CHEMICAL SYNTHESIS OF THE O-LINKED OLIGOSACCHARIDE OF P-SELECTIN GLYCOPROTEIN LIGAND-1

by

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(Under the Direction of Geert-Jan Boons)

ABSTRACT

P-Selectin Glycoprotein Ligand-1 (PSGL-1) is the key ligand for selectins involved in the inflammatory cascade. This glycoprotein is necessary for the initial steps of the inflammation process, and the absence of this ligand from leukocytes eliminates transmigration of white blood cells into injured tissue. Also blocking P-selectins on the endothelial cell wall leads to decreases in transmigration of leukocytes. The key feature of PSGL-1's binding to selectins is an 18 amino acid signal sequence that contains 3 tyrosine sulfate residues and a glycosylated threonine residue. This sulfated glycoprotein has been chemoenzymatically synthesized on several occasions, but has never before been completely chemically synthesized. Chemical synthesis will give this compound on a preparative scale that will allow for increased and improved biological studies on the blocking of P-selectins.

INDEX WORDS: Inflammatory cascade, Chronic inflammation, P-selectins, P-selectin glycoprotein ligand-1, Leukocyte transmigration

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INTRODUCTION

Inflammation in tissue is recognized by fever and swelling in the site of injury. The body responds to injury by recruiting white blood cells flowing in the blood stream to this site. Here selectin proteins capture the white blood cells, macrophages and lymphocytes, and transmigrate them into the tissue. When this transmigration process continues for a prolonged duration, this condition is known as chronic inflammation. This inflammatory response will inevitably cause tissue damage and is accompanied by simultaneous attempts at healing and repair[1]. The exact nature, extent and time course of chronic inflammation is variable and depends on a balance between the causative agent and the attempts of the body to remove it. Figure 1[2] shows the chronically inflamed thyroid which is known as Hashimoto's disease.

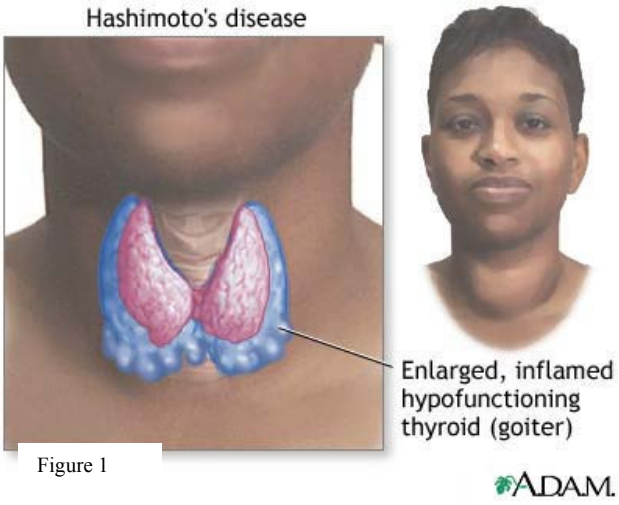


Figure 1

There are a number of ways that aetiological agents can cause chronic inflammation. Some infectious organisms can avoid or resist defenses that the host presents to the injury, and therefore, persists in the tissue for a prolonged period. This group includes Mycobacterium tuberculosis, Actinomycetes, and a number of fungi, protozoa, and metazoal parasites. Other infectious organisms are not innately resistant but will persist in damaged regions where they are protected from the host's defenses. These include bacteria that may grow in the puss inside an

undrained abcess cavity. Here they are protected from host immunity and blood-borne therapeutic agents. Irritant non-living foreign material such as wood splinters, metals and plastics that cannot be removed by enzymatic breakdown may cause chronic inflammation. Sometimes the inflammatory response is activated and maintained due to the body's immune response to its own tissues. Finally, many diseases characterized by the inflammatory response remain unknown such as Crohn's disease of the intestine[3].

The accumulation of macrophages and lymphocytes in the areas of chronic inflammation can occur in at least three ways. These include the continued recruitment from blood circulation, local proliferation in the tissue, and prolonged survival and immobilization in the inflamed area. The development, maintenance and termination of the inflammatory response is controlled by chemical mediators that are released from the damaged tissue, inflammatory cells, and enzyme systems in blood plasma. The process of healing and repair are also controlled by chemical interactions between the cells and extracellular tissue elements that are involved. Some examples of chronic inflammatory diseases include tuberculosis, chronic cholecystitis, bronchiectasis, rheumatoid arthritis, and Hashimoto's thyroiditis[4]. Figure 2[5] is a picture of stained neutrophils migrating from the blood vessel into the tissue area of injury.

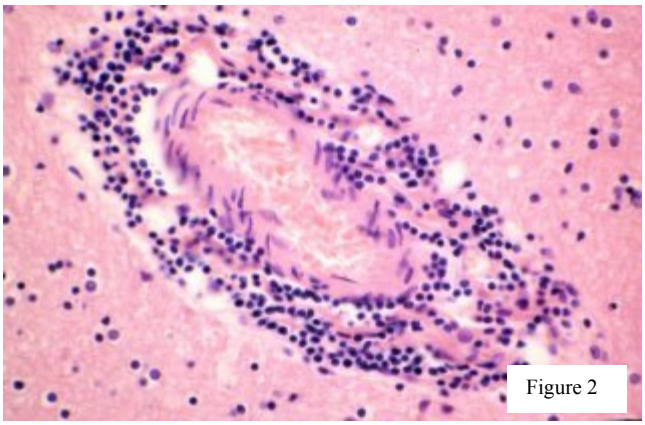


Figure 2

The main goal of inflammation is to localize and eliminate the irritant, and to repair the damaged tissue. Inflammation is a beneficial process and necessary for the continued existence of the host[6]. There are three major stages to the inflammatory response starting with the

dilation of capillaries which increases the flow of blood. This is followed by changes in the microvascular structure and escape of plasma proteins from the bloodstream. Finally, leukocytes are captured and transmigrated through the endothelium to the site of the injured tissue where the cell exerts its effects on the inflamed site. The leukocyte adhesion cascade is a five step process of capture, rolling, slow rolling, firm adhesion, and transmigration[7,8]. Each of these steps are necessary because when any of the steps are blocked, a severe reduction in leukocyte migration occurs. The goal of inflammation research is to develop anti-inflammatory drugs that function as blockers, suppressors, or modulators of the inflammatory response.

Endothelium is the interface between the flowing blood and the vessel wall where the leukocyte is captured. This layer prevents interactions between blood cells and the vessel wall as the blood flows through the vessel lumen[9]. The endothelium is constructed of simple squamous epithelium that lines the lumen of all blood vessels. This layer is critical in the mechanics of blood flow, coagulation regulation, adhesion of leukocytes, and vascular smooth muscle cell growth. It also serves as a barrier to the transvascular diffusion of liquids and solutes. Figure 3[10] is a diagram of the blood vessel which includes the lumen, the basement membrane, and the endothelium which is the layer between the blood flow and the outer layer of the vessel. This tissue also performs other active functions such as the secretion and modification of vasoactive substances and the contraction and relaxation of vascular smooth muscle.

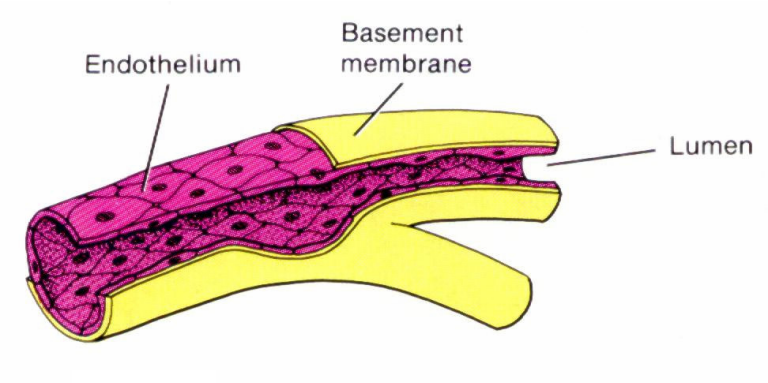


Figure 3

Figure 4

Blood is composed of erythrocytes (red blood cells), leukocytes (white blood cells), and platelets. Leukocytes are captured by the endothelium and are transmigrated into the site of the injured tissue. The five classes of leukocytes are neutrophils, eosinophils, basophils, monocytes and lymphocytes. Neutrophils, eosinophils, and basophils contain granules in their cytoplasm, and monocytes and lymphocytes are known as mononuclear cells. Neutrophils migrate into the extravascular tissue where they are activated by chemoattractants at the site of injury. Neutrophils ingest bacteria by phagocytosis and then release enzymes that destroy the bacteria. Monocytes mature into macrophages once they are released in the bloodstream, and migrate to tissues where they are actively phagocytic and ingest particulate matter[11].

INFLAMMATORY RESPONSE

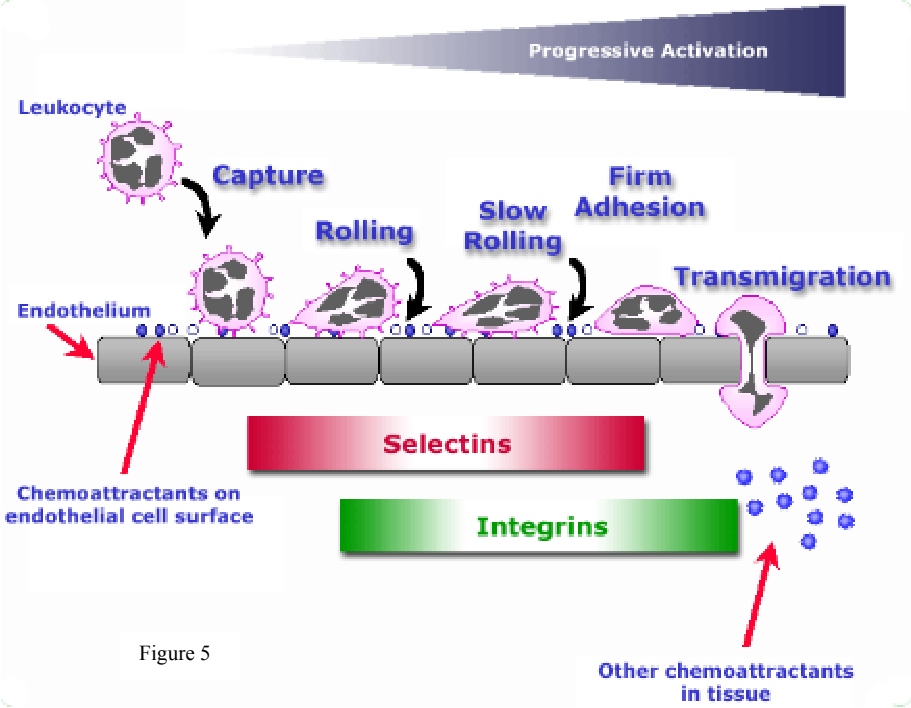


Figure 5

Figure 5[8] is an overview of the leukocyte's five step journey from the blood flow until it inters the injured tissue. The first step in the inflammation cascade is the initial contact between the endothelial cell wall and the leukocyte known as capture or tethering. Chemoattractants activate selectins that are expressed on the endothelium cell wall at the site of injury, and flail within the blood flow[12]. This activation of selectins, known as margination, is required to initiate the tethering of leukocytes to the endothelium cell wall. These selectins interact with glycoproteins located on the leukocyte surface forming bonds that decrease the velocity of the leukocytes in blood flow[13]. P-selectin is the primary adhesion molecule necessary to initiate the process of rolling located on the endothelial cells[14]. P-selectin glycoprotein ligand-1 (PSGL-1) located on leukocytes is the main ligand for selectins[15]. L-selectins are also important in the role of capture, but its ligand on endothelial cells is still unknown.

