentasaccharide conjugate was prepared.

Finally, a route to functionalise and anomerise common monosaccharides and disaccharides with carrying a methylseleno label was investigated. The same selenium functional group was introduced to a disaccharide and β selectivity was obtained despite the absence of participating group.

Experimental

The experimental is not presented in chronological order but follows the thesis order, explaining the lack of characterisation for some compounds.

Part of the experimental is included in paper I and paper II.

General methods

Proton magnetic resonance (δ_H) spectra reported were recorded on Bruker Avance 500 (500 MHz) or Varian 500 MHz. All chemical shifts are quoted on the δ-scale, with $CDCl_3$, CD_3OD, D_2O or Me_4Si as internal standard. Mass spectra were recorded on a Bruker Reflex VI using THAP MALDI matrix and TOF technique. High resultion mass spectra were recorded on Bruker micro-TOF. Thin layer chromatography (TLC) was carried out on fluka glass-backed sheets, precoated with $60F_{264}$ silica. Plates were developed using AMC (ammonium molybdate 10g, cerium sulfate 2g dissolved in 10% H_2SO_4 (200 mL)), H_2SO_4 8-10%in H_2O or ninhydrine in EtOH followed by heating. Flash chromatography was carried out using 0.040-0.063 μm silica. Ion exchange chromatography was carried out using Dowex resin (-COOH). Reverse phase chromatography was carried out using C_{18}, 60Å, 40-43 μm.

Chapter 2:

General procedure for enzyme substrate

DSS (440 mg, 1.2 mmol) and 1-hydroxybenzotriazole (150 mg) were dissolved in DMSO (1.5 mL, heat). After the mixture was cooled at room temperature, chitosylamine was added and the mixture stirred for 3h. After complete conversion (TLC, 4 BuOH, 2 EtOH, 1 EtOAc, 3 H_2O), the excess of DSS and HOBt were precipated with ice-cold water. After filtration, ethylene diamine (162 µL, 2.4 mmol) was added to the filtrate and the mixture stirred at room temperature for 3h. After concentration *in vacuo*, the residue is purified by ion exchange chromatography and reverse phase chromatography to give the expected product

N-(2-aminoethyl)-*N*'-(chitobiosylacetamido)octanediamide 12

Chitobiosylamine **19** (50 mg, 0.12 mmol) was treated as described for the general enzyme substrate procedure

The expected product is a white crystalline compound (44mg, 57%, overall yield).

m/z (MALDI-TOF) 644.4 (M^+ + Na), $C_{26}H_{47}N_5O_{12}$ requires 644.3.

N-(2-aminoethyl)-*N*'-(chitotriosylacetamido)octanediamide 13

Chitotriosylamine **20** (50 mg, 0.08 mmol) was treated as described for the general enzyme substrate procedure.

The expected product is a white crystalline compound (38 mg, 57%, overall yield).

m/z (MALDI-TOF) 824.5 (M^+ + Na), $C_{34}H_{60}N_6O_{17}$ requires 824.4.

_N_-(2-aminoethyl)-_N'_-(chitotriosylacetamido)octanediamide 14

Chitotetraosylamine **21** (50 mg, 0.06 mmol) was treated as described for the general enzyme substrate procedure.

The expected product is a white crystalline compound (28 mg, 44%, overall yield).

m/z (MALDI-TOF) 1050.5 (M^+ + Na), $C_{42}H_{73}N_7O_{22}$ requires 1050.5.

Disuccimidyl suberate 18[83]

Suberic acid (430mg, 2.5 mmol), *N*-hydroxysuccinimide (630 mg, 5.5 mmol) and DCC (1.1g, 5.5 mmol) were sitirred in DCM/THF (1:1) at 0°C. After 1h, the mixture was allowed to attain room temperature and the stirring was continued for 2h. The reaction mixture was then filtered through a pad of celite with additional DCM. TH solvent was removed in vaccuo and the white crystalline residue recrystallised from EtOH to give the expecting product as white crystals (759 mg, 84%).

^{1}H NMR (250 MHz, $CDCl_3$) δ = 2.84 (s, 8H), 2.63 (t, 4H), 1.78 (m, 4H), 1.47 (m, 4H).

General procedure for glycosylamine

Chitooligomer (50 mg) and ammonium carbonate (250 mg, 5 fold excess) were suspended in DMSO (0.5 mL) and was heated at 40°C over night. The mixture was freeze dried several days to remove the excess of ammonium carbonate and DMSO to afford chitobiosylamine as a white solid. The amine was used without further purification because of its instability.

Chitobiosylamine 19[70]

Chitobiose **9** (50 mg, 0.12 mmol) was treated as described for the general glycosylanime procedure.

Chitotriosylamine 20[145]

Chitotriose **10** (50 mg, 0.08 mmol) was treated as described for the general glycosylanime procedure.

Chitotetraosylamine 21

Chitotetraose **11** (50 mg, 0.06 mmol) was treated as described for the general glycosylanime procedure.

Chitopentaosylamine 23

Chitopentaose **22** (10 mg, 12 μmol) was treated as described for the general glycosylanime procedure.

Chapter 3:

Tolyl 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside 25 [100]

Ethyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 26 [146]

Ethyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4-*O*-acetyl-6-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 27 [94, 92]

3-Azidopropyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 28

Paper I, compound 4

Ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside 29[146]

3-Azidopropyl (3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 30

Paper I, compound 11

3-Aminopropyl (α-L-fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-[(α-L-fucopyranosyl)-(1→4)]-(β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside 31

Paper I, compound 1

Tolyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 32[103]

3-Azidopropyl (2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 33

Paper I, 8

3-Azidopropyl β-D-galactopyranoside 34[147]

3-Azidopropyl 3-*O*-allyl-β-D-galactopyranoside 35[148]

3-Azidopropyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 36

Paper I, compound 3

3-Azidopropyl 2,4-di-*O*-benzyl-β-D-galactopyranoside 37

Not charactarised

3-Azidopropyl 2,4-di-*O*-benzyl-3,6-di-*O*-benzoyl-β-D-galactopyranoside 38

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.04-7.61 (m, 20H, aromatic H), 5.18 ppm (dd, $J_{2,3}$=10.2 Hz, $J_{3,4}$=3.1 Hz, H-3), 4.76 (d, 2H, J=11.5 Hz, OCH_2Ph), 4.66 (d, 1H, J=11.5 Hz, OCH_2Ph), 4.63 (d, 1H, J=11.5 Hz,OCH_2Ph), 4.44-4.48 (m, 2H, H-1 and OCH_2Ph), 4.27 (dd, 1H, $J_{6,6'}$=11 Hz, $J_{5,6}$=7 Hz H-6), 4.02 (ad, 1H, J=2.6 Hz, H-4), 3.90-3.96 (m, 2H, H-2, H-6'), 3.84 (at, 1H, H-5), 3.57-3.62 (m, 2H, OCH_2CH$_2$CH$_2$N$_3$), 3.38 (t, 2H, J=6.8 Hz, OCH$_2$CH$_2$CH_2N$_3$), 1.77-1.90 (m, 2H, OCH$_2$CH_2CH$_2$N$_3$)

3-Azidopropyl 3,4-*O*-isopropyledene-β-D-galactopyranoside 39

Paper I, compound 5

3-Azidopropyl 2,6-di-*O*-benzyl-3,4-*O*-isopropyledene -β-D-galactopyranoside 40

Paper I, compound 5

3-Azidopropyl 2,6-di-*O*-benzyl-β-D-galactopyranoside 41

Paper I, compound 5

3-Azidopropyl 2,6-di-*O*-benzyl-3,4-*O*-benzylidene-β-D-galactopyranoside 42

Paper I, compound 5

3-Azidopropyl 3-*O*-benzoyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 43

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.23-7.36 (m, 20H, aromatic H), 5.05 (dd, 1H, $J_{2,3}$=10.2 Hz, $J_{3,4}$=3.1 Hz, H-3), 4.94 (d, 2H, *J*=11.7 Hz, OCH_2Ph), 4.79 (d, 2H, *J*=11.7 Hz, OCH_2Ph), 4.65 (d, 2H, *J*=11.4 Hz,OCH_2Ph), 4.51 (d, 2H, J=11.7 Hz,OCH_2Ph), 4.49 (d, 2H, *J*=11.8 Hz,OC*H*2Ph), 4.35 (d, 1H, $J_{1,2}$=7.6 Hz, H-1), 3.98-4.02 (m, 1H, H-6), 3.88 (d, 1H, $J_{3,4}$=3.2 Hz, H-4), 3.56-3.68 (m, 5H, H-2,5,6 and OC$H_2$$CH_2$$CH_2$$N_3$), 3.41 (t, 2H, J=6.8 Hz, $OCH_2CH_2CH_2N_3$), 1.85-1.97 (m, 2H, $OCH_2CH_2CH_2N_3$)

Ethyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside 44[94, 95]

3-Azidopropyl (3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 47

Paper I, compound 7

3-Azidopropyl (2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 48

Paper I, compound 10

Tolyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranoside 50[99]

3-Azidopropyl (2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside 51

^{1}H NMR (500 MHz, $CDCl_3$) δ = 1.73 (s, 3H, NCOCH_3), 1.76-1.85 (m, 2H, $OCH_2CH_2CH_2N_3$), 3.28-3.78 (m,15H), 3.86-3.96 (m, 6H), 4.25 (d, 1H, J=7.5 Hz), 4.29 (dd, 1H,J=10.3 Hz, 4.8 Hz), 4.37-4.63 (m, 8H), 4.67 (d, 1H, J=11.5 Hz, OCH_2Ph), 4.74 (d, 1H, J=11.5 Hz,OCH_2Ph) 4.85 (d, 1H, J=11.9 Hz, OCH_2Ph), 4.95 (d, 1H, J=11.6 Hz,OCH_2Ph), 5.30 (d, 1H, J=7.0 Hz), 5.32 (d, 1H, J=8.3 Hz),5.50 (s, 1H, CHPh), 5.60 (dd, 1H, J=9.9 Hz, 8.2 Hz) 7.05-8.05 (m, 40H, aromatic H)

^{13}C NMR (126 MHz, $CDCl_3$) δ = 171.36, 170.76 (C=O), 125.4-139.0 (ArC), 103.89, 101.35, 101.05, 100.69 (C-1, C-1^{I},C-1^{II}, CHPh), 82.08, 81.29, 80.04, 78.91, 75.85, 74.76, 74.60, 74.50, 73.60, 73.50, 73.48, 73.39,

72.91, 72.60, 72.17, 71.63, 68.98, 68.91, 68.26, 66.54, 65.54 (C-2,3,4,5,6, C-3^{I},4^{I},5^{I},6^{I}, C-2^{II},3^{II},4^{II},5^{II},6^{II}, O$CH_2CH_2CH_2N_3$ and 6xCH_2Ph), 59.09 (C-2^{II}), 48.35($OCH_2CH_2CH_2N_3$), 29.21($OCH_2CH_2CH_2N_3$), 22.44 ($NHCOCH_3$).

3-Azidopropyl (2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(2-acetamido-6-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-O-benzyl-β-D-galactopyranoside 52

Not characterised

3-Azidopropyl (2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-(3,4,6-tri-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)]-(2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-2,4,6-tri-O-benzyl-β-D-galactopyranoside 53

Paper I, compound 13

3-Aminopropyl (α-L-fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-[(α-L-fucopyranosyl)-(1→4)]-(β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside DSS conjugate 54

Not characterised

3-Aminopropyl (α-L-fucopyranosyl)-(1→2)-(β-D-galactopyranosyl)-(1→3)-[(α-L-fucopyranosyl)-(1→4)]-(β-D-glucopyranosyl)-(1→3)-β-D-galactopyranoside BSA conjugate 55

See appendix for incorporation calculation

Chapter 4:

3-Azidopropyl-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 60 [149]

Tolyl 3-*O*-allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranoside 61

Crude tolyl 3-*O*-allyl-4,6-di-*O*-benzyl-β-D-galactopyranoside **71** (approx. 1.07 mmol) and DMAP (cat. amount) were dissolved in pyridine, benzoyl chloride was added and the reaction mixture stirred at room temperature for 30 min. The solvent was co-evaporated with toluene and the residue was dissolved in ethyl acetate, washed with brine and water, dried and concentrated. The crude product was purified by column chromatography (toluene→toluene/ethyl acetate 4:1) to give tolyl 3-*O*-allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranoside **61** (438 mg, 0.68 mmol, 64%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.09 (d, 2H, ArH), 7.47 (t, *J*=7.7 Hz, 2H, ArH), 7.39 – 7.28 (m, 13H, ArH), 7.01 (d, *J*=7.9 Hz, 2H, ArH), 5.77 – 5.68 (m, 1H, OCH_2CHCH_2), 5.62 (at, *J*=9.8 Hz, 1H, H-2), 5.18 (dd, *J*=17.2 Hz, 1.5 Hz, 1H, OCH_2CHCH_2), 5.07 (dd, *J*=10.4 Hz, 1.5 Hz, 1H, OCH_2CHCH_2), 4.99 (d, *J*=11.6 Hz, 1H, CH_2Ph), 4.76 (d, *J*=9.9 Hz, 1H, H-1), 4.61 (d, *J*=11.6 Hz, 1H, CH_2Ph), 4.49 (d, *J*=11.6 Hz, 1H, CH_2Ph), 4.45 (d, *J*=11.6 Hz, 1H, CH_2Ph), 4.14 – 4.08 (m, 1H, H-6_b), 4.00 (d, *J*=2.7 Hz, 1H, H-4), 4.03 – 3.96 (m, 1H, H-6_b), 3.73 – 3.64 (m, 4H, H-3, H-5, OCH_2CHCH_2), 2.30 (s, 3H, $ArCH_3$).