The next step in the inflammatory cascade is rolling. When leukocytes are captured, they will transiently adhere to the venular endothelium and begin to roll. The rolling process occurs at velocities less than that of the flow of blood. This decreased velocity, known as critical velocity or hydrodynamic velocity, separates rolling from free flowing cells. This rolling process is mediated by the selectin family (P-, E-, and L-selectins) of transmembrane adhesion

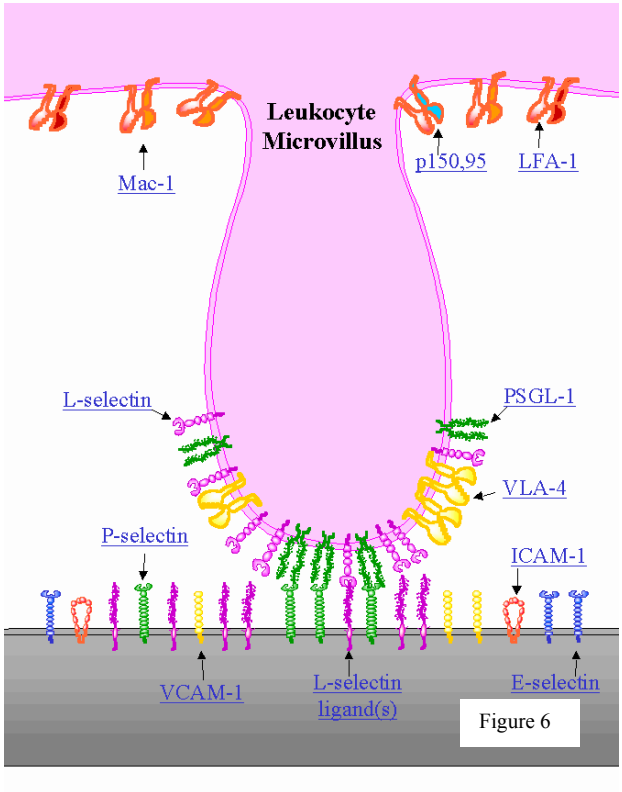


Figure 6

molecules. Figure 6[8] denotes the selectins and their ligands where non-covalent interactions are formed and broken between the leukocyte and the endothelial cell wall causing the leukocyte to roll along the endothelium. The most important selectin for the rolling process, P-selectin, can support capture and rolling in the absence of other selectins[16]. P –selectins are rapidly surface-expressed on venular endothelium that is stimulated by trauma, and increases the affinity of leukocytes to the endothelial cell wall. The selectin binds with PSGL-1 which is located on the leukocyte, and as bonds are formed and broken, the velocity continuously decreases. The leading edge of the leukocyte is where the bonds are formed between P-selectin of the endothelium and PSGL-1 of the leukocyte, and the trailing edge is where the bonds are breaking.

After the pro-inflammatory cytokine like TNF-α is injected to induce inflammation of the injured tissue the velocity of the traveling leukocyte drastically decreases[17]. The cells average velocity drops to between 5 to 10 μm per second, and this part of the inflammatory response is know as slow rolling as shown in figure 7. E-selectin expression on the endothelial cells and interaction with leukocytes as well as expression of C-18 integrins on the rolling leukocytes are necessary for process of slow rolling. Slow rolling is not based on a unique property of E-selectin, but the expression of E-selectin and its ligands in vivo shows that it supports this process[18].

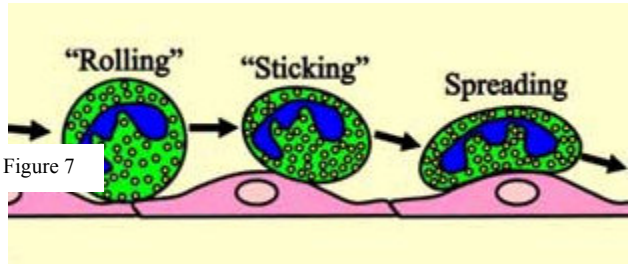


Figure 7

The leukocyte will continuously decrease in speed until the point of firm adhesion, the forth major step of the inflammatory response. Firm adhesion will occur almost always in the company of rolling, because direct adhesion is extremely rare. E-selectins are necessary for firm adhesion because in the absence of the specific selectins leads to increased critical velocities and

decreased firmly adheared leukocytes[18]. One of the most efficient ways of curbing leukocyte recruitment occurs during interfering with CD18 integrins. The deceleration to the point of firm adhesion is strictly dependant on these integrins, but they have little to no effect on capture or rolling[19].

Finally, after the leukocyte has come to rest on the endothelium, the cell will migrate across the membrane into the injured tissue. Exogenous chemoattractants must be present in order for transmigration to occur[20]. In, studies, when a gradient of IL-8 or fMLP is added, the result is transmigration, and this transmigration is dose dependant and can cause 50-90% of neutrophils to migrate to the tissue. Transmigration is considered "leukocyte driven" or chemotactic transmigration. Concentrations of secreted chemoattractants are significant amounts, usually enough to produce maximal chemotactic responses[21]. Figure 8[22] exhibits the transmigration of the leukocytes to their destined site in order to destroy the cause of injury and to repair the damaged tissue. There are a number of other adhesion molecules that have been implicated in transmigration such as PECAM-1, ICAM-1, VE-cadherin, CD11a/CD18, IAP, and VLA-4, but confidence in each's actual involvement varies[23].

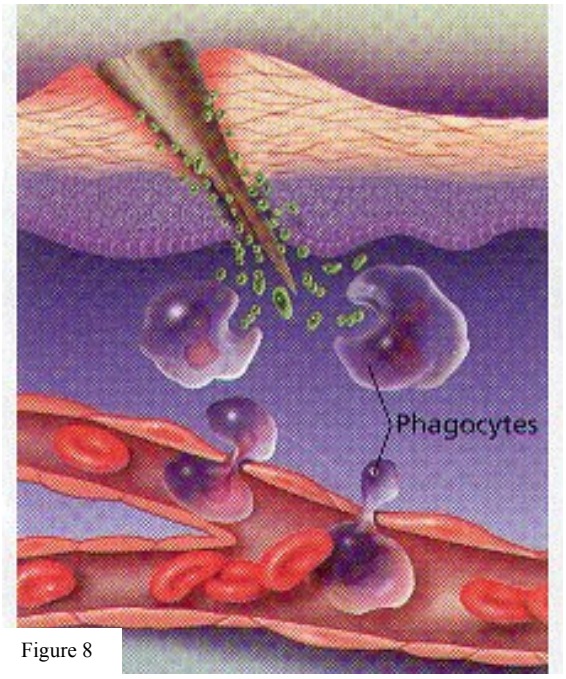


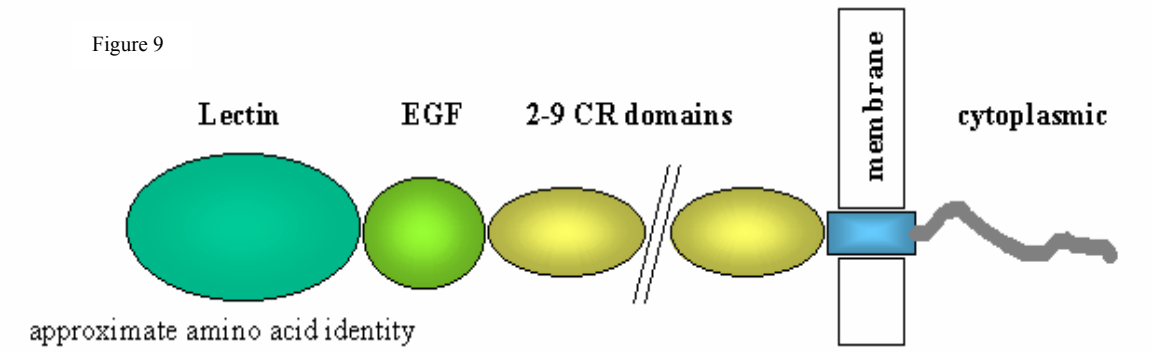
Figure 8

SELECTINS AND LIGANDS

There are three known selectins (P-, E-, and L-) which are Ca^{2+} dependant binding proteins that play roles in the inflammatory response. P-selectin is the most important of the

three selectins for this process, and these transmembrane proteins are located on α-granules of activated platelets and granules of endothelial cells[24]. P-selectins are 140 kDa, the largest of the three known selectins. Like P-selectins, E-selectins are located on the endothelium whereas the L-selectins are found on the leukocyte surface[8]. L-selectins play a role in the inflammatory response, but they cannot independently support rolling at typical in vivo velocities. P-selectin has the ability to capture leukocytes from the flowing blood even in the absence of L-selectins. Each of the selectins contain a lectin domain at the extreme amino terminal of the protein,

Figure 9



followed by an EFG domain, and a number of consensus repeat domains before the transmembrane domain. The number of consensus repeats distinguishes each selectin. P-selectins contain 9 repeats, E-selectins have 6, and there are 2 for the L-selectins. Figure 9[8] is a general example of the selectins where the only difference in the three is the number of consensus repeats found between the EGF domain and the transmembrane domain. Upon endothelial activation by inflammatory mediators such as histamine, thrombin or phorbol esters, the P-selectins extend approximately 40 nm from the endothelial surface within minutes and are available for interaction with leukocytes. P-selectins are also known as CD62-P, Granule Membrane Protein 140 (GMP-140), and Platelet Activation-Dependant Granule to External Membrane Protein (PADGEM).

During inflammatory response, adhesion of leukocytes to endothelial cells is controlled by the binding of vascular selectins to complementary glycoprotein ligands. The oligosaccharide units of the selectin ligands are very important for the binding interactions between the selectins and their ligands, and these bond formations are important in the mediation of the early steps of the inflammatory response. Each selectin has a number of ligands that they bind, and all selectins recognize glycoproteins and glycolipids that contain the tetrasaccharide sialyl-Lewis^{x}. L-selectin, the smallest of the three selectins, binds with four different ligands, GlyCAM-1, MAdCAM-1, CD34, and PSGL-1. P-selectin will bind with CD24, but the primary ligand for this selectin is PSGL-1. The ligands for E-selectin are not certain, but it is estimated that it interacts with both ESL-1 and PSGL-1. Figure 10 shows each selectin and the ligand(s) they may bind[13].

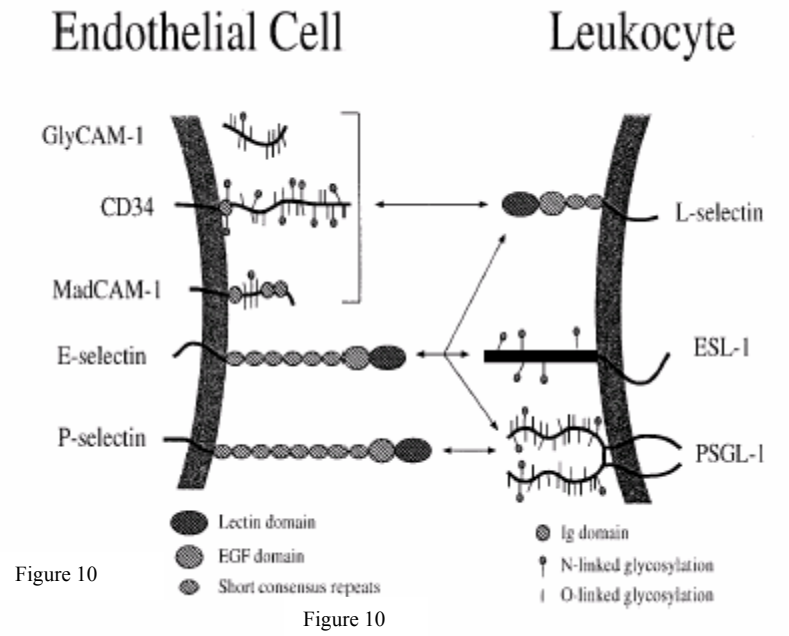


Figure 10

Figure 10

P-SELECTIN GLYCOPROTEIN LIGAND-1 (PSGL-1)

P-selectin glycoprotein ligand-1 is a ligand for each of the selectins, but it is the primary ligand for the P-selectin. This ligand is a 240 kDa homodimer that consists of two 120 kDa polypeptide chains, and is expressed on all leukocytes[25,26,27,28]. The transmembraneous polypeptide is rich in proline, serine, and threonine residues which is typical of mucin-type glycoproteins. This dimer contains a disulfide bond located near the transmembraneous domains

that binds the two peptides. Each of the peptides contains many O-glycans and only a few N-glycans. Figure 11 is the dimer of PSGL-1 that binds with each selectin. The post-translational modifications necessary for receptor interactions with P-selectins are (1,3) fucosylation and α(2,3) sialylation[29]. These glycosylations are located on the O-linked oligosaccharide of the peptide usually on the sialyl Lewis^x tetrasaccharide moiety.

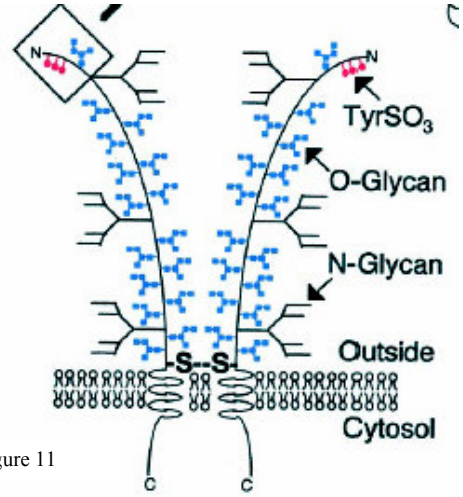


Figure 11

PSGL-1 is predicted to be constructed of 412 amino acids and the binding signal sequence of this protein is an 18 amino acid peptide chain. This signal sequence spans residues 42-59 of the extreme N-ternimus, and it contains the oligosaccharide necessary for binding to selectins. This oligosaccharide is linked to the peptide via a threonine residue at position 57. This signal sequence also contains three sulfated tyrosine residues at positions 46, 48 and 51 which are key for binding. The oligosaccharide consists of the sialyl Lewis^x tetrasaccharide (NeuAcα2->3Galβ1->4[Fucα1->3]GlcNAcβ1->R) which is linked to the peptide via a core-2 motif. The core 2 is constructed of a galactose monosaccharide linked (β1->3) to galactosamine which contains an α linked threonine residue. Sometimes the core-2 motif contains another sialic acid residue (α2->3) linked to the galactose monosaccharide. This 18 amino acid signal sequence with the threonine linked hexasaccharide and three sulfated tyrosine residues is the binding sequence of the ligand with each of the selectins. Figure 12 shows the

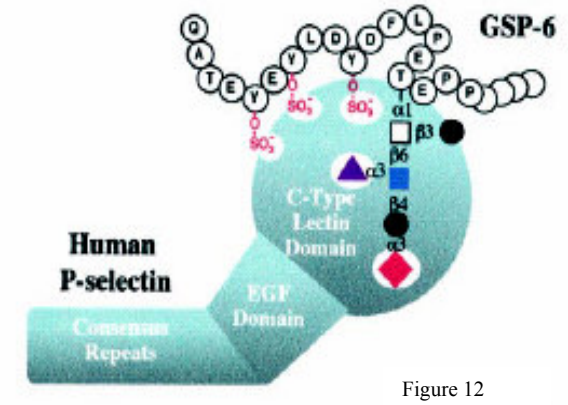


Figure 12

key features of PSGL-1 that are required for binding to human P-selectins. This includes the three sulfates, sialic acid (red diamond), and fucose (purple triangle).

There are several keys to binding of PSGL-1 to the selectins. Sialic acid linked to the Lewis^x trisaccharide is necessary for binding, because treatment of purified PSGL-1 with sialidase abolishes its binding ability with P-selectins. Studies have proven the N-glycans of PSGL-1 are unnecessary for binding interactions when all N-glycans were remove with N-glycosidase F. Also treatment of the ligand with O-sialoglycoprotease, the enzyme that degrades sialylated mucins, demolishes the binding properties. Also like sialic acid, when the fucose is removed, the binding is abolished. Sulfation of the tyrosine residues is also necessary, but not all three have to be sulfated in order to assist in binding. Upon removal of all sulfates with bacterial

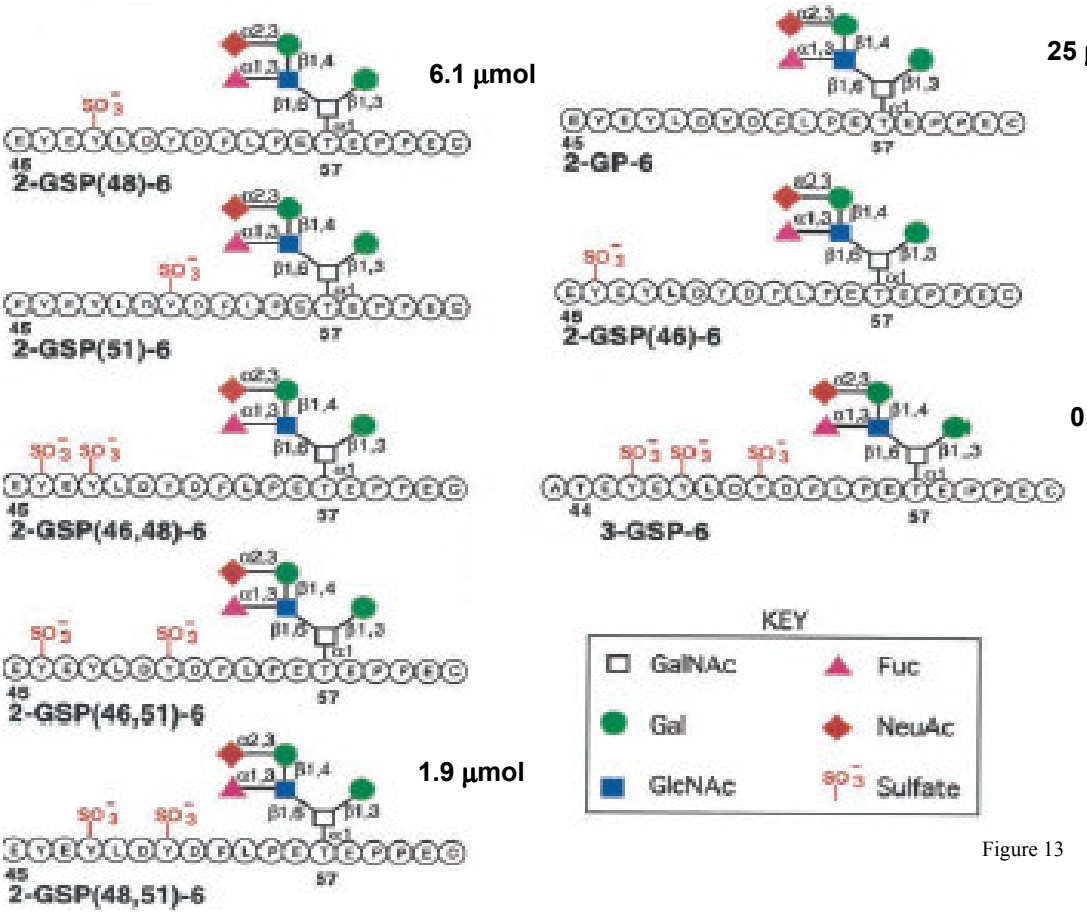


Figure 13

aryl sulfatases, binding properties were ceased. When one of the tyrosine residues is sulfated, binding with selectins can occur, but it increases when two are sulfated and even more with all three as shown in figure 13.

The binding pocket of the selectins is located on the lectin domain and interacts with the signal sequence peptide chain of PSGL-1. Binding interactions are Ca^{2+} dependant, and this ion networks with the three and four hydroxyls of fucose in the pocket. The fucose also binds with Glu80, Asn82, Asn83, and Glu107 of the selectin. The sialic acid residue binds with Arg97 and Tyr48 and the galactose of SLex interacts with Glu92 at the sixth hydroxyl and Tyr94 at the forth hydroxyl. The sulfated tyrosine residue at position 51 and the proline residue at 59 both bind with Arg85 in the pocket. The tyrosine sulfate at position 48 binds with Ser46, Ser47 and His114 of the selectin[30].

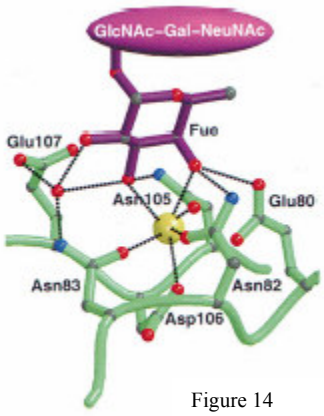


Figure 14

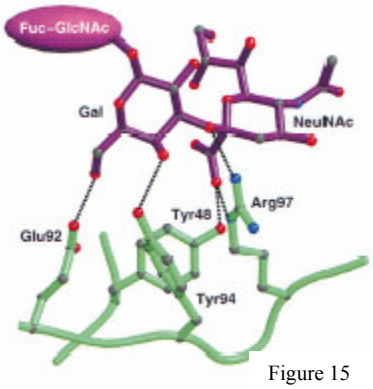


Figure 15

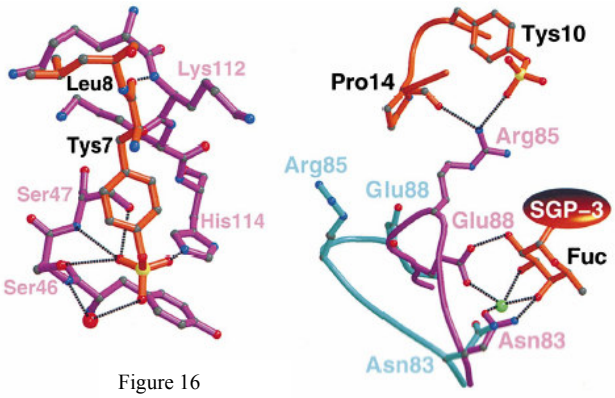


Figure 16

The 18 amino acid signal sequence of PSGL-1 has been synthesized in several cases, but it has never before been entirely chemically synthesized. In most cases, the sulfated peptide

containing the O-glycan has been chemoenzymatically synthesized. Wong et al. produced the glucosamine-galactosamine-threonine amino disaccharide and extended the peptide to Tyr51 from this point by solid phase peptide synthesis (SPPS)[31,32]. Chemical deprotection and sulfation produced the precursor that was extended to the SLex with first β1,4-Galactose Transferase (β1,4-GalT), then with α2,3-Sialic Acid Transferase (α2,3-SiaT) and finally with α1,3-Fucose Transferase (α1,3-FucT). Cummings et al. utilized SPPS to produce the 23 amino acid peptide sequence with an alpha linked galactosamine at Thr57[27]. Then by utilizing the necessary transferases, the hexasaccharide was completed followed by sulfation also by enzymatic synthesis.