^{13}C NMR (126 MHz, $CDCl_3$) δ = 165.26 (C=O), 138.58x2, 137.94, 137.61 (ArC), 134.38 (OCH_2CHCH_2), 132.97, 132.69, 130.28, 129.82, 129.49,

128.44, 128.36, 128.19, 128.05, 127.94, 127.81, 127.48(ArC), 117.28 (OCH_2CHCH_2), 87.28(C-1), 81.45(C-5), 77.75 (C-3), 74.35, 73.64(CH_2Ph, 2C), 73.19 (C-4), 71.34 (C-6), 70.66 (C-2), 68.92 (OCH_2CHCH_2), 21.13 ($ArCH_3$).

HR-ESI calcd for $C_{37}H_{38}O_6S$ $[M+Na]^+$ 633.2287. Found 633.2281.

3-Azidopropyl (4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside 62

Paper II, compound 6.

Tolyl 2,3,4,6-tetra-*O*-benzyl-1thio-β-D-galactopyranoside 63[144]

3-Azidopropyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside 64

Paper II, compound 9.

3-Azidopropyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside 65

Paper II, compound 12.

3-Aminopropyl α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl)-(1→4)-2-β-D-glucopyranoside 66

Paper II, compound 1.

Tolyl-1-thio-β-D-galactopyranoside 67[150]

Tolyl 2,3-*O*-(2',3'-dimethoxy-2',3'-diyl)-1-thio-β-D-galactopyranoside 68

Tolyl thio-β-D-galactopyranoside **67** (6.30 g, 22 mmol) was dissolved in dry methanol (50 mL) with 2,3-butanedione (2.1 mL, 24.2 mL) and trimethylorthoformate (12 mL, 110 mmol). Camphorsulfonic acid (0.5 g, 2.2 mmol) was added and the solution was heated to reflux. After 4h, the reaction was quenched with Et_3N and the solvent was removed under reduced pressure and the resulting crude tolyl 2,3-*O*-(2',3'-dimethoxy-2',3'-diyl)-1-thio-β-D-galactopyranoside **68** was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.45 (d, *J*=8.0 Hz, 2H, ArH), 7.10 (d, *J*=8.0 Hz, 2H, ArH) 4.72 (d, *J*=8.6 Hz, 1H, H-1), 4.05 (at, *J*=8.6 Hz, 9.9 Hz, 1H, H-2), 4.00 (d, *J*=2.7 Hz, 1H, H-4), 3.96 (m, 1H, H-6b), 3.80 (m, 1H, H-6a), 3.78 (dd, *J*=2.7 Hz, 9.9 Hz, 1H, H-3), 3.59 (t, 1H, H-5), 3.27 (s, 3H, OCH_3), 3.22 (s, 3H, OCH_3), 2.33 (s, 3H, $ArCH_3$), 1.34 (ad, 6H, 2x CCH_3).

13C NMR (126 MHz, CDCl3) δ = 129.5, 129.6, 132.4, 137.6 (Aromatic C), 100.4 ($OCOCH_3$), 85.9 (C-1) 78.8 (C-5), 71.7 (C-3), 68.4 (C-4), 65.5 (C-2), 62.6 (C-6), 48.1 (OCH_3), 21.1 ($ArCH_3$), 17.6, 17.7 (2x CCH_3).

HR-ESI calcd for $C_{19}H_{28}O_7SK$ $[M+K]^+$ 439.1187. Found 439.1203.

Tolyl 4,6-*O*-di-benzyl-2,3-*O*-(2',3'-dimethoxy-2',3'-diyl)-1-thio-β-D-galactopyranoside 69

Crude tolyl 2,3-*O*-(2',3'-dimethoxy-2',3'-diyl)-1-thio-β-D-galactopyranoside **68** (approx. 22 mmol) was dissolved in DMF and cooled down at 0°C. NaH (60%, 3.52 g, 88 mmol) was added portionwise and stirred until no more

gas evolved. Benzyl bromide (7.8 mL, 66 mmol) was then added and the stirring was continued for additional 1h. The reaction mixture was poured onto an ice/toluene mixture and the water phase was extracted twice with toluene. The combined organic layers were washed with brine, dried with $MgSO_4$ and concentrated. The crude tolyl 4,6-*O*-di-benzyl-2,3-*O*-(2',3'-dimethoxy-2',3'-diyl)-1-thio-β-D-galactopyranoside **69** underwent a flash column chromatography (toluene/ethyl acetate 20:1) and used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.43– 7.25 (m, 12H, ArH), 7.00 (d, *J*=8.0 Hz, 2H, ArH), 5.00 (d, *J*=11.4 Hz, 1H, C*H*$_2$Ph), 4.72 (d, *J*=10.0 Hz, 1H, H-1), 4.61 (d, *J*=11.4 Hz, 1H, C*H*$_2$Ph), 4.47 (d, *J*=11.6 Hz, 1H, C*H*$_2$Ph), 4.42 (d, *J*=11.6 Hz, 1H, C*H*$_2$Ph), 4.21 (at, *J*=9.5 Hz, 10.0 Hz, 1H, H-2), 3.85 – 3.79 (m, 2H, H-3, H-4), 3.72 – 3.63 (m, 3H, H-5, H-6a, H-6b), 3.28 (s, 3H, OCH_3), 3.20 (s, 3H, OCH_3), 2.29 (s, 3H, $ArCH_3$), 1.34 (ad, 6H, 2x CCH_3).

^{13}C NMR (126 MHz, $CDCl_3$) δ = 138.93, 138.06, 137.06, 132.12, 129.42, 128.43, 128.39, 128.08, 127.89, 127.73, 127.42 (Aromatic C), 100.11, 99.80 (2x O*C*OCH$_3$), 86.12 (C-1), 78.18 (C-5), 73.96, 73.64 (2x *C*H$_2$Ph), 73.62, 73.38 (C-3, C-4), 69.08(C-6), 65.88 (C-2), 47.99, 47.95 (2x O*C*H$_3$), 21.07 (Ar*C*H$_3$), 17.79, 17.72 (2x *C*CH$_3$).

HR-ESI calcd for $C_{33}H_{40}O_7SK$ $[M+K]^+$ 619.3065. Found 619.3013.

Tolyl 4,6-*O*-di-benzyl-1-thio-β-D-galactopyranoside 70

Tolyl 4,6-*O*-di-benzyl-2,3-*O*-(2',3'-dimethoxy-2',3'-diyl)-1-thio-β-D-galactopyranoside **69** (approx. 22 mmol) was dissolved in

dichloromethane (150 mL) and TFA/H_2O (10:1, 22 mL) added. The reaction mixture was heated to reflux for 2h and then poured onto ice, diluted with dichloromethane and neutralised with $NaHCO_3$. The organic layer was separated, washed with water and concentrated. The crude product was purified by column chromatography (toluene/ethyl acetate, 2:1) to give tolyl 4,6-*O*-di-benzyl-1-thio-β-D-galactopyranoside **70** (7.18g, 15.4 mmol, 70% over 3 steps).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.46 (d, *J*=8.0 Hz, 2H, ArH), 7.37 – 7.28 (m, 10H, ArH), 7.07 (d, *J*=8.0 Hz, 2H, ArH), 4.74 (d, *J*=11.7 Hz, 1H, C*H*$_2$Ph), 4.69 (d, *J*=11.7 Hz, 1H, C*H*$_2$Ph), 4.54 (d, *J*=11.7 Hz, 1H, C*H*$_2$Ph), 4.49 (d, *J*=11.7 Hz, 1H, C*H*$_2$Ph), 4.46 (d, *J*=9.3 Hz, 1H, H-1), 3.93 (d, *J*=3.1 Hz, 1H, H-4), 3.76 – 3.62 (m, 5H, H-2, H-3, H-5, H-6a, H-6b), 2.33 (s, 3H, ArC*H*$_3$).

^{13}C NMR (126 MHz, $CDCl_3$) δ = 138.43, 138.03, 137.80, 132.83, 129.70, 128.45, 128.41, 127.85, 127.84, 127.72, 127.62 (Aromatic C), 88.75 (C-1), 77.60 (C-5), 76.08 (C-4), 75.34 (C-3), 74.99, 73.56 (2x *C*H_2Ph), 70.33, 68.51 (C-2, C-5), 21.15 (Ar*C*H_3).

HR-ESI calcd for $C_{27}H_{30}O_5SK$ $[M+K]^+$ 505.1446. Found 505.1408.

Tolyl 3-*O*-allyl-4,6-di-*O*-benzyl-β-D-galactopyranoside 71

Tolyl 4,6-*O*-di-benzyl-1-thio-β-D-galactopyranoside **70** (500 mg, 1.07 mmol) was added to a suspension of Bu_2SnO (533 mg, 2.14 mmol) in dry methanol (15 mL) and the reaction mixture was heated to reflux overnight. After removal of the solvent under reduced pressure, the residue was

dissolved in dry acetonitrile (5 mL). CsF (203 mg, 1.3 mmol) and allyl bromide (463µL, 5.4 mmol) were added and the reaction mixture stirred at room temperature for a day. The solvent was then removed under reduced pressure and the residue was dissolved in ethyl acetate, washed with KF (aq 10%) and water, dried and concentrated. The crude tolyl 3-*O*-allyl-4,6-di-*O*-benzyl-β-D-galactopyranoside **71** was used without further purification.

^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.46 (d, *J*=8.0 Hz, 2H, ArH), 7.37 – 7.27 (m, 10H, ArH), 7.03 (d, *J*=7.9, 2H Hz, ArH), 5.97 – 5.88 (m, 1H, OCH_2CHCH_2), 5.32 (dd, *J*=17.2 Hz, 1.5 Hz, 1H, OCH_2CHCH_2), 5.21 (d, *J*=10.4 Hz, 1H, OCH_2CHCH_2), 4.88 (d, *J*=11.5 Hz, 1H, CH_2Ph), 4.56 (d, *J*=11.5 Hz, 1H, CH_2Ph), 4.50 (d, *J*=11.7 Hz, 1H, CH_2Ph), 4.49 (d, *J*=9.5 Hz, 1H,H-1), 4.46 (d, *J*=11.7 Hz, 1H, CH_2Ph), 4.17 (m, 2H, H-6_a, H-6_b), 3.97 – 3.90 (m, 2H, H-2, H-4), 3.70 – 3.63 (m, 3H, H-5, OCH_2CHCH_2), 3.40 (dd, *J*=9.3, 2.7, 1H, H-3), 2.31 (s, 3H, $ArCH_3$).

^{13}C NMR (126 MHz, $CDCl_3$) δ = 138.72, 137.91, 137.81 (ArC, 3C), 134.52 (OCH_2CHCH_2), 132.87, 129.60, 128.44, 128.36, 128.13, 127.93, 127.82, 127.76, 127.41 (ArC), 117.34 (OCH_2CHCH_2), 88.82 (C-1), 82.96 (C-3), 77.59 (C-5), 74.30, 73.61 (CH_2Ph, 2C), 73.00 (C-4), 71.21 (C-6), 68.94 (C-2), 68.74 (OCH_2CHCH_2), 21.13 ($ArCH_3$).

HR-ESI calcd for $C_{30}H_{34}O_5S$ $[M+Na]^+$ 529.2025. Found 529.2019.

3-Azidopropyl (3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside 72

Paper II, compound 5.

3-Azidopropyl (3-*O*-allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside 73

Paper II, compound 8.

3-Azidopropyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside 74

Paper II, compound 10.

3-Azidopropyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside 75

Paper II, compound 11.

3-Azidopropyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)]-(4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)]-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside 76

Paper II, compound 13.

General procedure for the Zemplén deacetylation procedure

Methyl seleno glycoside (50 mg) was dissolved in MeOH (2 mL). MeONa was added until pH>7 and the mixture was stirred at room temperature for 2h. After complete conversion, the solution was neutralised with Dowex resin to pH=7, filtrated and concentrated *in vaccuo* to give the expected product

Methyl 1-seleno-β-D-glucopyranoside 77

Methyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-β-D-glucopyranoside **95** was treated as described for the general Zemplén deacetylation procedure.

The expected product is a white crystalline compound (29 mg, 96%).

^{1}H NMR (500 MHz, D_2O) δ = 4.72 (d, *J*=9.2 Hz, 1H); 3.91 (d, *J*=12.0 Hz, 1H); 3.73 (dd, *J*=4.8 Hz, 12.3 Hz, 1H); 3.51-3.41 (m, 4H); 2.15 (s, 3H).

Methyl 1-seleno-β-D-galactopyranoside 79

Methyl 2,3,4,6-*O*-tetra-acetyl-1-seleno-β-D-galactopyranoside **96** (50 mg, 0.12 mmol) was treated as described for the general Zemplén deacetylation procedure.

The expected product is a white crystalline compound (28 mg, 93%)

^{1}H NMR (500 MHz, D_2O) δ = 4.66 (d, 1H, *J*=9.2 Hz); 4.01 (d, *J*=3.2 Hz, 1H); 3.78-3.63 (m, 5H); 2.15 (s, 3H).

Methyl 1-seleno-α-D-mannopyranoside 82

Methyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-α-D-mannopyranoside **97** (38 mg, 0.09 mmol) was treated as described for the general Zemplén deacetylation procedure.

The expected product is a white crystalline compound (18 mg, 78%)

^{1}H NMR (500 MHz, D_2O) δ = 5.10 (s, 1H); 4.12 (d *J*=3.5 Hz, 1H,); 3.93 (dd, 1H, *J*=1.3 Hz, 12.3 Hz); 3.74 (dd, *J*=6.0 Hz, 12.3 Hz, 1H); 3.62 (at, *J*=9.5 Hz, 9.8, 1H); 4.41 (ddd, *J*=2.2 Hz, 6.3 Hz, 8.5 Hz, 1H); 3.68 (dd, 1H, *J*=3.5 Hz, 9.8 Hz); 2.19 (s, 3H).

Penta-*O*-acetyl-β-D-glucopyranose 85 [151]

Penta-*O*-acetyl-β-D-galactopyranose 86 [48]

Penta-*O*-acetyl-α-D-mannopyranose 87[152]

Tetra-*O*-acetyl-β-L-fucopyranose 88[153]

Tetra-*O*-acetyl-β-L-fucopyranose 89[153]

2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide 90[151]

2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide 91[153]

2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl bromide 92[152]

2,3,4-tri-*O*-acetyl-α -L-fucopyranosyl bromide 93[153]

2,3,6,2',3',4',6'-hepta-*O*-acetyl-α-lactopyranosyl bromide 94[153]

General procedure for the methyl seleno glycosides

Me_2Se_2 (86 µL, 0.91 mmol) was added to a slurry of $NaBH_4$ (34 mg, 0.91 mmol) in dry MeCN (2mL). The mixture was refluxed for 30 min under argon atmosphere and then cooled down to room temperature glycoside bromide (250 mg, 0.6 mmol) dissolved in MeCN (2mL) was added and the reflux continued for 15 min. The mixture was neutralised with acetic acid (0.5 mL) and stirred for 10 min. Water (10 mL) was added and the different phases were separated. The aqueous phase was extracted twice with ethyl acetate. The combined organic phases were washed with saturated hydrogen carbonate solution and water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (toluene/ethyl acetate, 6:1) to give the expected product.

Methyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-β-D-glucopyranoside 95-β[119]

.2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide **90** was treated as described for the general selenoglycoside procedure.The expected product is a colourless crystalline compound (237 mg, 91%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.21 (at, 1H, *J*=9.2 Hz, 9.5 Hz); 5.09 (at, 2H, *J*=9.8 Hz); 4.64 (d, 1H, *J*=10.1 Hz); 4.24 (dd, 1H, *J*=5.1 Hz, 12.6 Hz); 4.14 (dd, 1H, *J*=2.2 Hz, 12.3 Hz); 3.72 (ddd, 1H, *J*=2.2 Hz, 4.7 Hz, 9.8 Hz); 2.09, 2.08, 2.06, 2.02, 2.01 (5x s, 5x 3H).

Methyl 2,3,4,6-*O*-acetyl-1-seleno-α-D-glucopyranoside 95-α

Methyl 2,3,4,6-*O*-acetyl-1-seleno-β-D-glucopyranoside **95** (25 mg, 0.2 mmol) was dissolved in dry DCM (2 mL) with 4Å molecular sieve pellets and $BF_3 \cdot OEt$ (100 µL, 0.8 mmol) was added to obtain a 0.4 M solution. The reaction was stirred at room temperature for 4 h. The mixture was quenched using Et_3N, filtrated, concentrated *in vaccuo* and the residue was purified by flash column chromatography (toluene/ethyl acetate, 9:1) to give the expected product as colourless crystals (6 mg, 24%).

^1H NMR (500 MHz, $CDCl_3$) δ = 5.72 (d, *J*=3.5 Hz, 1H); 5.25 (at, *J*=9.5 Hz, 1H); 5.16-5.12 (m, 2H); 4.30 (dd, *J*=4.5 Hz, 12.3 Hz, 1H); 4.12 (dd, *J*=1.9 Hz, 12.3 Hz, 1H); 3.85 (ddd, *J*=2.2 Hz, 4.7 Hz, 10.1 Hz, 1H); 2.10, 2.08, 2.05, 2.02, 2.02 (5x s, 5x 3H).

Methyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-β-D-galactopyranoside 96

2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **91** (250 mg, 0.6 mmol) was treated as described for the general selenoglycoside procedure. The expected product is a colourless crystalline compound (250 mg, 96%).

^1H NMR (500 MHz, $CDCl_3$) δ = 5.46 (d, *J*=3.2 Hz, 1H); 5.31 (at, *J*=9.8 Hz, 10.1 Hz, 1H); 5.05 (dd, *J*=3.5 Hz, 10.1 Hz, 1H); 4.66 (d, *J*=10.1 Hz, 1H); 4.16 (dd, *J*=6.6 Hz, 11.4 Hz, 1H); 4.12 (dd, *J*=6.3 Hz, J 11.0 Hz, 1H); 3.95 (at, *J*=6.6 Hz, 1H); 2.16, 2.12, 2.08, 2.05, 2.00 (5x s, 5x 3H).

Methyl 2,3,4,6-tetra-*O*-acetyl-1-seleno-α-D-mannopyranoside 97

2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide **92** (250 mg, 0.6 mmol) was treated as described for the general selenoglycoside procedure.The expected product is a colourless crystaline compound (38 mg, 15%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.54 (d, *J*=3.5 Hz, 1H); 5.25 (at, *J*=10.1 Hz, 1H); 5.07 (dd, *J*=3.5 Hz, 10.1 Hz, 1H); 4.97 (bs, 1H); 4.27 (dd, *J*=6 Hz, 12.3 Hz, 1H); 4.14 (dd, *J*=2.5 Hz, 12.3 Hz, 1H); 3.63 (ddd, *J*=2.6 Hz, 5.7 Hz, 9.5 Hz, 1H); 2.19, 2.17, 2.08, 2.04, 1.98 (5x s, 5x 3H)

Methyl 2,3,4-tri-*O*-acetyl-1-seleno-β-L-fucopyranoside 98

2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl bromide **93** (250 mg, 0.71 mmol) was treated as describe for the general selenoglycoside procedure.The expected product is a colourless crystalline compound (212 mg, 81%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.30-5.26 (m, 2H); 5.04 (dd, *J*=2.5 Hz, 10.1 Hz, 1H); 4.63 (d, *J*=10.1 Hz, 1H), 3.84 (dd, *J*=6.0 Hz, 12.6 Hz,1H); 2.18, 2.12, 2.07, 1.99 (4x s, 4x 3H); 1.22 (d, *J*=6.7 Hz, 3H)

Methyl 2,3,6,2',3',4',6'-hexa-*O*-acetyl-1-seleno-β-lactopyranoside 99

2,3,6,2',3',4',6'-hepta-*O*-acetyl-α-lactopyranosyl bromide **94** (500 mg, 0.7 mmol) was treated as described for the general selenoglycoside procedure.The expected product is a colourless crystalline compound (300 mg, 57%).

^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.36 (d, *J*=3.2 Hz, 1H); 5.21 (at, *J*=9.5 Hz, 9.1 Hz, 1H); 5.12 (dd, *J*=6.9 Hz, 10.1 Hz, 1H), 5.01 (at, *J*=9.5 Hz, 9.8 Hz, 1H), 4.96 (dd, *J*=3.5 Hz, 10.5 Hz, 1H); 4.63 (d, *J*=10.1 Hz, 1H); 4.49 (d,

J=11.1 Hz, 1H); 4.48 (d, *J*=7.9 Hz, 1H); 4.12 (m, 3H), 3.88 (at, *J*=6.3 Hz, 7.00 Hz, 1H); 3.81 (at, *J*=9.5 Hz, 9.8 Hz, 1H); 3.36, 2.16, 2.13, 2x 2.07, 2.06, 2.05, 1.97 (8x s, 8x 3H).

Methyl 2-*O*-(α-L-fucopyranosyl)-1-seleno-β-D-galactopyranoside 100

methyl 2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-3,4,6-tri-*O*-acetyl-1-seleno-β-D-galactopyranoside **107** was dissolved in methanol, sodium methanolate was added until pH>7 and the mixture was stirred at room temperature for 2h. After complete conversion, the solution was neutralised with Dowex resin to pH=7, filtered and concentrated *in vacuo* to give the expected methyl 2-*O*-(α-L-fucopyranosyl)-1-seleno-β-D-galactopyranoside **100** (16 mg, 96%).

$[\alpha]_D$ −12° (c 1, MeOH)

^{1}H NMR (500 MHz, CD_3OD) δ = 5.19 (d, $J_{1',2'}$=3.8, 1H, H-1'), 4.63 (d, $J_{1,2}$=9.6 Hz, 1H, H-1), 4.38 (d, $J_{5',6'}$=6.6 Hz, 1H, H-5'), 3.93 (d, J=2.1, 1H), 3.84–3.62 (m, 7H), 3.53 (t, J=6.0, 1H), 2.11 (s, 3H, $SeCH_3$), 1.20 (d, $J_{5',6'}$=6.6 Hz, 3H, H-6').

^{13}C NMR (125 MHz, cd3od) δ = 102.87(C-1'), 81.87, 80.81, 79.37, 77.12, 73.79, 71.72, 70.58, 70.47, 68.68, 62.55 (10x carbohydrate C), 16.51 (C-6'), 1.76 ($SeCH_3$).

HR-ESI calcd for $C_{25}H_{36}O_{15}Se$ $[M+Na]^+$ 427.0483. Found 427.0512.

Tolyl 2,3,4-tri-O-benzyl-1-thio-β-fucopyranoside 101[144]

1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose 102[143]

2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside 103

2-*O*-(α-L-fucopyranosyl)-1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside **105** (642 mg) was dissolved in pyridine (20 ml) and Ac_2O (5 ml) was added to the reaction mixture. The reaction was stirred at room temperature overnight. The mixture was co-evaporated three times with toluene and purified by silica gel column chromatography (toluene/EtOAc 20:1→3:7) yielding to the expected 2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside **103** as a white foam (0.7215 g, 1.1637 mmol, 60% two steps).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.34 (d, $J_{1,2}$=3.9 Hz, 1H, H-1), 5.41 (d, $J_{3,4}$=3.2 Hz, 1H, H-4),. 5.31 (d, $J_{1',2'}$=3.9 Hz, 1H, H-1'), 5.27 (dd, $J_{2,3}$=10.4 Hz, $J_{3,4}$=3.4 Hz, 1H, H-3), 5.24 (d, $J_{3',4'}$=3.1 Hz, 1H, H-4'), 5.21 (dd, 1H, $J_{2',3'}$=10.9 Hz, $J_{3',4'}$=3.3 Hz, H-3'), 4.96 (dd, $J_{1',2'}$=3.9 Hz, $J_{2',3'}$=10.9 Hz, 1H, H-2'), 4.30 (t, $J_{5,6}$ = 6.8 Hz, 1H, H-5), 4.15 (m, 2H, H-2, H-5'), 4.08 (d, $J_{5,6}$=6.8 Hz, 2H, H-6), 2.22 (s, 3H, COCH_3), 2.14 (s, 6H, 2x COCH_3), 2.03 (s, 3H, COCH_3), 1.99 (s, 6H, 2x COCH_3), 1.97 (s, 3H, COCH_3), 1.14 (d, 2H, $J_{5',6'}$=6.5 Hz, H-6').