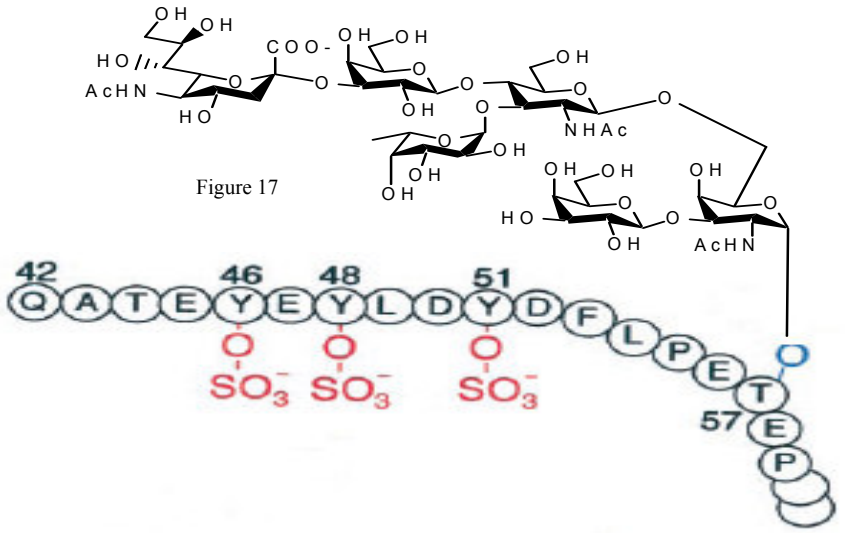


Figure 17

SYNTHESIS

It is envisioned that a completely chemical synthetic route to produce the signal sequence of PSGL-1 will produce the glycopeptide in a more efficient process. Enzymes can sometimes be costly and during the reaction, their decomposition rate can at times be problematic. It is more probable that synthesizing the sequence chemically will allow production of the compound on a more preparative scale. Usually enzymatic synthesis is limited in the amount of compound that can be prepared per reaction. With the chemical synthesis, more manipulations of the ligand are possible such as sulfation in the place of the sialic acid of SLe^x,

or addition of a sialic acid on the Core-2 disaccharide. An efficient chemical synthesis of the 18 amino acid signal sequence including three tyrosine sulfates and the hexasaccharide linked via threonine will produce the compound in efficient quantities necessary for further biological studies.

The synthetic scheme of this signal sequence has to be thoroughly examined to eliminate possible complications in the latter stages especially for deprotection. Utilizing acetyls as protecting groups on the oligosaccharide is necessary because they can be removed under mildly basic conditions. Protecting groups that require more harsh basic conditions can be problematic with beta elimination of the oligosaccharide, unintentional removal of the sulfates, and degradation of the peptide. Specific amino protecting groups are utilized that can be transferred to acetamido groups in only one efficient step. Protecting groups for the peptide cannot require strongly acidic conditions for removal because this can lead to elimination of the fucose moiety, the sialic acid moiety or the sulfates. Most protecting group manipulations necessary for the glycosylations are done at an early stage in the synthesis in order to decrease the steps on the more expensive, larger compounds.

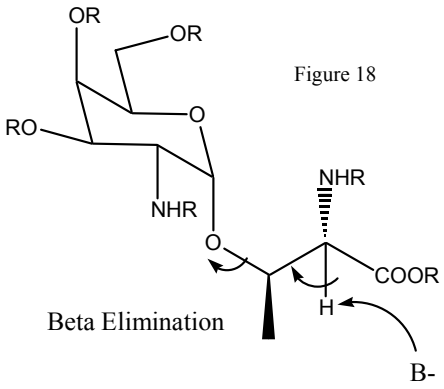


Figure 18

Synthesis of carbohydrates is unlike synthesizing most organic molecules because it is necessary to select protecting groups that can be activated for glycosylations, participating for α and β selectivity and selectively removable in the presence of other protecting groups. Utilizing acetyl groups on the fucose moiety

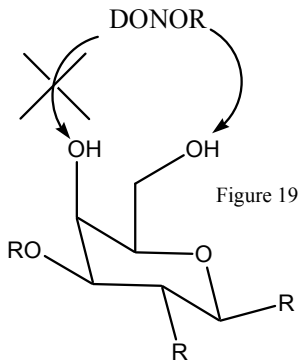
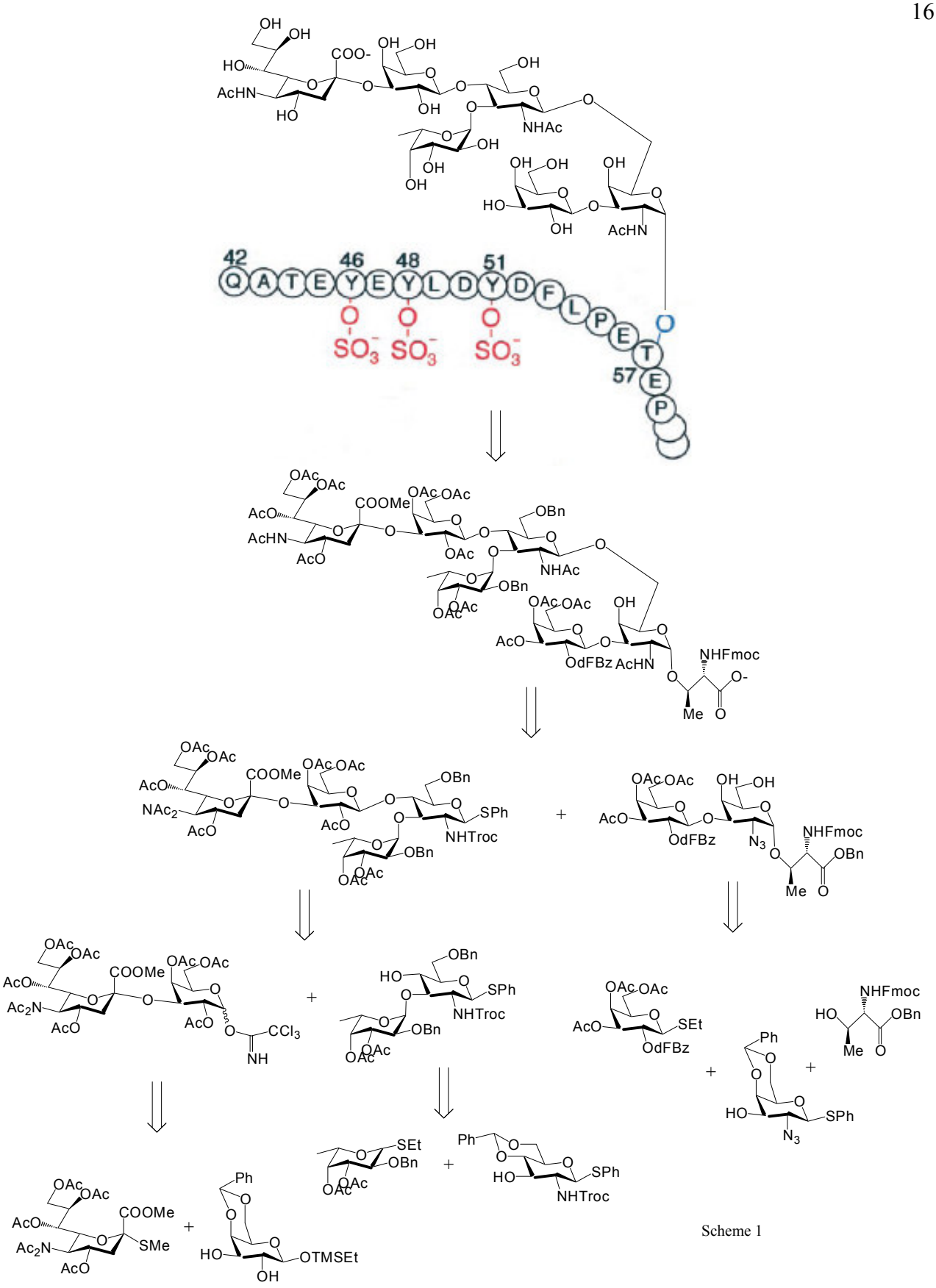


Figure 19

Regioselective Glycosylation

decreases the acid lability of this moiety thereby making it more stable during glycosylation reactions that can sometime be quite acidic. Other key ingredients of the synthetic scheme include stereoselective glycosylations and chemoselective activations. In cases where the two hydroxyl and three hydroxyl of galactose are unprotected, the glycosylation will occur at the three hydroxyl due to its increased reactivity compared to the other available hydroxyl. This is also the case with the four and six hydroxyls of galactose where the six hydroxyl will be more reactive due to less steric hindrance and the lower reactivity of the axial hydroxyl. Chemoselective activation is an efficient tool because one protecting group can be activated for glycosylation in the presence of a similar anomeric group due to greater reactivity, and the latter group can be activated under the same conditions without necessary manipulations in future reactions. Thio derivatives are usually efficient anomeric protecting groups because of the stability towards other protecting group manipulations, yet they can be activated under somewhat mild glycosylation conditions. For example, a thioethyl moiety located on a fucose monosaccharide can be activated in the presence of a thiophenyl moiety on glucosamine because of the increased reactivity of the fucose and of the more electron dense thioethyl anomeric group. Then, the thiophenyl of the disaccharide can be activated for glycosylation without any other necessary manipulations to prepare the donor.

The retrosynthesis of the peptide signal sequence of PSGL-1 begins with the strategically protected monosaccharides. In coupling sialic acid to galactose, a regioselective glycosylation occurs at the three hydroxyl. Chemoselective glycosylations utilizing thio anomeric protecting groups was used for fucose coupling to glucosamine, and for the one-pot synthesis of galactose to galactosamine then the disaccharide to threonine. In each case for the disaccharides, very few manipulations are necessary to provide the donors and acceptors necessary to produce the



Scheme 1

necessary tetrasaccharide then hexasaccharide. Another regioselective glycosylation will occur at the six hydroxyl of galactosamine when the sialyl Lewis^x tetrasaccharide is coupled to give the hexasaccharide. Finally, the peptide is added via SPPS, and complete deprotection is done on the compound to give the sulfated glycopeptide.

The dimer of this glycoprotein binds to each side of the Lectin domain of selectins. As stated before, the key features necessary for binding are the oligosaccharide and three tyrosine sulfates at the extreme amino terminus of the protein. The crystal structure as seen in Rasmol provides a picture of the bound dimer to the selectin in color. In figures 20 and 21, the hexasaccharide is colored blue, the tyrosine sulfates are teal, the remainder of the peptide is red, and the selectin protein is green. The fully synthesized glycopeptide signal sequence will be studied biologically to determine if it contains therapeutic properties that decrease the effects of chronic inflammation. A recombinant truncated form of this sulfated glycopeptide

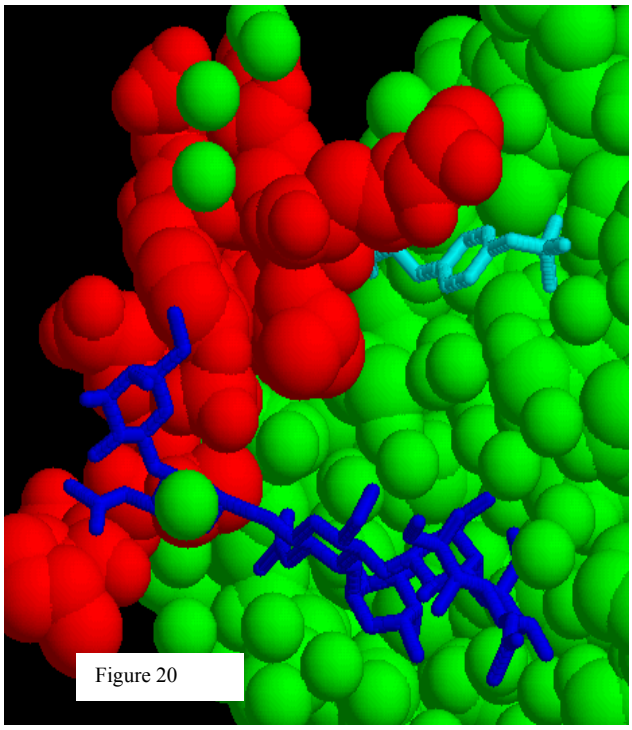


Figure 20

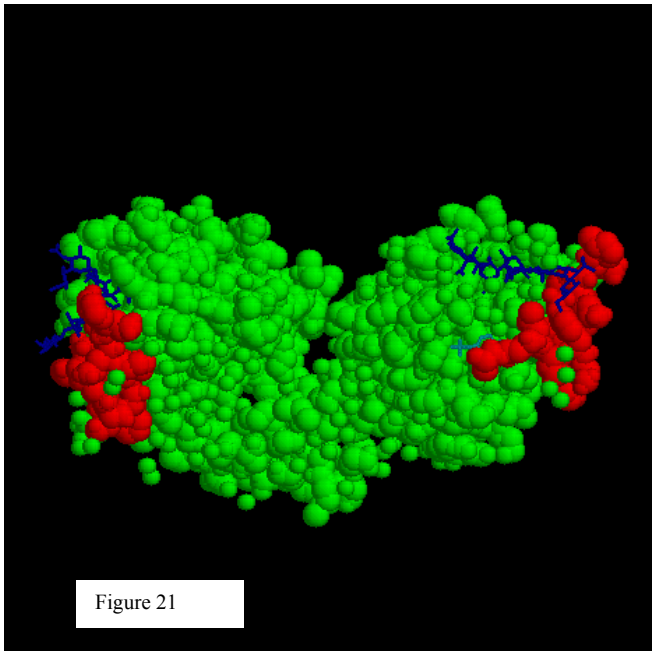


Figure 21

has been determined to be an inhibitor to leukocyte binding to selectins. Chemically synthesizing this inhibitor will give more preparative quantities that will be more suitable for biological studies and clinical trials. Synthesis of the oligosaccharide is a key starting point and major challenge for preparation of the complete 18 amino acid sulfated glycopeptide signal sequence.