^{13}C NMR (125 MHZ, $CDCl_3$): δ = 169.77, 170.09, 170.35, 170.41, 170.69, 170.71, 170.83 (7x $COCH_3$), 91.18, 97.20 (2x anomeric C), 61.54, 65.61, 67.39, 67.82, 68. 53, 68.94, 70.02, 70.08, 71.38 (9x carbohydrate C), 20.95, 20.97, 20.99, 21.23, 21.78 (5x $COCH_3$), 20.93 (2x $COCH_3$), 15.20 (C-6').

2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-1-,3,4,6-tetra-*O*-α-D-galactopyranoside 104

1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside **102** (502 mg, 1.44 mmol), 2,3,4,-tri-*O*-benzyl-β-L-fucopyranoside **101** (1.167 g, 2.26 mmol) and Et_4NBr (0.454 g, 2.16 mmol) were dissolved DCM/DMF (10:1, 2mL). Molecular sieves MS-3 Å powder was added and the reaction was stirred under argon for 1 hour at room temperature. Br_2 (100 µL, 1.95 mmol) was added to the solution and the reaction was kept on stirring overnight at room temperature. The reaction completion was monitored by TLC (toluene/EtOAc 3:1) and mass spectrometry and the reaction was quenched with Et_3N (400 µL). The reaction mixture diluted with DCM, washed with brine and water and the organic layer was separated, dried over MgSO4 and concentrated under vacuum. Flash column chromatography (petrol/EtOAc 20:1→5:2) gave the expected 2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-1-,3,4,6-tetra-*O*-α-D-galactopyranoside **104** as a white foam (880 mg, 1.15 mmol, 80%).

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.27-7.35 (m, 15H, ArH) , 6.28 (d, $J_{1,2}$=3.8 Hz, 1H, H-1), 5.47 (d, $J_{3,4}$=3.5 Hz, 1H, H-4), 5.34 (dd, $J_{2,3}$=10.7 Hz, $J_{3,4}$=3.5 Hz, 1H, H-3), 5.05 (d, $J_{1',2'}$=3.8 Hz, 1H, H-1'), 4.94 (d, *J*=11.4 Hz, 1H, CH_2Ph), 4.76 (d, *J*=11.7 Hz, 1H, CH_2Ph), 4.73 (d, *J*=11.7 Hz, 1H, CH_2Ph), 4.69 (d, *J*=12.0 Hz, 1H, CH_2Ph), 4.65 (d, *J*=12.0 Hz, 1H, CH_2Ph), 4.63 (d, *J*=11.7 Hz, 1H, CH_2Ph), 4.27 (m, 1H, H-5), 4.14 (dd, $J_{1,2}$=3.8 Hz, $J_{2,3}$=10.8 Hz, 1H, H-2), 4.07 (d, $J_{5,6}$=6.6 Hz, 2H, H-6_a, H-6_b), 4.03 (dd, $J_{1',2'}$=3.8 Hz, $J_{2',3'}$=10.4 Hz, 1H, H-2'), 3.83 (q, $J_{5',6'}$=6.6 Hz, 1H, H-5'), 3.77

(dd, $J_{3',4'}$=2.6 Hz, $J_{2',3'}$=10.4 Hz, 1H, H-3'), 3.63 (d, $J_{3',4'}$=1.6 Hz, 1H, H-4'), 1.73, 2.03, 2.10, 2.15 (4s, 12H, 4x COCH_3),1.12 (d, 3H, $J_{5',6'}$=6.5 Hz, H-6'). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.51 (*C*OCH_3), 170.21 (2x *C*OCH_3), 169.50 (*C*OCH_3),127.41, 127.60, 127.65, 127.78, 128.37, 128.44, 128.50, 138.61, 138.77, 138.82 (aromatic C), 91.52, 99.84 (2x anomeric C), 61.45, 67.51, 67.88, 68.57, 69.54, 71.61, 72.89, 72.98, 74.91, 75.98, 77.52, 78.72 (9x carbohydrate C, 3x CH_2Ph), 21.14 (CO*C*H_3), 20.80 (2x CO*C*H_3), 20.63 (CO*C*H_3), 16.68 (C-6').

MALDI-TOF calcd for $C_{41}H_{48}O_{14}$ $[M+Na]^+$ 787.29; Found 787.61.

2-*O*-(α-L-fucopyranosyl)-1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside 105

2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside **104** (1.4914 g, 1.95 mmol) was dissolved in MeOH (10 ml). A catalytic amount of Pd/C (10% on carbon) was added to the solution and the reaction was stirred under H_2 at room temperature for 2 days. The reaction was monitored by TLC (DCM/MeOH 9:1) and mass spectrometry until completion. The reaction mixture was filtered and concentrated under vacuum. The residue was poored over silica gel column chromatography (DCM/MeOH 20:1→3:1) yielding 2-*O*-(α-L-fucopyranosyl)-1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside **105** as a white foam.

^{1}H NMR (500 MHz, CD_3OD): δ = 6.31 (d, $J_{1,2}$=3.7 Hz,1H, H-1), 5.50 (d, $J_{3,4}$=3.1 Hz, 1H, H-4), 5.35 (dd, $J_{2,3}$=10.6 Hz, $J_{3,4}$=3.3 Hz, 1H, H-3),. 4.93 (d, $J_{1',2'}$=4.0 Hz, 1H, H-1'), 4.41 (t, $J_{5,6}$=6.5 Hz, 1H, H-5), 4.05-4.19 (m, 3H, H-2, H-6_a, H-6_b), 3.93 (q, $J_{5',6'}$=6.5 Hz, 1H, H-5'), 3.73 (dd, $J_{1',2'}$=4.0 Hz,

$J_{2',3'}$=10.2 Hz, 1H, H-2'), 3.65 (d, $J_{3',4'}$=3.0 Hz, 1H, H-4'), 3.62 (dd, $J_{2',3'}$=10.2 Hz, $J_{3',4'}$=3.2 Hz, 1H, H-3'), 2.03, 2.04, 2.17, 2.20 (4s, 12H, 4x COCH_3),1.21 (d, $J_{5',6'}$=6.6 Hz, 3H, H-6').

^{13}C NMR (125 MHz, CD_3OD): δ = 171.12, 171.94, 171.97, 172.11 (4x *C*OCH_3), 92.64, 103.28 (2x anomeric C), 62.60, 68.61, 69.37, 69.66, 69.79, 70.82, 71.30, 73.53, 73.82 (9x carbohydrate), 20.53, 20.57, 20.84 (4x CO*C*H_3), 16.64 (C-6').

MALDI-TOF calcd for $C_{20}H_{30}O_{14}$ $[M+Na]^+$ 517.15; Found 517.14.

Methyl 2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-3,4,6-tri-*O*-acetyl-1-seleno-β-D-galactopyranoside 107

2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside **103** (47 mg, 0.076 mmol) and Et_4NBr (13 mg, 0.114 mmol) were dissolved in dry DCM (0.5 ml). Ac_2O (72 µl, 0.758 mmol) and HBr-HOAc 33% (75 µl, 0.455 mmol) were added to the solution and the reaction was stirred for 2 hours at room temperature. In the meantime, $NaBH_4$ (43 mg, 0.137 mmol) was dissolved in CH_3N (0.5 ml), Me_2Se_2 (0.012 ml, 0.114 mmol) was added to the solution and the reaction was stirred at 50° C for 1 hour.

The mixture containing the bromide intermediate **106** was co-evaporated twice with toluene and the crude compound was added to the sodium methylselenoate reaction mixture. The combined reaction mixture was stirred for 10 minutes at 50° C. After completion, the reaction was quenched with HOAc (100 µl). The reaction mixture was extracted with EtOAc, washed with water, aqueous saturated $NaHCO_3$ and water, dried

overs $MgSO_4$, filtered and concentrated under vacuum. Flash column chromatography (toluene/EtOAc 20:1→ 3:1) was performed to give methyl 2-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-3,4,6-tri-*O*-acetyl-1-seleno-β-D-galactopyranoside **107** (31 mg, 0.048 mmol, 63%).

$[\alpha]_D$ -57° (c 1, CH_2Cl_2)

^{1}H NMR (500 MHz $CDCl_3$): δ = 5.47 (d, $J_{1',2'}$=4.2 Hz, 1H, H-1'), 5.36 (d, $J_{3,4}$=2.8 Hz, 1H, H-4), 5.29 (bs, 1H, H-4'), 5.27 (d, $J_{2',3'}$=10,3 Hz, 1H, H-3'), 5.09 (dd, 1H, $J_{1',2'}$=4.0 Hz, $J_{2',3'}$=10.3 Hz, H-2'), 4.96 (dd, 1H, $J_{2,3}$=9.3 Hz, $J_{3,4}$= 3.4 Hz, H-3), 4.86 (q, $J_{5,6}$=6.4 Hz, 1H, H-5), 4.71 (d, 1H, $J_{1,2}$=9.7 Hz, H-1), 4.05-4.15 (m, 2H, H-6_a, H-6_b), 4.04 (at, 1H, $J_{1,2}$=9.7 Hz, $J_{2,3}$=9.3 Hz, H-2), 3.88 (t, $J_{5',6'}$=6.6 Hz, 1H, H-5'), 2.00, 2.03, 2.12, 2.15, 2.17 (s, 15H, 7x COCH_3), 1.98 (s, 6H, 2 x COCH_3), 1.14 (d, 3H, $J_{5',6'}$=6.5 Hz, H-6').

^{13}C (125 MHz $CDCl_3$): δ = 170.11, 170.32, 170.35, 170.43, 170.62, 170.80 (6x *C*OCH$_3$), 96.32 (C-1'), 77.68 (C-1), 75.51 (C-3), 75.13 (C-5), 72.04 (C-2), 71.38 (C-4'), 67.78 (C-3'), 67.77 (C-2'), 67.51 (C-4), 66.14 (C-5'), 61.58 (C-6), 20.78, 20.80, 20.96 (3 x COCH$_3$),20.75 (2 x COCH$_3$), 20.73 (COCH$_3$), 15.98 (C-6'), 2.13 (SeCH$_3$).

HR-ESI calcd for $C_{25}H_{36}O_{15}Se$ $[M+Na]^+$ 679.1117. Found 679.1122.

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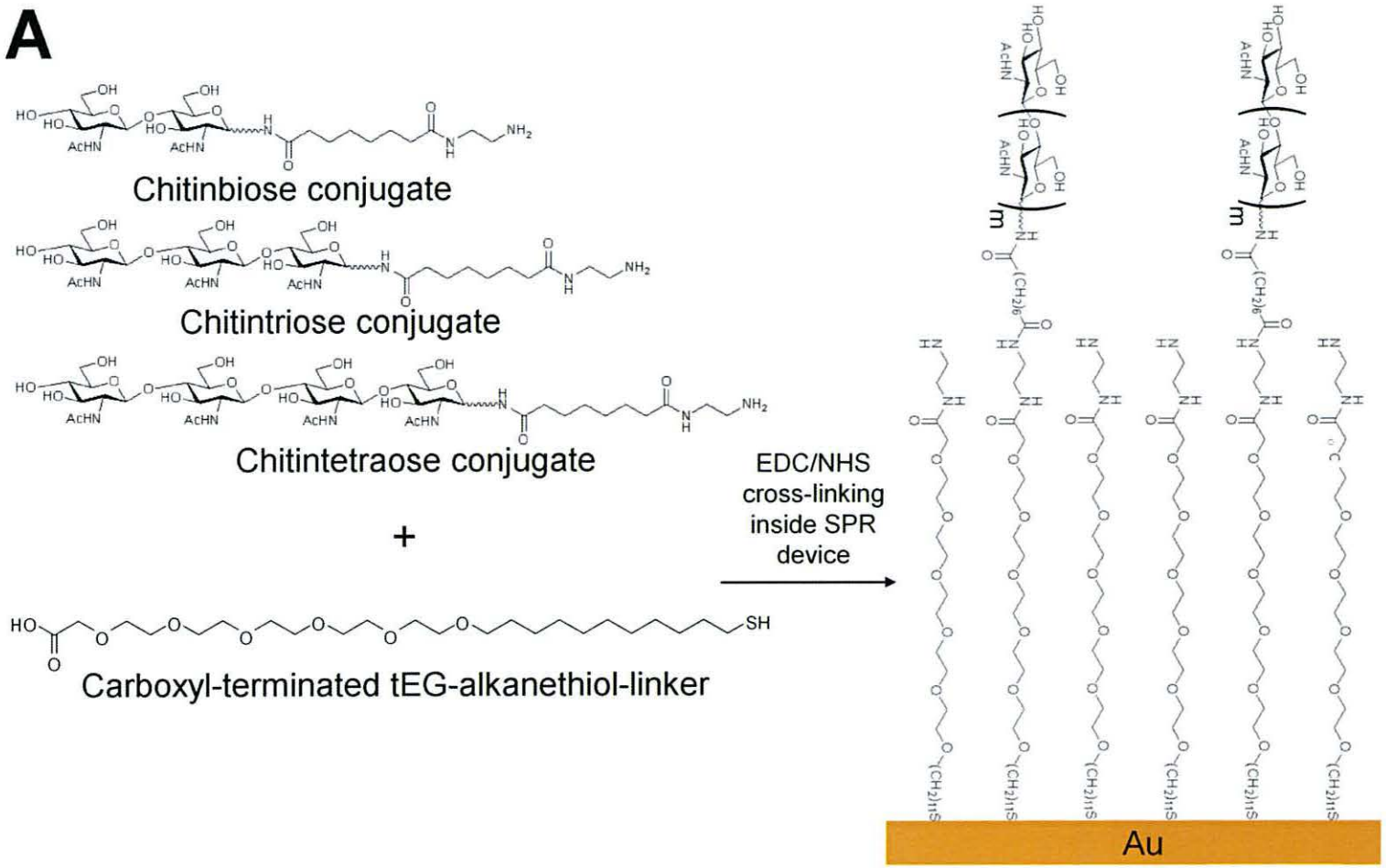
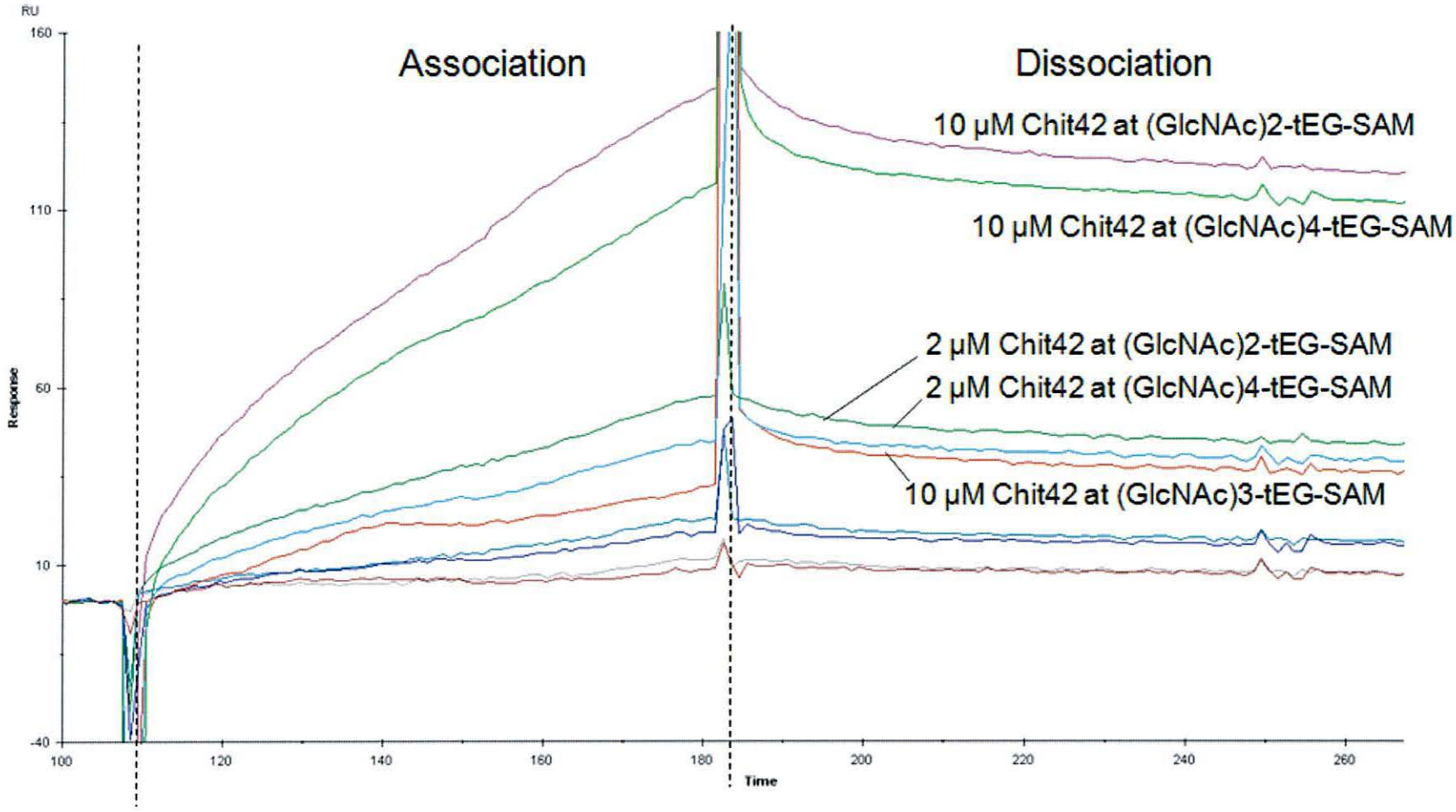
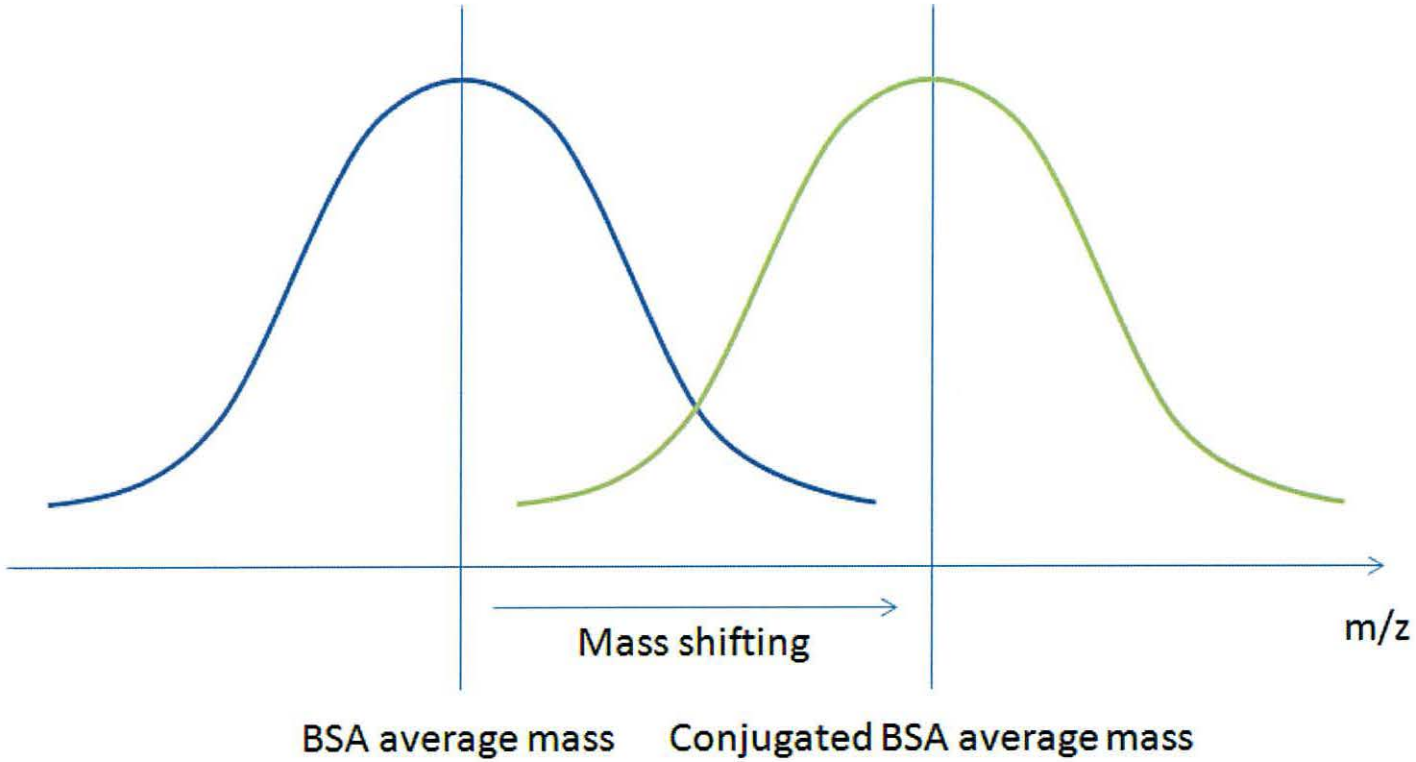
Appendix

Results of chito-oligosaccharides SPR analyse


A
Chitinbiose conjugate
Chitintriose conjugate
Chitintetraose conjugate
+
Carboxyl-terminated tEG-alkanethiol-linker
EDC/NHS
cross-linking
inside SPR
device
Au


RU
160
110
60
10
-40
Response
100
120
140
160
180
200
220
240
260
Time
Association
Dissociation
10 μM Chit42 at (GlcNAc)2-tEG-SAM
10 μM Chit42 at (GlcNAc)4-tEG-SAM
2 μM Chit42 at (GlcNAc)2-tEG-SAM
2 μM Chit42 at (GlcNAc)4-tEG-SAM
10 μM Chit42 at (GlcNAc)3-tEG-SAM

BSA incorporation analyse



$$\text{Average incorporation} = \frac{(\text{BSA average mass} - \text{conjugated BSA average mass})}{\text{conjugate exact mass}}$$

Paper 1: Synthesis of the Lewis b pentasaccharide and a HSA-conjugate thereof

Paper 2: Synthesis of the B-Lewis b heptasaccharide using a versatile lacto-N-tetraose intermediate (draft)

Synthesis of the B-Lewis b heptasaccharide using a versatile lacto-N-tetraose intermediate

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Abstract

A straightforward synthetic route to the B-Lewis b heptasacharide is presented. The key element is a spacer-equipped suitably protected lacto-*N*-tetraose (LNT) intermediate. Its versatile protection group pattern gives access to a variety of blood-group determinants containing the LNT as common core.

Introduction

The gastric pathogen *Helicobacter pylori* induces chronic inflammation, which may progress to severe forms of gastroduodenal diseases such as peptic ulceration and gastric adenocarcinoma[1]. *H. pylori* expresses attachment proteins, so-called adhesins that *inter alia* recognise fucosylated blood group antigens present in the gastric mucosa[2]. This

binding process, mediated by the **B**lood group **a**ntigen **b**inding **A**dhesin, BabA, which belongs to a large and divergent family of *H. pylori* outer membrane proteins with conserved N- and C-terminal domains[2], is essential for colonization. It has been found that most disease associated

Table 1 The table below shows seven blood group determinants which can be ascribed to the LNT structure.

Position	LNT	H-1	A	B	Le^a	Le^b	A-Le^b	B-Le^b
R^1	H	α-L-Fuc	α-L-Fuc	α-L-Fuc	H	α-L-Fuc	α-L-Fuc	α-L-Fuc
R^2	H	H	α-D-GalNAc	α-D-Gal	H	H	α-D-GalNAc	α-D-Gal
R^3	H	H	H	H	α-L-Fuc	α-L-Fuc	α-L-Fuc	α-L-Fuc

[a] R^1, R^2, R^3 referring to the residues in Fig. 1.

strains express BabA[e.g. 3]. Binding studies[4] with the South American *H. pylori* strain P466 suggested a conserved BabA recognition limited to the monofucosylated H1 and the difucosylated Lewis b (Le^b) antigens – antigens generating a blood group O phenotype – well in agreement with the observation that individuals carrying blood group O phenotype are particularly susceptible to peptic ulcer disease[5]. Unexpected binding behaviour of a *H. pylori* strain (CCUG17875) initiated the detailed inspection of the BabA binding prevalence from 373 *H. pylori* strains, collected from various geographic regions, against several blood group antigens, *i.e.* H1, A, Le^b, A-Le^b and B-Le^b. Only small amounts of these complex carbohydrate structures are obtained by isolation from natural sources and, although numerous procedures are known for the preparation of the H1 and the Le^b[e.g.6,7,8,9] oligosaccharides, no synthetic routes for the structurally related A, A-Le^b and B-Le^b heptasaccharides (Fig.1, Tab.1) have been published so far. As the B-Le^b heptasaccharide **1** (Fig. 2) was not accessible from any commercial sources, its synthetic preparation was necessary.