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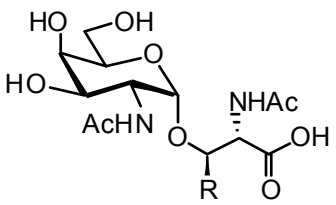
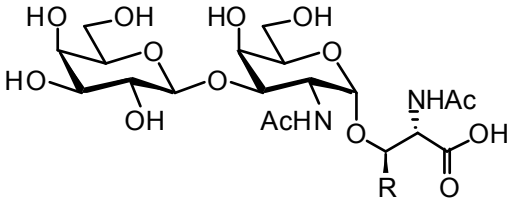
CHAPTER 2

Highly Efficient Stereospecific Preparation of Tn and TF Building Blocks Using Thioglycosyl Donors and the Ph_2SO/Tf_2O Promotor System.



R= H or CH_3

1 Tn



R= H or CH_3

2 TF

Figure 1. Tn and TF

INTRODUCTION

Mucins are a family of highly glycosylated proteins that are expressed on most epithelial cells.[1] The polypeptide backbone of mucins consists of highly conserved tandem repeats that are heavily O-glycosylated through clustered serine and threonine residues. The large and complex mucin-type carbohydrate structures serve as important recognition motifs for interactions with proteins. Although the glycoforms of mucins display extreme heterogeneity in regards to saccharide composition, length and linkages, the biosynthesis of mucins is initiated by the addition of N-acetylgalactosamine to serine and threonine residues. Further elongation of this structure leads to the large family of O-glycans, classified into eight groups depending on their

core stucture. This elongation takes place at the 3-O and/or 6-O-position by addition of galactose and/or poly-lactosamine residues followed by chain terminations with sialic acids, fucoses or sulfation.

In malignant cells, altered expressions levels of glycosyl transferases such as the down-regulation of glucosaminyltransferases and concomitant up- regulation of sialyltransferases, lead to simpler truncated forms of the glycans. This aberrant glycosylation has been correlated with specific disease states. For example, it is known that the presence of the Tn antigen and the related structure Gal*p*β(1-3)Gal*p*Nacα-Ser/Thr, also known as the TF antigen (figure 1), is common on human epithelial tumor cells,[2] such as colon and prostate cancers.[3] The presence of these antigens has spurred intense studies aimed at the development of immunotherapy for cancer.[4, 5] However, the enormous structural diversity that is introduced by glycosylation renders the isolation of well-defined glycopeptides from natural sources an almost impossible task, thus presenting a major obstacle to the study of the structure-activity relationship of these compounds. It is thus not surprising, that homogeneous synthetic glycopeptides would be most valuable tools for unraveling the specific roles of glycopeptides derived from mucins in biological processes.[6]

Our goal is to develop synthetic carbohydrate-based anti-cancer vaccines and to construct complex glycosulfopeptides derived from mucins. With this goal in mind, we needed a facile route to substantial quantities of Tn and TF building blocks that could be used for Fmoc solid phase synthesis. To this end, the formation of the α-glycosidic linkage between N-acetylgalactosamine and serine or threonine is a key step. This particular glycosylation has garnered much attention and has been extensively reviewed in the literature.[7-9] Here we report

the use of the van Boom/van der Marel promotor system for the activation of thio-glycosides in the synthesis of Tn and TF derivatives useful for solid phase glyco-peptide synthesis.

RESULTS AND DISCUSSION

Despite recent advances, the chemical synthesis of glycopeptides remains a difficult task.[10, 11] A crucial step in any glycopeptide synthesis is the incorporation of the saccharide part to the peptide backbone. Currently, the most general synthetic methodology employs preformed glycosylated amino acids for the stepwise solid phase synthesis of peptides. The protecting groups for these glycosylated amino acids must be carefully chosen and are rather limited, as the *O*-glycosidic bond is acid labile and the *O*-linked glycopeptide can undergo β-elimination upon treatment with strong bases. Presently, the use of acetyl esters as hydroxyl protection for the oligosaccharide part and N^{α}-Fmoc protected amino acids is a standard technique in solid phase glycopeptide synthesis. The formation of the α-glycosidic linkage between N-acetylgalactosamine and serine or threonine is a key step in the preparation of suitable glycosylated amino acid derivatives. For the installment of the 1,2-*cis* linkage, the non-participating 2-azido-2-deoxy group is commonly employed to mask the amino function. Recent reports have shown that 2-acetamido-2-deoxy galactose derivatives carrying a 4,6-benzylidene groups give α-selectivity in the preparation of Tn,[12] TF[13] and sialyl-TF[14] building blocks. Anomeric halides[4, 15-18] and trichloroacetimidates[19, 20] are the most commonly used glycosyl donors to prepare the Gal*p*NAcα-Ser/Thr linkage.[8, 9] A typical example of this glycosylation is the activation of fully acetylated 2-azido-2-deoxy bromides by $AgClO_4/Ag_2CO_3$ in the presence of serine or threonine derivatives (Scheme 1).[21] The reported yields and stereoselectivities are highly dependent on the protecting group patterns of both the saccharide donor and amino acid acceptor. Anomeric fluorides have also been utilized for the preparation of Tn derivatives with

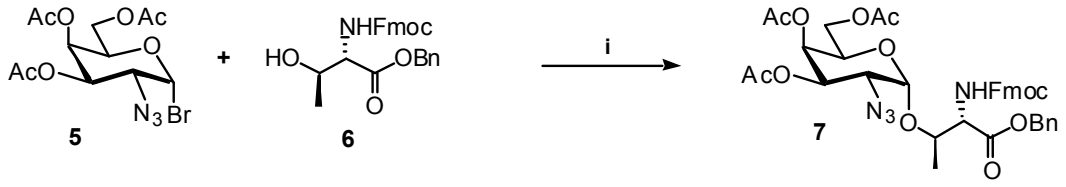
$Cp_2ZrCl_2/AgClO_4$ as promoters.[4, 20, 22] Although high yielding in glycosylations of both serine and threonine derivatives, the observed stereoselectivity was markedly decreased for the N^{α}-Fmoc-Ser-OBn derivative.[20]

The resulting poor stereoselectivities from the use of various TF disaccharide donors[4, 23-25] for the glycosylation of serine and threonine derivatives led Danishefsky and co-workers to implement the "cassette method".[4] This method involves the use of a properly protected Tn derivative as a general acceptor in the synthesis of practically any O-linked glycopeptide. Most reported methods depend on conventional labile donors that must be prepared just prior to glycosylation, thus clearly diminishing synthetic flexibility. More stable donors such as the *n*-pentenyl-[26], and seleno-glycosides,[27] are capable of withstanding protecting group manipulations and may be directly activated for glycosidations, but have not found widespread use for the synthesis of Tn and TF building blocks.[28-32]

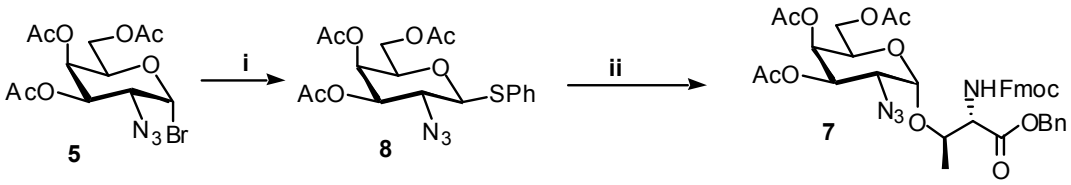
A new generation of thioglycosyl promoters has recently been introduced by Crich and co-workers.[33, 34] In the most successful promoter system, 1-benzenesulfinylpiperidine (BSP) was reacted with triflic anhydride (Tf_2O) to form a sulfonium species which, could convert disarmed thioglycosides into reactive triflates at very low temperatures.[34] However, it was found that the BSP/Tf_2O promoter pair was unable to successfully activate the disarmed phenylthioglycosides of 2-azido-2-deoxy-mannose and 2-azido-2-deoxy-glucose. Thus, building upon the foundation of the Crich discovery, this promoter system was further refined by van Boom and van der Marel.[35, 36] Reacting diphenylsulfoxide (Ph_2SO) with Tf_2O led to a highly electrophilic species capable of activating highly unreactive donors. This potent promoter resulted in high yields and excellent stereoselectivities. By exploiting differences in reactivity, it

was shown to be useful in chemo-selective glycosylation sequences with the BSP/Tf_2O promoter system.

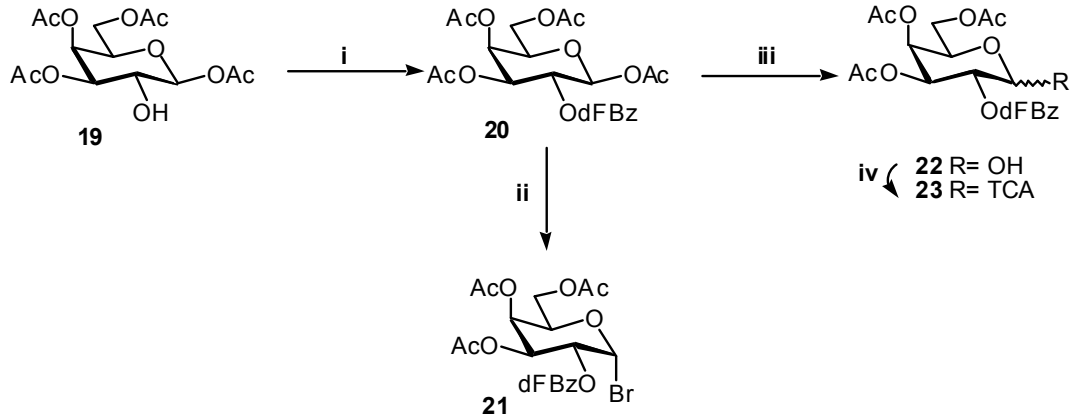
In light of this discovery, we wished to explore the use of thioglycosides as donors in the formation of the α-glycosidic linkage between 2-azido-2-deoxy derivatives of the Tn and TF saccharides.