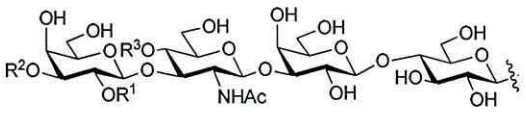


Fig. 1 The LNT is the common core in several blood group structures. The assignment of the locator R^1, R^2 and R^3 is shown in Table 1.

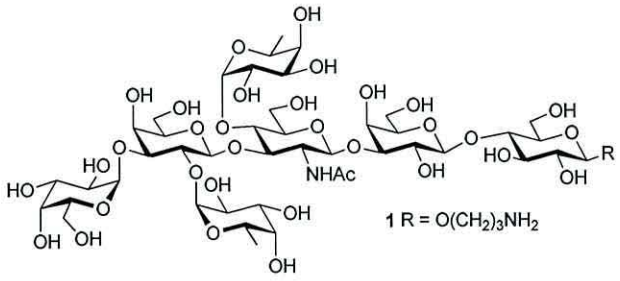
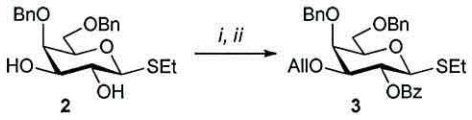


Fig. 2 The spacer-equipped target B-Le^b heptasaccharide.

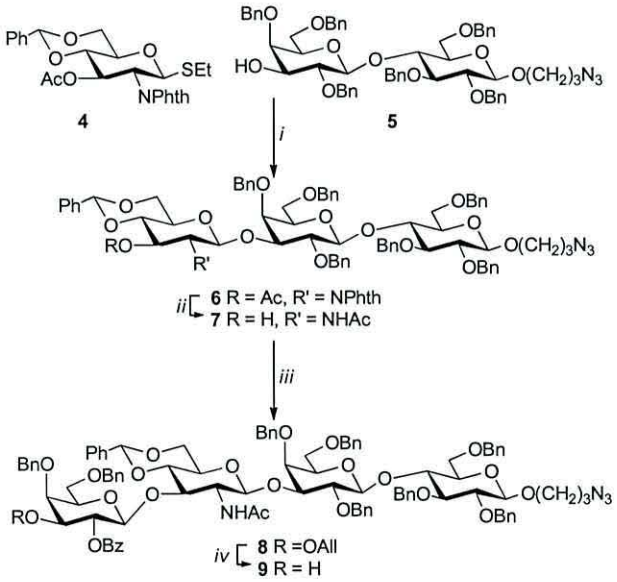
Result and discussion

The B-Le^b heptasaccharide differs from the Le^b hexasaccharides only by the additional blood group B determining non-reducing end (1→3)-α-D-galactosyl residue. When designing the synthesis of the B-Le^b heptasaccharide **1** we made use of the experience gained from our syntheses of the Le^b hexasaccharide[8,9]. The plan was to construct a versatile protected lacto-*N*-tetraose (LNT) intermediate first, then to introduce the non-reducing end (1→3)-α-D-galactosyl residue and finally the two fucosyl residues. To allow the introduction of the B (or A) determining monosaccharide, a temporary protecting group had to be introduced into the 3-*O*-position orthogonal to a 2-*O*-ester protecting group of the non-reducing end galactose unit of the LNT tetrasaccharide (Scheme I).



Scheme I *i*: 1.Bu_2SnO, dry MeOH, 2. CsF, allyl bromide, MeCN, *ii*: benzoyl chloride, DMAP, pyridine, 55%.

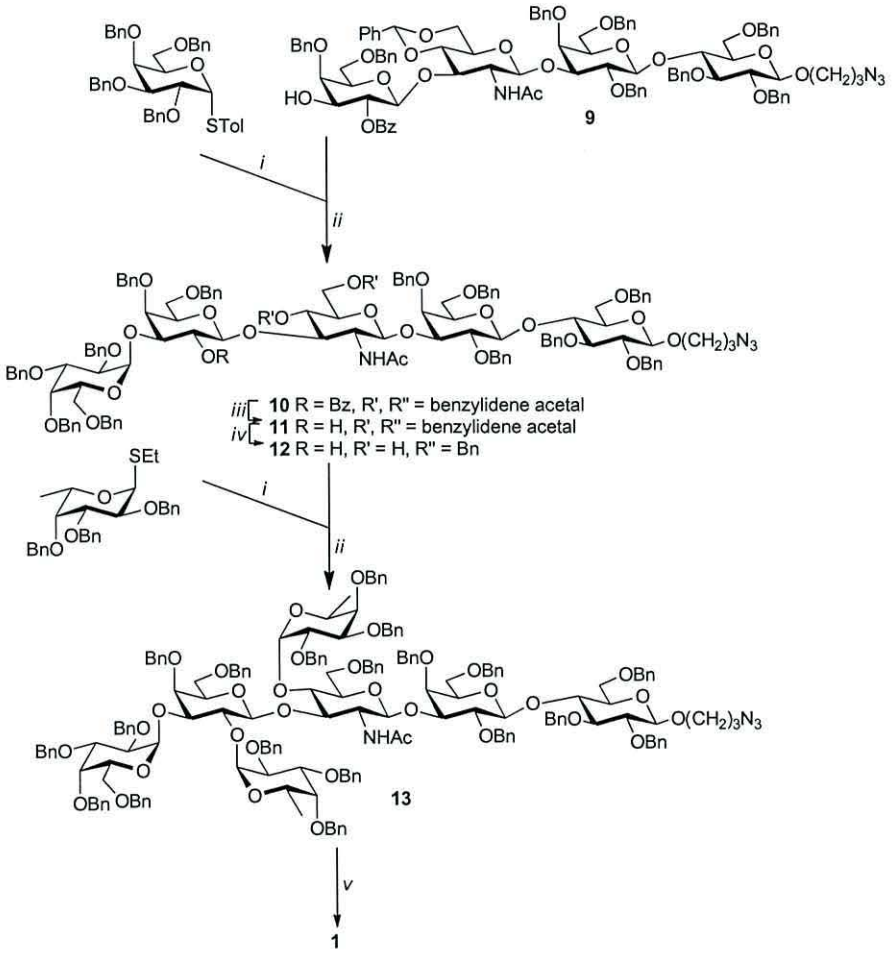
Therefore, the known ethyl thiogalactoside **2**[10] was regioselectivly 3-*O*-allylated using activation by Bu_2SnO in methanol, followed by reaction with allyl bromide in presence of CsF. The crude product was benzoylated to afford **3** in 55% yield over two steps.



Scheme II *i*: **3**, NIS, AgOTf, DCM, rt, 91%, *ii*: 1. ethylenediamine, EtOH, reflux; 2. NaOMe; 3. Ac_2O, MeOH/toluene, 74%, *iii*: **3**, NIS, AgOTf, DCM, 0 °C, 85% *iv*: 1,5-cyclooctadienebis(methyldiphenylphosphine) iridium(I) PF_6, THF, NIS, H_2O, 89%.

The tetrasaccharide building block **9** (Scheme II) was synthesised from the donor-acceptor pair **4**[8] and **5**[11] in a NIS/AgOTf promoted coupling giving trisaccharide **6** in 91% yield followed by conversion of the phthalimido group into the NHAc group and concomitant removal of the acetate group producing acceptor trisaccharide **7** (74 %). NIS/AgOTf promoted coupling with the 3-*O*-allylated donor **3** produced the fully protected tetrasaccharide

8 (85%). This LNT derivative is designed to allow the preparation of several blood group determinants (Table 1) by removal of appropriate temporary protecting group(s) followed by sequential or simultaneous introduction of the various α-linked sugar residue(s). Isomerisation of the allyl protecting group by 1,5-cyclooctadiene-bis(methyldiphenylphosphine) iridium(I) PF_6 in THF followed by hydrolysis of the formed vinyl ether with NIS and H_2O, afforded the deallylated tetrasaccharide acceptor **9** in 89% yield.



Scheme III *i*: Br_2, DCM, *ii*: TEAB, DCM, 56%, *iii*: NaOH, THF/EtOH,H_2O, 35 °C, 97%, *iv*: $NaBH_3CN$, HCl/Et_2O, 52%, *v*: TEAB, DCM/DMF (3:2), 43%, *vi*: 1. Pd/C, THF, 2. Pd/C, 1 M HCl (1 eq), THF, EtOH, H_2O, H_2, 1 atm, RT, 46%.

The α-linked galactosyl residue was introduced under halide-assisted conditions using 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl bromide[13] as donor to give pentasaccharide **10** in acceptable yield (56 %). Cleavage of the benzoate ester was extremely slow under Zemplén conditions, but when compound **10** was treated with NaOH at 35 °C overnight an almost quantitative yield (97 %) of the mono-hydroxy pentasaccharide **11** was obtained. Subsequent opening of the benzylidene acetal using $NaBH_3CN$/HCl resulted in diol acceptor **12**, which was glycosylated with 2,3,4-tri-*O*-benzyl-L-fucopyranosyl bromide[14] under halide assisted conditions using TEAB as promoter to afford heptasaccharide **13** (43%). Debenzylation, with concomitant reduction of the azide, by hydrogenolysis using Pd/C as catalyst in the presence of 1 molar equivalent of HCl (1M in EtOH 95%) gave the unprotected target heptasaccharide **1** (46%). The heptasaccharide was conjugated to HSA using squarate ester methodology and used in *H. pylori* BabA binding studies[5].

In summary, a straightforward synthetic route to the B-Lewis b heptasacharide has been developed. A linear approach is utilized featuring a suitably protected lacto-*N*-tetraose derivative as a key intermediate, which also enclose the possible synthesis of a variety of other structurally related blood group structures.

Experimental

General methods

Methylene chloride was distilled before use and dry DMSO was kept over molecular sieves 3 Å, all other chemicals were used as purchased. Organic solutions were dried over $MgSO_4$ before concentration, which was performed under reduced pressure at <40 °C (bath temperature). NMR spectra were recorded at 300 MHz (Varian) or 400 MHz (Varian or Jeol) (^{1}H) or at 75 MHz or 100MHz (^{13}C), respectively, in $CDCl_3$, D_2O or CD_3OD at ambient temperature if not other stated. TMS was used as internal

standard (δ = 0) for ^{1}H-spectra in chloroform and methanol. NMR experiments in D_2O were referred to the water signal (δ = 4.80). ^{13}C-spectra were referred to the chloroform signal (δ = 77.17) or methanol signal (δ = 49.15). Silica gel Normasil (40-63 µm, Prolabo, VWR International) was used for flash chromatography. TLC was performed on silica gel 60 F254 (Merck) glass plates with detection by UV-light and/or charring with 8% sulfuric acid, ninhydrine (0.1 % in ethanol) or AMC-solution (ammonium molybdate, cerium (IV) sulfate, 10 % sulfuric acid [5: 0.1: 100, w/w/v]). MALDI-TOF spectra were recorded on a Bruker Biflex III using 2',4',6'-trihydroxy-acetophenone monohydrate (THAP) as matrix.

Ethyl 3-*O*-allyl-2-*O*-benzoyl-4,6-*O*-di-benzyl-1-thio-β-D-galactopyranoside (3)

Ethyl thio-β-D-galactopyranoside (11 g, 49 mmol), 2,3-butanedione (4.7 ml, 54 mmol) and trimethylorthoformate (17.7 ml, 162 mmol) were dissolved in dry methanol (100 ml). Camphorsulphonic acid (1.25 g, 5.4 mmol) was added and the solution was refluxed for 2 h. The reaction was quenched with triethyl amine and the solvent was evaporated giving crude ethyl 2,3-*O*-(2',3'-dimethoxy-2',3'-diyl)-1-thio-β-D-galactopyranoside which was used without further purification in the next step. The crude material (approx. 49 mmol) was dissolved in DMF and cooled to 0 °C. NaH (7.0 g, 175 mmol) was added in small portions. The mixture was stirred for 1 h at 0 °C and 30 min at room temperature. Benzyl bromide (17.5 ml, 147 mmol) was added dropwise. The solution was stirred for 1 h and was then poured on an ice/toluene mixture. The water phase was extracted twice with toluene. The organic layer was then washed with water, dried and the solvent was evaporated. ethyl 4,6-*O*-di-benzyl 2,3-*O*-(2',3'-dimethoxy-2',3'-diyl)-1-thio-β-D-galactopyranoside (18 g, 35 mmol, 71% over two steps) was obtained by column chromatography (toluene-ethyl acetate, 6:1). δ_H(400 MHz, $CDCl_3$) 1.28 (t, $SCH_2C\mathbf{H_3}$, 3H), 1.32 (s, CH_3, 6H), 2.71 (m, $SC\mathbf{H_2}CH_3$, 2H), 3.27 (s, OCH_3, 3H), 3.31 (s, OCH_3, 3H), 3.63 (m, H-5,6,

3H), 3.81 (H-2, 1H), 4.07 (H-3, 1H), 4.45 (dd, OC**H**$_2$Ph, 2H), 4.67 (m, OC**H**$_2$Ph and H-1, 2H), 4.96 (d, OC**H**$_2$Ph, 1H), 7.29 (m, CH phenyl, 10 H) δ_C(100 MHz, $CDCl_3$) 15 (SCH$_2$**C**H$_3$, 1C), 18 (CH_3 acetal, 2C), 24 (S**C**H$_2$CH$_3$, 1C), 48 (OCH_3, 2C), 66-74 (C-2,3,4,5,6 and O**C**H$_2$Ph, 6C), 78 (O**C**H$_2$Ph, 1C) 83 (C-1, 1C), 100 (**C** acetal, 2C), 128 (**C**H phenyl, 10C), 138 (**C** phenyl, 2C). TFA:H_2O (10:1, 20 ml) was added to a solution of ethyl 4,6-*O*-di-benzyl 2,3-*O*-(2',3'-dimethoxy-2',3'-diyl)-1-thio-β-D-galactopyranoside (9.8 g, 18.9 mmol) in dichloromethane (120 ml). The solution was refluxed for 2 h, then poured on ice diluted with dichloromethane and neutralised with $NaHCO_3$ (s). The organic layer was separated, washed with water and the solvent was evaporated. The crude product was purified by column chromatography (toluene-ethyl acetate 2:1) to give **2** (6.6 g, 16.3 mmol, 86%). A sample of **2** was acetylated for characterisation. **2** (580 mg, 1.43 mmol) was dissolved in pyridine. Acetic anhydride (0.6 ml, 6.4 mmol) and DMAP (cat. amount) were added and the solution was stirred overnight. The solvent was evaporated and the residue was coevaporated with toluene, followed by flash chromatography (petroleum ether-ethyl acetate, 5:1) yielding ethyl 2,3-O-di-acetyl-4,6-*O*-di-benzyl-1-thio-β-D-galactopyranoside (560 mg, 1.15 mmol, 80%). Bu_2SnO (7.77 g, 31.2 mmol) was added to a solution of **1** (6.31 g, 15.6 mmol) in dry methanol (150 ml). The reaction mixture was refluxed overnight and the solvent was subsequently evaporated. The residue was dissolved in MeCN (50 ml), CsF (3.08 g, 20.3 mmol) and allylbromide (6.75 ml, 78 mmol) were added and the reaction mixture was stirred at room temperature for 1 d. After removal of the solvent, the residue was dissolved in ethyl acetate and washed with KF (aq. 10%) followed by water. The product was dried over $MgSO_4$ and the solvent was evaporated. The crude ethyl 3-*O*-allyl-4,6-di-*O*-benzyl-β-D-galactopyranoside was dissolved in pyridine. Benzoyl chloride (3.6 ml, 31 mmol) and DMAP (cat. amount) were added and the solution was stirred overnight. After concentration of the reaction mixture under reduced pressure, the residue was dissolved in ethyl acetate and washed with

water and brine. The solvent was removed and the product was co-evaporated with toluene to yield **3** as white crystals (4.23 g, 8.6 mmol, 55% over 2 steps). δ_H(400 MHz, $CDCl_3$) 1.21 (t, $SCH_2C\mathbf{H}_3$, 3H), 2.72 (m, $SC\mathbf{H}_2CH_3$, 2H), 3.64 (m, H-3,H-5,H-6_a,H-6_b, 4H), 4.00 (dd ~ m, H-4, 1H), 4.12 (m, $OC\mathbf{H}_2CHCH_2$, 2H), 4.45 (d, H-1, 1H), 4.45-4.54 (d ~ m $OC\mathbf{H}_2Ph$, 2H), 4.60 ($OC\mathbf{H}_2Ph$, 1H), 4.99 ($OC\mathbf{H}_2Ph$, 1H), 5.06 (dd, $OCH_2CHC\mathbf{H}_2$, 1H), 5.18 (dd, $OCH_2CHC\mathbf{H}_2$, 1H), 5.64 (dd ~ t, H-2), 5.75 (m, $OCH_2C\mathbf{H}CH_2$) 7.2-7.5 (m, CH phenyl, 13 H), 8.0-8.1 (m, CH benzoyl, 2 H) δ_C(100 MHz, $CDCl_3$) ($SCH_2\mathbf{C}H_3$, 1C), 24 ($S\mathbf{C}H_2CH_3$, 1C), 68-75 (C-2,3,4,5,6 and allyl, 6C), 78 ($\mathbf{C}H_2Ph$, 1C), 81 (C-1, 1C), 84 ($\mathbf{C}H_2Ph$, 1C), 117 (allyl, 1C), 128-131 (CH phenyl, benzoyl 15C), 135 (allyl, 1C),138 (**C** phenyl, 2C), 162, 165 (C=O, 2C).

3-Azidopropyl (3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (6)

A solution of ethyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**4**, 212 mg, 439 µmol) and 3-azidopropyl-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5**, 360 mg, 361 µmol) in dry CH_2Cl_2 (10 mL) was stirred with powdered molecular sieves (4Å) under nitrogen for 1 h when NIS (160 mg, 0.71 mmol) and a catalytic amount of AgOTf were added. After 10 min stirring at room temperature, the reaction was quenched by addition of Et_3N (100 µl), and the mixture diluted with CH_2Cl_2 (20 mL), filtered through Celite. The filtrate was washed with $Na_2S_2O_3$ (10 % aqu.) and water, dried and concentrated. The residue was applied onto a silica gel column and eluted (toluene (0.5 % Et3N)→toluene/EtOAc 10:1) to give **6** (455 mg, 328 µmol, 91 %). $[\alpha]_D$ –42.4° (c 0.5,$CHCl_3$)[11]; δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.83 (2 H, m), 1.88 (3 H, s), 2.92 (1 H, m), 3.32-3.65 (m), 3.76-3.92 (m), 4.06 (1 H, d *J* 12.1), 4.19 (1 H, d *J* 7.3), 4.24-4.99 (m), 5.56 (1 H, s), 5.69 (1 H, d *J*

8.4), 5.96 (1 H, m), 6.91, 7.06-7.50, 7.67, 7.77 and 7.89 (m); δ_c(100 MHz; $CDCl_3$; Me_4Si) 20.6, 29.3, 48.4, 55.9, 66.1, 66.4, 67.7, 68.2, 68.8, 69.6, 73.0, 73.1, 73.4, 74.8, 75.1, 75.5, 75.8, 76.6, 77.3, 78.9, 79.4, 81.7, 81.9, 83.0, 100.0, 101.8, 102.4, 103.5, 125-128, 134.5, 137.0, 138.3, 138.4, 138.5, 138.7, 139.1, 139.3 and 170.1; m/z (HR-ESI) 1409.5573 (M^+ + Na) $C_{80}H_{82}N_4NaO_{18}$ requires 1409.5522; Anal. Calcd. For $C_{74}H_{82}N_4O_{17}$: C,96.25; H, 5.96. Found: C, 96.18; H, 6.10.

3-Azidopropyl (4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (7)

A solution of **6** (450 mg, 324 µmol) and ethylene diamine (1.2 mL) in EtOH (99.5 %, 12 mL) was brought to reflux. After 5 h, NaOMe (1M, 200 µl) was added to cleave remaining acetates. The mixture was left over night at ambient temperature, then diluted with toluene and concentrated. After co-evaporation with toluene, the residue was dissolved in MeOH/toluene (2:1, 10 ml) and Ac_2O (1 ml) was added. After additional 30 min, the mixture was concentrated and co-evaporated with toluene. The residue was treated with water (20 ml) and extracted with ethyl acetate (2x25 ml), dried, filtered and concentrated. Purification on a silica gel column (toluene→toluene/EtOAc 2:1) gave **7** (300 mg, 0.24 mmol, 74 %). $[\alpha]_D$ −12.0° (c 1,CH_2Cl_2); δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.56 (3 H, s), 1.94 (2 H, m), 3.37 (1 H, m), 3.42-3.76 (13 H, m), 3.80-3.90 (4 H, m), 3.98 (1 H, d *J* 3.3), 4.00 (1 H, m), 4.10 (1 H, t *J* 9.4), 4.34 (1 H, d *J* 11.7), 4.41 (1 H, d *J* 7.7), 4.46 (3 H, m), 4.54 (1 H, d *J* 7.7), 4.62 (1 H, d *J* 11.4), 4.68 (1 H, d *J* 13.2), 4.72 (1 H, d *J* 12.4), 4.79 (1 H, m), 4.83 (1 H, d *J* 11.1), 4.91 (1 H, d *J* 10.8), 4.97 (1 H, d *J* 11.7), 5.01 (1 H, *J* 2.4), 5.11 (1 H, d *J* 5.1), 5.49 (1 H, d *J* 5.1), 5.64 (1 H, s), 7.22-7.46 (m) and 7.60 (2 H, m); δ_c(100 MHz; $CDCl_3$; Me_4Si) 22.7, 29.3, 48.4, 59.2, 66.6, 68.1 (2 x), 68.7, 72.9, 73.3, 73.5 (2 x), 74.2, 75.0, 75.1, 75.5, 76.2, 76.3, 80.0, 81.6, 81.7 (2 x), 82.9, 102.0, 102.6 (2 x), 103.6, 127-129, 137.1, 138.1, 138.3, 138.5, 138.6,

139.0, 139.1 and 172.5; m/z (HR-ESI) 1257.5627 (M^+ + H) $C_{72}H_{81}N_4O_{16}$ requires 1257.5642.

3-Azidopropyl (3-*O*-allyl-2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (8)

A solution of **7** (260 mg, 207 µmol) and **3** (170 mg, 310 µmol) in dry CH_2Cl_2 (15 mL) was stirred at 0 °C under nitrogen when NIS (90 mg, 0.40 mmol) and a catalytic amount of AgOTf were added. After 10 min stirring at room temperature, the reaction was quenched by addition of Et3N (50 µl).After concentration, the residue was applied onto a silica gel column and eluted (toluene (0.5 % Et3N)→toluene/EtOAc 10:1) to give **8** (307 mg, 176 µmol, 85 %); $[\alpha]_D$ +5.4° (c 1,CH_2Cl_2); δ_H(400 MHz; $CDCl_3$; Me_4Si) 1.85 (2 H, m), 2.36 (3 H, s), 3.17 (1 H, m), 3.35-3.80 (m), 3.85-4.00 (m), 4.18-4.40 (m), 4.46-4.81 (m), 4.89 (2 H, m), 4.99 (1 H, m), 5.00 (1 H, dd), 5.27 (2 H, m), 5.48 (1 H, s), 5.51 (1 H, dd *J*), 5.64 (1 H, m) 7.2-7.6 (m) and 8.01 (2 H, m); δ_c(100 MHz; $CDCl_3$; Me_4Si) 22.4, 29.3, 48.4, 59.2, 65.5, 66.5, 68.1, 68.2, 68.3, 69.0, 71.2, 72.5, 72.7, 72.9, 73.2, 73.3, 73.5, 74.6, 74.8, 74.9, 75.1 (2 x), 75.4, 76.2, 76.3, 79.4, 80.2, 81.3, 81.7, 82.4, 82.9, 100.6, 101.0, 101.4, 102.5, 103.6, 117.3, 126-129, 133.2, 134.4, 137.1-139.1, 165.3 and 170.7; m/z (HR-ESI) 1765.7482 (M^+ + Na.) $C_{102}H_{110}N_4NaO_{22}$ requires 1765.7509.