Scheme 1. i: $AgClO_4/Ag_2CO_3$, DCM/toluene, 48h, 64%



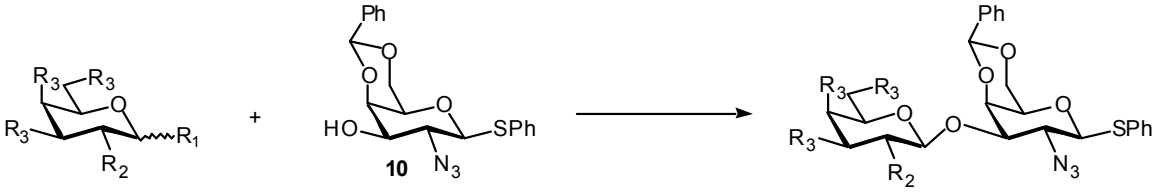
Scheme 2. i: NaSPh, EtOH/DCM, 3h, % ; **ii**: **6**, Ph_2SO/Tf_2O, DCM, -60°C, 1h, 85%



Scheme 3. **i**: dFBzCl, DMAP, pyridine, 18 h, 96%; **ii**: 30% HBr/HOAc, Ac_2O, 50 °C, 89%; **iii**: NH_2NH_2-HOAc, DMF, 60°C, 3h, 92%; **iv**: Trichloroacetonitrile, DBU, DCM, 0°C, 96%

To streamline the synthesis of Tn and TF derivatives, we wanted to explore the use of the Ph_2SO/Tf_2O promoter system for the direct activation of 2-azido-2-deoxy thiogalactoside donors

in glycosylations with threonine derivatives. In an attempt to accomplish this, bromide **5**[37] was converted into the corresponding thiophenyl derivative by reaction with sodium thiophenolate in a mixture of dichloromethane and ethanol (Scheme 2).[38] Activation of thiophenyl glycoside **8**[39] with the Ph_2SO/Tf_2O promoter system for the glycosylation of N^{α}-Fmoc-Thr(OH)-OBn (**6**) proceeded with high efficiency and produced the glycosyl amino acid **7** in an excellent yield of



Entry	Donor	R_1	R_2	R_3	Promotor/Solvent/Temp.	Product
1	**11**	α-Br	Ac	Ac	AgOTf /DCM/-40°C-RT	Orthoester/**12**
2	**11**	α-Br	Ac	Ac	$HgO/HgCl_2$/DCM/50°C	Orthoester/**12**
3	**13**	α/β-OC(NH)CCl_3	Ac	Ac	TMSOTf/DCM/-20°C-RT	Orthoester/**12**
4	**14**	β-SEt	Ac	Ac	NIS/TMSOTf/DCM/-20°C-RT	Orthoester/**12**
5	**14**	β-SEt	Ac	Ac	Ph_2SO/Tf_2O/DTBMP/DCM/-60°C-RT	Orthoester
6	**14**	β-SEt	Ac	Ac	Ph_2SO/Tf_2O/DCM/-60°C-RT	Orthoester
7	**15**	α-Br	Bz	Bz	AgOTf/DCM/-40°C-RT	**16** (76%)
8	**17**	α-Br	dFBz	dFBz	AgOTf/DCM/-40°C-RT	**18** (51%)
9	**21**	α-Br	dFBz	Ac	AgOTf/DCM/-40°C-RT	**25** (63%)
10	**23**	α/β-OC(NH)CCl_3	dFBz	Ac	TMSOTf/DCM/-20°C-RT	**25** (74%)

85%. The reaction proceeded with complete stereochemical control as the α-anomer was formed exclusively. Compared to similar glycosylations using MeOTf,[28] DMTST,[28, 29] or NBS/TBAOTf,[30-32] the Ph_2SO/Tf_2O promoter provides far superior yield and stereoselectivity. Encouraged by this result, we directed our attention to the preparation of the TF antigen. By deacetylation and the introduction of a 4,6-benzylidene group, thioglycoside **8** was easily converted into the known acceptor **10**.[39] For the galactosylation, several glycosyl donors were investigated. The results are summarized in Table 1. The commonly used donor per-O-acetylated galactosyl bromide **11** activated by AgOTf[40] (entry 1) gave in our experiments unreliable results. Disaccharide **12** was often accompanied by a formation of substantial amounts of the

corresponding orthoester. Changing the promoter system or anomeric leaving group did not improve the outcome (entries 2-4).

The fact that the Ph_2SO/Tf_2O promoter system uses nearly stoichiometric quantities of activator, and the thioglycoside is converted into the corresponding triflate before the glycosyl acceptor is added to the reaction mixture,[35] prompted us to explore the possibility of using the readily available thiogalactoside **14**. Activation of **14** by Ph_2SO/Tf_2O in the presence of 2,5-di-*t*butyl-4-methylpyridine (DTBMP) and subsequent reaction with acceptor **10** (entry 5) gave mainly the corresponding orthoester. We were encouraged to find only trace amounts of the disaccharide corresponding to the activation and self-coupling of acceptor **10**. In an attempt to avoid the orthoester formation, a similar reaction was performed with the omission of the base. Unfortunately, this reaction gave the same disappointing result (entry 6).

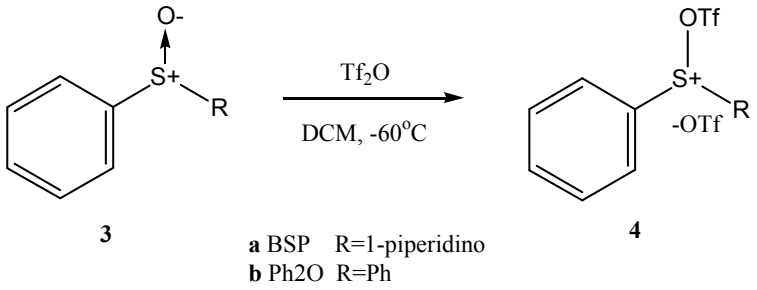


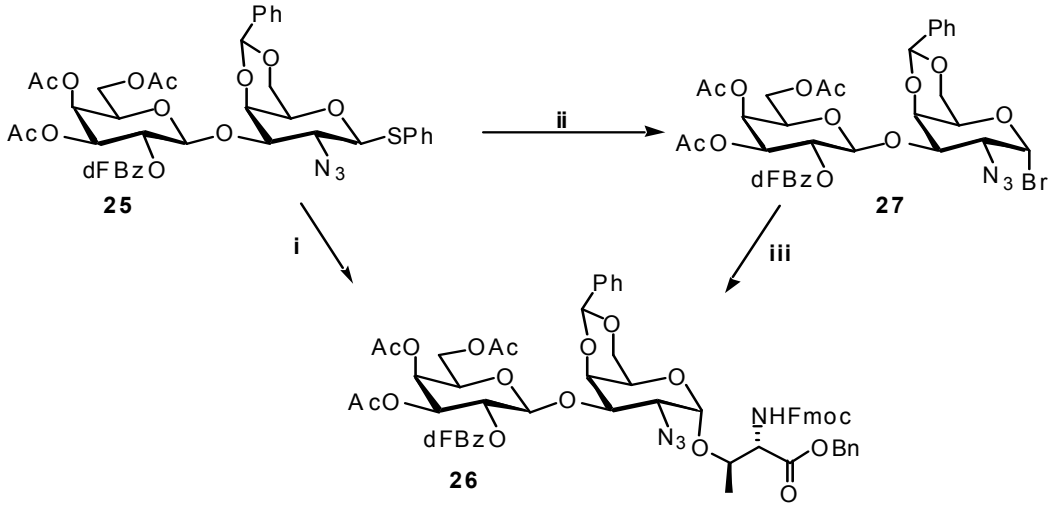
Figure 3

Replacing a 2-O-acetyl by a 2-O-benzoyl is a common way to avoid orthoester formation. Indeed, using fully benzoylated bromide **15** in an AgOTf activated glycosylation of acceptor **10** gave disaccharide **16** in 76% yield (entry 7). However, due to the more severe basic conditions required for the removal of *O*-benzoates and in particular a 2-*O*-benzoate of galactose, this derivative would not be suitable for use in glycopeptide synthesis. Instead, we turned our attention to the 2,5-di-fluorobenzoyl group (dFBz) that was recently introduced for glycopeptide synthesis by Kihlberg and co-workers. [41] An advantage of this protecting group is that the difluorobenzoyl possesses a combination of the positive qualities associated with 2-O-benzoyl

groups in glycosylations and the ease of removal of acetyl esters. Using the easily accessible fully difluorobenzoylated galactosyl bromide **17**[41] in a AgOTf mediated reaction, the desired disaccharide **18** was obtained, but only in 51% yield (entry 8). The donor was found to be very unreactive and the reaction sluggish. An attempt to improve the reaction by gentle heating was unsuccessful. A positive feature of this donor was that no orthoester was isolated. The mediocre yield prompted us to prepare glycosyl donors **21** and **23** (Scheme 3). Carrying a C-2 dFBz and acetyl esters at the C-3, C-4, and C-6 position, it was believed that these donors would exhibit a higher reactivity. To accomplish this goal, alcohol **19**[42] was acylated with difluorobenzoyl chloride in the presence of 4-dimethylaminopyridine (DMAP), which afforded C-2 dFBz derivative **20** in 96% yield. Conversion of **20** into glycosyl donor **21** was achieved by treatment with 30% hydrogen bromide in acetic acid at 50°C. Selective cleavage of the anomeric acetate of **20** gave hemiacetal **22**, which was transformed into trichloroacetimidate **23** using standard conditions.[43] AgOTf activation of bromide **21** at –40°C in the presence of glycosyl acceptor **10** provided disaccharide **25** in 63% yield (entry 9). The superior reactivity of bromide **21** as compared to that of fully difluorobenzoylated bromide **17** was reflected by a slightly increased yield. The condensation of trichloroacetimidate **23** activated by TMSOTf at –20°C and alcohol **10** furnished disaccharide **25** in a further improved yield of 74%.

Having established a reliable and efficient route to the thiophenyl TF disaccharide, we chose to evaluate this glycosyl donor with construction of the α-O-linkage to threonine in mind. As depicted in Scheme 4, thioglycoside **25** was activated by Ph_2SO/Tf_2O in the presence of DTBMP at –60°C for the glycosylation of threonine derivative **6**. Remarkably, the reaction yielded exclusively the α-anomer product **26** in an excellent yield of 82%. It should be noted that

these results are not only the best results reported for the TF disaccharide thioglycosides, but



Scheme 4. i: **6**, Ph_2SO/Tf_2O, DCM, -60°C, 1h, 82%; **ii**: Br_2, DCM, 0°C; **iii**: **6**, $AgClO_4$, DCM, rt, 48h, 68% yield over two steps.

the selectivity of this reaction is more superior than what is observed in most procedures using halides and trichloroacetimidates as glycosyl donors. To illustrate this feature, thiophenyl **25** was converted into the corresponding bromide **27** by treatment with molecular bromine. Subsequently activation of the bromide with $AgClO_4$ in a glycosidation with threonine acceptor **6** gave **26** in an acceptable overall yield, but as expected, the α/β-selectivity was lowered and isolation of **26** required careful chromatography.

In conclusion, we have described an efficient route for Tn and TF antigen building blocks that are useful in the solid phase synthesis of glycopeptides derived from mucins. Using the promotor system introduced by van Boom and van der Marel for the activation of disarmed thioglycosides, we found that the activation of Tn and TF thioglycoside donors proceeded smoothly and provided the α-O-glycosidic bond to N^{α}-Fmoc-Thr benzyl ester in high yields and with exclusive formation of the α-product. Additionally, the TF derivative may serve as an intermediate for further extension in the synthesis of other mucin derived glycopeptides.