3-Azidopropyl (2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (9)

Compound **8** (300 mg, 172 µmol) was dissolved in freshly distilled THF (6 mL) and stirred 10 min with a catalytic amount of Pd/C. The filtered solution was degassed and set under nitrogen atmosphere. After addition of 1,5-cyclooctadienebis (methyldiphenylphosphine) iridium(I) PF_6 (16.5 mg, 20 µmol), the solution was saturated with hydrogen until the intensive red colour of the mixture had disappeared (approx. after 20 min). The solution was stirred for 1 h under nitrogen at room temperature, then NIS (260 mg, 1.16 mmol) and H_2O (1.1 mL) were added. After 18 h the reaction mixture was diluted with EtOAc, washed consecutively with 10% aqueous $Na_2S_2O_3$, $NaHCO_3$ and brine, dried and concentrated. The crude product was purified by flash chromatography (toluene/EtOAc 3:1) to give **9** (240 mg, 153 µmol, 89%). $[\alpha]_D$ +7.2° (c 1,CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.87 (2 H, m), 2.36 (3 H, s), 3.05 (1 H, m), 3.20 (1 H, m), 3.35-4.00 (m), 4.22-4.41 (m), 4.50-4.84 (m), 4.91 (1 H, d), 5.00 (1 H, d), 5.22 (2 H, m), 5.53 (1 H, s), 7.2-7.6 (m) and 8.01 (2 H, m); δ_c(100 MHz; $CDCl_3$; Me_4Si) 22.4, 29.4, 48.4, 58.9, 65.6, 66.5, 68.1, 68.2, 69.0, 72.9 (2 x), 73.0, 73.2, 73.4, 73.5, 74.6, 74.8, 74.9, 75.1, 75.2, 75.5 (2 x), 76.3, 76.4 (2 x), 77.3, 79.6, 81.3, 81.8, 82.3, 83.0, 100.6, 100.8, 101.5, 102.6, 103.6, 125-130, 133.4, 137.6, 137.7, 138.1, 138.3, 138.4, 138.7, 138.9, 139.1, 139.5, 166.3 and 170.6; m/z (HR-ESI) 1725.7230 (M^+ + Na.) $C_{99}H_{106}N_4NaO_{22}$ requires 1725.7191.

3-Azidopropyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(2-*O*-benzoyl-4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (10)

Tolyl 2,3,4-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside[15], (170 mg, 0.29 mmol) in CH_2Cl_2 (2 ml) was treated with bromine (18 µl, 0.35 mmol) at 0 °C for 10 min. After concentration and co-evaporation with dry toluene, the crude 2,3,4-tetra-*O*-benzyl-D-galactopyranosyl bromide was dissolved in

CH_2Cl_2 (1 ml) and added to a mixture of **9** (230 mg, 135 µmol mmol), Et_4NBr (67 mg, 0.32 mmol) and powdered molecular sieves (4 Å, 200 mg) in CH_2Cl_2/DMF (3:2, 2 ml), which had been stirred under nitrogen for 1 h. After 24 h and 48 h an additional amount bromide (80 mg, 0.14 mmol) was added to complete the reaction. The stirring was continued for 24 h, then MeOH (1 mL) was added, followed by filtration through Celite. Purification on silica gel (toluene →toluene/EtOAc 2:1) gave **10** (170 mg, 76 µmol, 56 %). $[\alpha]_D$ +32.2° (c 1,CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.61 (3 H, s),1.85 (2 H, m), 2.99 (2 H, m), 3.14-4.00 (m), 4.12-4.99 (m), 5.20 (1 H, d *J* 7.0), 5.27 (1 H, d *J* 7.7), 5.46 (1 H, s), 5.50 (1 H, m), 5.57 (1 H, dd *J* 8.1 *J* 10.2) 7.1-7.4 (m) and 7.92 (2 H, d *J* 7.0); δ_c(100 MHz; $CDCl_3$; Me_4Si) 22.4, 29.4, 48.4, 59.2, 65.6, 66.5, 68.0, 68.1, 68.3, 68.5, 69.0, 69.7, 72.3, 72.7, 73.0 (2 x), 73.2, 73.3, 73.4 (2 x), 73.6, 74.4, 74.8, 74.9, 75.1, 75.2, 75.5, 76.3, 76.5 (2 x), 77.0, 77.4, 79.0, 79.4, 79.7, 81.2, 81.8, 82.5, 98.5 (*J* 167 α), 100.7 (*J* 176, 4,6-Bn), 100.9 (*J* 165 β), 101.4 (*J* 161 β), 102.6 (*J* 153 β), 103.6 (*J* 155 β), 127-130, 133.1, 137.7, 138.0, 138.1, 138.3, 138.4, 138.5, 138.7 (2 x), 138.8, 138.9, 139.0, 139.2, 139.5, 165.2 and 170.6; m/z (HR-ESI) 2247.9632 (M^+ + Na.) $C_{133}H_{140}N_4NaO_{27}$ requires 2247.9603.

3-Azidopropyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(4,6-*O*-benzylidene-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (11)

Compound **10** (170 mg, 76 µmol) was dissolved in THF/EtOH/H_2O (5:2:1, 4 ml) and NaOH (300 µL, 10 M) was added. The mixture was stirred at 35 °C overnight, then neutralised by adding ion exchange resin (Dowex). After filtration and concentration, the crude material was purified by flash chromatography (toluene(Et_3N)→toluene/EtOAc 2:1) to yield **11** (158 mg, 74 µmol, 97 %). $[\alpha]_D$ +7.2° (c 1,CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.61

(3 H, s),1.85 (2 H, m), 2.99 (2 H, m), 3.14-4.00 (m), 4.12-4.99 (m), 5.20 (1 H, d *J* 7.0), 5.27 (1 H, d *J* 7.7), 5.46 (1 H, s), 5.50 (1 H, m), 5.57 (1 H, dd *J* 8.1 *J* 10.2) 7.1-7.4 (m) and 7.92 (2 H, d *J* 7.0); δ_c(100 MHz; $CDCl_3$; Me_4Si) 22.4, 29.4, 48.4, 59.2, 65.6, 66.5, 68.0, 68.1, 68.3, 68.5, 69.0, 69.7, 72.3, 72.7, 73.0 (2 x), 73.2, 73.3, 73.4 (2 x), 73.6, 74.4, 74.8, 74.9, 75.1, 75.2, 75.5, 76.3, 76.5 (2 x), 77.0, 77.4, 79.0, 79.4, 79.7, 81.2, 81.8, 82.5, 98.5 (*J* 167 α), 100.7 (*J* 176, 4,6-Bn), 100.9 (*J* 165 β), 101.4 (*J* 161 β), 102.6 (*J* 153 β), 103.6 (*J* 155 β), 127-130, 133.1, 137.7, 138.0, 138.1, 138.3, 138.4, 138.5, 138.7 (2 x), 138.8, 138.9, 139.0, 139.2, 139.5, 165.2 and 170.6; m/z (HR-ESI) 2143.9388 (M^+ + Na.) $C_{126}H_{136}N_4NaO_{26}$ requires 2143.9341.

3-Azidopropyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-(4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (12)

$NaBH_3CN$ (30 mg, 0.48 mmol) and powdered molecular sieves (3 Å) were added to a solution of **11** (155 mg, 73 μmol) in THF (5 mL). After 30 min, HCl/Et_2O was added dropwise until evolution of gas ceased. The reaction was stirred for an additional 6 h and then quenched with Et_3N (0.5 mL). The mixture was filtered through Celite, concentrated and purified on a silica gel column (toluene →toluene/EtOAc 3:1) to give **12** (78 mg, 38 μmol, 52 %). $[\alpha]_D$ +18.9° (c 1,CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.61 (3 H, s),1.85 (2 H, m), 3.2-4.15 (m), 4.20-5.05 (m) and 7.1-7.4 (m) ; δ_c(100 MHz; $CDCl_3$; Me_4Si) 23.1, 29.4, 48.4, 56.7, 66.5, 68.0, 68.2, 68.6, 68.9, 69.5, 69.8, 70.1, 70.2, 70.4, 72.9, 73.3, 73.4, 73.7, 73.8, 74.2, 74.6, 74.8, 75.0, 75.1, 75.2, 75.3, 75.5, 76.4, 76.5, 77.4, 78.8, 79.7, 81.7, 82.3, 83.0, 84.4, 85.8, 99.3, 101.6, 102.5, 103.6, 104.8, 127-130, 133.1, 137.7-139.6 and 171.6; m/z (HR-ESI) 2145.9423 (M^+ + Na.) $C_{126}H_{138}N_4NaO_{26}$ requires 2145.9497.

3-Azidopropyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-(1→3)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)]-(4,6-di-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-[(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)]-(6-*O*-benzyl-2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-2-β-D-glucopyranoside (13)

Ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside[16] (100 mg, 0.21 mmol) in CH_2Cl_2 (1 ml) was treated with bromine (13 µl, 0.25 mmol) at 0 °C for 10 min. After concentration and co-evaporation with dry toluene, the crude 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide was dissolved in CH_2Cl_2 (250 µl) and added to a slurry of **12** (75 mg, 35 µmol), Et_4NBr (30 mg, 0.14 mmol) and powdered molecular sieves (4 Å, 150 mg) in CH_2Cl_2/DMF (3:2, 250 µl), which had been stirred under nitrogen for 1 h. After 24 h, an additional amount of bromine (prepared from 100 mg ethyl thio fucofuranoside as described above) and CH_2Cl_2 (400 µL) were added. The reaction was left for 8 h, then MeOH (1 mL) was added, followed by filtration through Celite. Purification on silica gel (two columns: toluene →toluene/EtOAc 2:1) gave **13** (45 mg, 15 µmol, 43 %). $[\alpha]_D$ −11.0° (c 1,CH_2Cl_2); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.27(6 H, s), 1.69 (3 H, s, br),1.86 (2 H, m), 2.89, 3.09, 3.22 (2 H, m), 3.30-5.08 (m), 5.27, 5.33 (1 H, d *J* 3.0), 5.65 (1 H, d *J* 3.0) and 7.1-7.4 (m) ; δ_c(100 MHz; $CDCl_3$; Me_4Si) 16.5, 16.6, 23.2, 29.3, 48.4, 56.7syns ej, 66.5, 66.7, 66.8, 67.7, 68.1, 68.4, 68.8, 69.8, 70.8, 71.6, 71.8, 71.9, 73.1-73.6, 74.2, 74.6-75.2, 75.5-75.8, 76.4, 76.6, 77.4, 77.7, 78.3, 79.2, 80.0, 80.3, 80.8, 81.7, 82.6, 83.1, 97.8, 97.9, 98.2, 101.7, 101.8, 102.6, 103.6, 127-129, 1, 138.1-139.7 and 169.3; m/z (HR-ESI) 2978.3425 (M^+ + Na.) $C_{180}H_{194}N_4NaO_{34}$ requires 2978.3472.

3-Aminopropyl α-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→3)-[α-L-fucopyranosyl-(1→4)]-2-

acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranosyl)-(1→4)-2-β-D-glucopyranoside (1)

The heptasaccharide **13** (42 mg, 14.2 µmol) was dissolved in THF (5 mL) and Pd/C (5 %, 5 mg) was added. The mixture was stirred for 5 h, filtered through a sandwich of filters (10 µm on top of a 5 µm filter pellet) and concentrated. The residue was dissolved in a mixture of THF (400 µL)/EtOAc (200 µL)/EtOH (95 %, 600 µL) and Pd/C (5 %, 30 mg) was added. Then the reaction mixture was buffered by adding HCl (1M, 14 µl in 400 µL EtOH, 95 %). The reaction mixture was set under an hydrogen atmosphere at atmospheric pressure. After 18 h water (225 µL) was added to keep the material in solution and stirring under hydrogen was continued for an additional 6 h. The suspension was buffered with $NaHCO_3$ (10 % aq., 75 µL), filtered through a sandwich of filters (as above), diluted with water (10 mL) and washed with Et_2O (2x5 mL). The water phase was collected and concentrated. Purification by reversed phase chromatography (C18, water) and size exclusion chromatography (P2) gave compound **1** (8 mg, 6.6 µmol, 46 %) as colourless syrup. $[\alpha]_D$ +1.4° (c 1,CH_2Cl_2); δ_H (400 MHz; D_2O) 1.27 (6 H, m), 1.69 (3 H, s, br),2.02 (2 H, m), 2.08 (3 H, s), 3.16 (2 H, t *J* 7.0), 3.33 (1 H, m), 3.53-4.09 (m), 4.16 (3 H, m), 4.26 (1 H, m), 4.42 (1 H, t *J* 7.9), 4.51 (1 H, t *J* 7.9), 4.60 (1 H, t *J* 8.6), 4.73 (1 H, t *J* 7.5), 4.82 (1 H, m), 5.04 (1 H, d *J* 3.3), 5.17 (1 H, d *J* 3.7) and 5.24 (1 H, d *J* 3.5); m/z (HR-ESI) 1241.4623 (M^+ + Na.) $C_{47}H_{82}N_4NaO_{34}$ requires 1241.4647.

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