EXPERIMENTAL

General: NIS was purchased from Fluka and re-crystallized from dioxane/CCl_4. All other chemicals were purchased from Aldrich, Acros, and Fluka and used without further purification. Molecular sieves were activated at 145 °C for 10 h. All solvents employed were of reagent grade and dried by refluxing over appropriate drying agents. TLC was performed using Kieselgel 60 F_{254} (Merck) plates, with detection by UV light (254 nm) and/or by charring with 8% sulfuric acid in ethanol. Column chromatography was performed on silica gel (Merck, mesh 70-230). Extracts were concentrated under reduced pressure at ≤ 40 °C (water bath). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova300 spectrometer, and a Varian Inova500 spectrometer equipped with Sun workstations. ^{1}H spectra recorded in $CDCl_3$ were referenced to residue $CHCl_3$ at 7.26 ppm or TMS, and ^{13}C spectra to the central peak of $CDCl_3$ at 77.0 ppm. Assignments were made using standard 1D and gCOSY, gHSQC and TOCSY 2D experiments. Positive ion matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were recorded using an HP-MALDI instrument using gentisic acid as a matrix.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-L-threonine Benzyl ester (7).** To a solution of compound **8** (43 mg, 101 μmol) and Ph_2SO (58 mg, 284 μmol) in dry CH_2Cl_2 (5 mL) was added, at -60°C, trifluoromethanesulfonic anhydride (24 μL, 141 μmol). The reaction mixture was stirred for 10 min, after which a solution of acceptor **6** (87 mg, 202 μmol) in CH_2Cl_2 (1 mL) was added. The mixture was stirred at -60°C for 1 h after which it was slowly warmed to rt and quenched by the addition of saturated aqueous $NaHCO_3$ (3 mL). The organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Purification of the residue by silica gel chromatography (Hexane/EtOAc 3:1) yielded **7** (64 mg, 86.0 μmol, 85%); TLC (Hexane/EtOAc 2:1) $R_f = 0.39$;

NMR data was in agreement with reported data. HR MALDI-TOF MS: m/z: calc for $C_{38}H_{40}N_4O_{12}$: 744.2643; found 767.2541 $[M+Na]^+$.

1,3,4,6-tetra-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-α-D-galactopyranose (20). To a solution of 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (700 mg, 2.01mmol) in dry pyridine (8 mL) was added 4-(dimethylamino)pyridine (49 mg, 0.402 mmol) and the solution was stirred at rt for 30 min. 2,5-difluorobenzoyl chloride (0.5 mL, 4.02 mmol) was added drop-wise over a 10 min period and the stirring was continued for 18 h. The reaction was quenched by addition of methanol (4 mL) and after stirring for 1 h, the solution was diluted with CH_2Cl_2 (120 mL) and washed with water (150 mL). The aqueous phase was extracted with CH_2Cl_2 (50 mL) and the combined organic phases were dried ($MgSO_4$), and evaporated to dryness. After purification by silica gel chromatography (Hexane/EtOAc 8:1), **20** (942 mg, 1.93 mmol, 96%) was afforded as a white solid; TLC (Hexane/EtOAc 1:1), $R_f = 0.68$; $[\alpha]_D + 20.0$ (c 2 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^{1}H, δ 7.55-7.05 (m, 3H, dFBz), 6.53 (d, 1H, $J_{1,2}$ 3.3 Hz, H-1), 5.60-5.41 (m, 3H, H-2, H-3, H-4), 4.39 (t, 1H, $J_{5,6}$ 6.6 Hz, H-5), 4.16-4.07 (m, 2H, H-6), 2.18, 2.14, 2.04, 2.03 (s, 12H, 4xCH_3CO); ^{13}C, δ 20.7, 20.8, 20.9, 21.0 (4xCH_3CO), 61.4 (C-6), 67.6 (C-5), 67.7 (C-3), 68.0 (C-4), 69.1 (C-2), 89.7 (C-1), 118.3-122.2 (aromatic C), 170.6, 170.3, 170.2, 170.1 (4xCH_3CO); HR MALDI-TOF MS: m/z: calc for $C_{21}H_{22}F_2O_{11}$: 488.1130; found 511.1029 $[M+Na]^+$.

3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-α-D-galactopyranosyl bromide (21). Compound **20** (300 mg, 614 μmol) was dissolved in a mixture of acetic acid (3 mL) and acetic anhydride (2 mL). 33% hydrogen bromide in acetic acid (4 mL) was added and the mixture was stirred at 50°C for 6 h. The solution was allowed to cool to rt, diluted with CH_2Cl_2 (100 mL) and washed with water (125 mL) and saturated aqueous $NaHCO_3$ (125 mL). The organic phase was dried ($MgSO_4$) and concentrated. Purification of the residue by silica gel chromatography

(Hexane/EtOAc 3:1) furnished **21** (278 mg, 545 μmol, 89%); TLC (Hexane/EtOAc 2:1), R_f = 0.51; $[\alpha]_D$ + 31.4 (c 2.0 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^1H, δ 7.61-7.08 (m, 3H, dFBz), 6.80 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.60-5.55 (m, 2H, H-3, H-4), 5.30 (dd, 1H, $J_{1,2}$ 2.2Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.54 (t, 1H, $J_{5,6}$ 6.6 Hz, H-5), 4.25-4.09 (m, 2H, H-6), 2.17, 2.06, 1.98 (s, 9H, 3xCH_3CO); ^{13}C, δ 20.7, 20.8, 20.9, (3xCH_3CO), 61.0 (C-6), 67.3 (C-5), 68.2 (C-2), 69.0 (C-4), 71.5 (C-3), 87.8 (C-1), 118.4-122.7 (aromatic C), 170.6, 170.1, 169.9 (3xCH_3CO); HR MALDI-TOF MS: m/z: calc for $C_{19}H_{19}BrF_2O_9$: 508.0181; found 531.0079 $[M+Na]^+$.

3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-D-galactopyranose (22). Hydrazine acetate (192 mg, 2.08 mmol) was added to a solution of **20** (925 mg, 1.89 mmol) in DMF (9 mL) heated at 60°C. The mixture was kept at 60°C for 3 h, allowed to return to rt, diluted with EtOAc (75 mL) and washed with 20% aqueous NaCl (75 mL). The aqueous phase was extracted with EtOAc (40 mL) and the combined organic layers were dried ($MgSO_4$), and concentrated under reduced pressure. Silica gel chromatography purification (Hexane/EtOAc 2:1) gave **22** (776 mg, 1.74 mmol, 92%); TLC (Hexane/EtOAc 2:1), R_f = 0.22; $[\alpha]_D$ + 7.4 (c 2.0 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^1H, δ 7.60-7.10 (m, 3H, dFBz), 5.68 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.59 (dd, 1H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.0 Hz, H-3), 5.52 (d, 1H, $J_{4,5}$ 2.5Hz, H-4), 5.36 (dd, 1H, $J_{1,2}$ 10.7 Hz, $J_{2,3}$ 3.6 Hz, H-2), 4.52 (t, 1H, $J_{5,6}$ 6.6 Hz, H-5), 4.20-4.10 (m, 2H, H-6), 3.10 (bs, 1H, OH), 2.18, 2.17, 2.06 (s, 9H, 3xCH_3CO); ^{13}C, δ 20.8, 20.9, 21.0 (3xCH_3CO), 62.0 (C-6), 66.7 (C-5), 68.5 (C-3), 69.9 (C-4), 71.5 (C-2), 90.8 (C-1), 118.2-122.2 (aromatic C), 170.2, 170.4, 170.7 (3xCH_3CO); HR MALDI-TOF MS: m/z: calc for $C_{19}H_{20}F_2O_{10}$: 446.1025; found 469.0920 $[M+Na]^+$.

3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-α-D-galactopyranosyl trichloroacetimidate (23). Compound **22** (250 mg, 560 μmol) was dissolved in dry CH_2Cl_2 (5 mL) at 0°C and trichloroacetonitrile (1.2 mL, 8.40 mmol) was added followed by 1,8-diazabicyclo[5.4.0] undec-

7-ene (DBU, 8 μL, 53.5 μmol). The mixture was stirred for 2 h at 0°C, concentrated to dryness and purified by silica gel chromatography (Hexane/EtOAc/TEA 5:1:0.01) to yield **23** (317 mg, 538 μmol, 96%); TLC (Hexane/EtOAc 2:1), R_f = 0.39; $[\alpha]_D$ + 26.25 (c 2.0 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^{1}H, δ 7.57-7.06 (m, 3H, dFBz), 6.74 (d, 1H, $J_{1,2}$ 2.2 Hz, H-1), 5.55-5.70 (m, 3H, H-2, H-3, H-4) 4.51 (t, 1H, $J_{5,6}$ 6.6 Hz, H-5), 4.24-4.08 (m, 2H, H-6), 2.20, 2.03, 1.99 (s, 9H, 3xCH_3CO); ^{13}C, δ 20.8, 20.9, 21.0 (3xCH_3CO), 61.5 (C-6), 67.7 (C-3), 67.8 (C-4), 68.2 (C-2), 69.4 (C-5), 93.6 (C-1), 118.2-122.5 (aromatic C), 161.0 (C=NH) 170.1, 170.3, 170.5 (3xCH_3CO); HR MALDI-TOF MS: m/z: calc for $C_{21}H_{20}Cl_3F_2NO_{10}$: 589.0121; found 612.0019 $[M+Na]^+$.

Phenyl 2-Azido-4,6-O-benzylidene-2-deoxy-3-O-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-1-thio-β-D-galactopyranoside (25). Trimethylsilyl trifluoromethane sulfonate (7 μL, 38.7 μmol) was added, at –20 °C and under argon, to a stirred mixture of **10** (35 mg, 91.1 μmol), **23** (75 mg, 128 μmol) and 4Å molecular sieves in CH_2Cl_2 (5 mL). The reaction was allowed to slowly return to rt and was quenched with triethylamine. The reaction was diluted with CH_2Cl_2 (50 mL), filtered through Celite, and evaporated to dryness. The residue was purified by silica gel chromatography (Hexane/EtOAc 2:1) to furnish **25** (55 mg, 67 μmol, 74%); TLC (Hexane/EtOAc 1:1) R_f =0.65; $[\alpha]_D$ + 10.6 (c 2 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^{1}H, δ 7.70-7.05 (m, 13H, dFBz, 2Ph), 5.51 (s, 1H, PhC*H*), 5.48 (t, 1H, $J_{1,2}$ 10.1 Hz, H-2'), 5.42 (d, 1H, $J_{3,4,5}$ 3.0 Hz, H-4'), 5.15 (dd, 1H, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.3 Hz, H-3'), 4.91 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1'), 4.42-4.34 (m, 2H, H-5', H-1), 4.26 (d, 1H, $J_{3,4,5}$ 2.5 Hz, H-4), 4.17-3.94 (m, 4H, H-5, H-6, 2H-6'), 3.73 (t, 1H, $J_{1,2,3}$ 9.9 Hz, H-2), 3.54 (dd, 1H, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 3.0 Hz, H-3), 3.46 (m, 1H, H-6), 2.14, 2.05, 1.93 (s, 9H, 3xCH_3CO); ^{13}C, δ 20.7, 20.9, 21.0 (3xCH_3CO), 60.0 (C-2), 61.7 (C-6), 67.2 (C-4'), 70.0 (C-2'), 70.1 (C-5') 71.2 (C-3'), 74.9 (C-4), 81.1 (C-3), 86.0

(C-1), 101.0 (Ph*C*H), 102.3 (C-1'), 126.6-137.9 (aromatic C), 170.3, 170.4, 170.5 (3xCH_3*C*O); HR MALDI-TOF MS: m/z: calc for $C_{38}H_{37}F_2N_3O_{13}S$: 813.2015; found 836.1913 $[M+Na]^+$.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-[2-Azido-4,6-O-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine Benzylester (26).** To a solution of compound **25** (64 mg, 79 μmol), Ph_2SO (45 mg, 221 μmol) and 2,5-di-*tert*.-butyl-3-methylpyridine (49 mg, 235 μmol) in dry CH_2Cl_2 (4 mL) was added trifluoromethanesulfonic anhydride (19 μL, 112 μmol) at -60°C. The mixture was stirred for 10 min, after which a solution of acceptor **6** (68 mg, 158 μmol) in CH_2Cl_2 (1.5 mL) was added. The reaction was stirred at -60°C for 1 h and then it was slowly warmed to rt and quenched by the addition of saturated aqueous $NaHCO_3$ (2 mL). The organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Purification by silica gel chromatography (Hexane/EtOAc 3:1) gave **26** (74 mg, 65 μmol, 82%); TLC (Hexane/EtOAc 1:1) R_f =0.67; $[\alpha]_D$ + 21.0 (c 2 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^{1}H, δ 7.60-6.97 (m, 21H, dFBz, Ph, Bn, Fmoc), 5.71 (d, 1H, J 9.3 Hz, NH), 5.54 (t, 1H, $J_{1,2,3}$ 9.8Hz, H-2'), 5.45 (d, 1H, $J_{4,5}$ 2.5 Hz H-4'), 5.18 (dd, 1H, $J_{2,3}$ 10.3 Hz, $J_{3,4}$ 2.9 Hz, H-3'), 5.13 (s, 1H, PhC*H*), 4.93 (d, 1H, $J_{1,2}$ 7.8 Hz H-1'), 4.89 (d, 1H, $J_{1,2}$ 2.9 Hz, H-1), 4.50-4.13 (m, 10 H, Fmoc 3H, H_α, H_β, H-4, H-5, H-6_{ax}, H-6'), 4.02-3.97 (m, 2H, H-3, H-5'), 3.73 (dd, 1H, $J_{1,2}$ 2.9 Hz, $J_{2,3}$ 10.3 Hz, H-2), 3.63 (m, 1H, H-6_{eq}), 2.18, 2.03, 1.93 (s, 9H, 3xC*H*$_3$CO), 1.27 (d, 3H, CH_3); ^{13}C, δ 19.2 (CH_3), 20.8, 20.9, 21.0 (3x*C*H_3CO), 59.3 (Cα), 59.8 (C-2), 63.6 (C-6), 69.2-66.2 (C-4', CH_2-Fmoc), 70.1 (C-2'), 71.3 (C-5', C-3'), 75.8 (C-3), 75.6 (C-4), 76.3 (Cβ), 99.2 (C-1), 101.0 (*C*HPh), 102.4 (C-1'), 117.9-130.2 (aromatic C), 169.2-171.1 (5xC=O); HR MALDI-TOF MS: m/z: calc for $C_{58}H_{56}F_2N_4O_{18}$: 1134.3558; found 1157.3450 $[M+Na]^+$.

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CHAPTER 3

Rapid Assembly of Oligosaccharides: a Highly Convergent Approach for the Synthesis of a Glycosylated Amino Acid Derived from PSGL-1

INTRODUCTION

The selectins are a family of three Ca^{2+} dependant membrane bound glycoproteins that mediate the adhesion of leukocytes and platelets to vascular surfaces[1]. Several studies have demonstrated that they play important roles in inflammation, immune responses, homeostasis and wound repair. Selectins also contribute to a broad spectrum of diseases such as arteriosclerosis, thrombosis, organ-transplant rejection, arthritis, sickle cell anemia and tumor metastasis.

Although that there are many candidates for selectin ligands, only P-selectin glycoprotein-1 (PSGL-1) has clearly been proven to mediate the adhesion of leukocytes to selectins under flow[2]. P-selectin binds to the amino-terminus of PSGL-1 through recognition of a sialyl $Lewis^x$ (SLe^x) moiety linked to a properly positioned core-2 O-glycan and three tyrosine sulfate residues.

Inhibitors of selectins may possess therapeutic properties, which may be applied for the treatment of a number of diseases. A recombinant truncated form of a PSGL-1 immunoglobulin fusion protein has already demonstrated effectiveness as such an inhibitor[3]. This glycoprotein can, however, only be produced in mammalian cells that are co-transfected with fucosyl- and core-2 GlcNAc transferases, making production of even small amounts of glycoprotein difficult.

The *N*-terminal glycosulfopeptide of PSGL-1 has also been obtained by chemo-enzymatic approaches[4]. In these procedures, a glycosulfopeptide of PSGL-1 that contains an *N*-acetyl galactosamine linked to a threonine moiety is chemically assembled. Subsequently, glycosyl transferases are employed to assemble the complete oligosaccharide. As membrane bound glycoproteins, mammalian glycosyltransferases generally can only be expressed in small quantities in eukaryotic cells[5]. Furthermore, their use in synthesis requires expensive sugar nucleotides or complicated *in-situ* recycling systems. The high selectivity of these enzymes also prevents the preparation of analogs exhibiting more desirable pharmacological properties. Recent progress in synthetic oligosaccharide chemistry[6] is beginning to provide opportunities for the efficient and large-scale synthesis of complex oligosaccharides.

As part of a program to prepare the N-terminal 18-amino acid signal sequence of PSGL-1 by a chemical approach, we report here a highly efficient and convergent synthesis of a properly protected oligosaccharide of PSGL-1 linked to a threonine that is appropriately protected for solid-phase peptide synthesis.

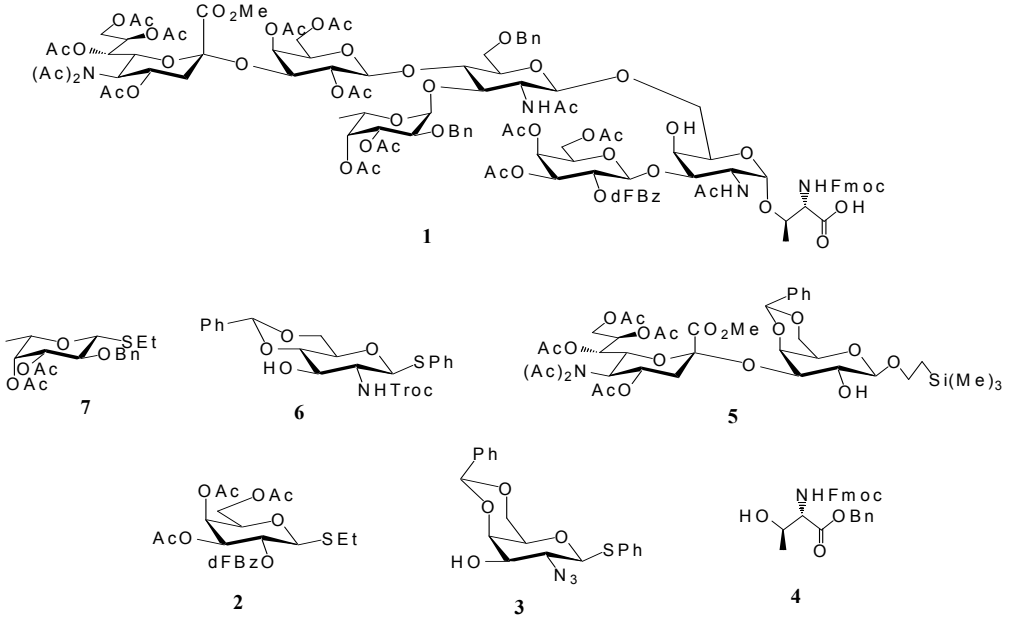


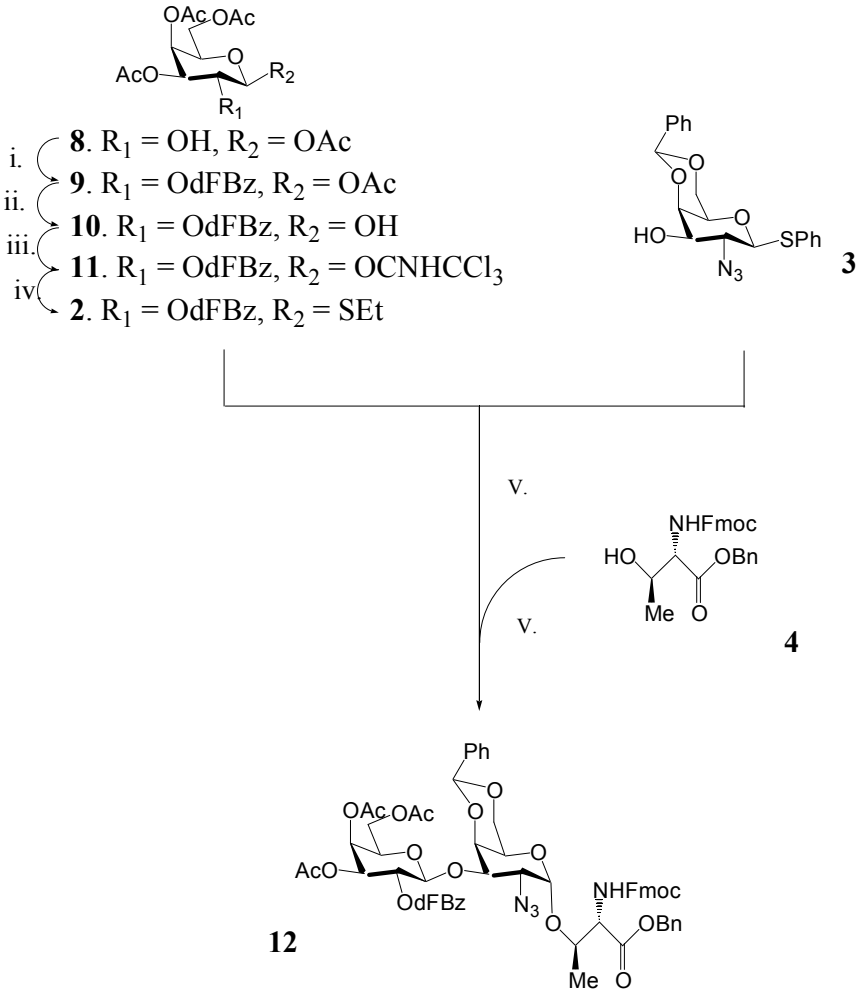
Figure 1

The synthesis of target compound **1** is complicated by the fact that *O*-glycosylated peptides are sensitive to acidic and basic conditions[7]. In addition, sufficient quantities of a precursor for glycosulfopeptide assembly can only be obtained by a highly convergent approach in which the target compound is assembled from properly protected monosaccharide building blocks employing a minimal number of synthetic steps. In this respect, the efficiency of classical oligosaccharide synthesis has been hindered by extensive protecting group manipulation of advanced synthetic intermediates[8]. However, strategies such as armed-disarmed, chemoselective and two-directional glycosylations have engendered an increased efficiency of oligosaccharide synthesis by minimizing the number of protecting group manipulations on advanced intermediates[9]. However, each of these methods has limitations complicating the preparation of highly complex compounds. Although unexplored, naturally occurring complex oligosaccharides can be best achieved by an orchestrated use of different glycosylation strategies.

It was envisaged that **1** could be prepared by employing a combination of armed-disarmed, chemo- and regioselective glycosylations[10]. Thus, the core-2 disaccharide linked to a properly protected threonine will be prepared by chemoselective one-pot glycosylation of thioglycoside **2** with thioglycoside **3**[11] to give the disaccharide, which is immediately activated to couple to the threonine acceptor **4** as shown in scheme 1. Removal of the benzylidine moiety of the galactoside of the resulting compound will give an acceptor that in a regioselective manner can be coupled with a properly protected SLe^x derivative obtained by armed-disarmed and chemoselective glycosylations using compounds **5**, **6**, and **7**.

Coupling of tetra-*O*-acetylated galactosyl bromide with thioglycosyl acceptor **3** in the presence of AgOTf led to a complex mixture of compound including the anticipated

Scheme 1



i. ClOdFBz, DMAP, pyr, 96%; ii. hydrazine acetate, THF, 60^{o}C, 92%; iii. trichloroacetonitrile, DBU, DCM, O^{o}C, 96%; iv. HSEt, BF_3Et_2O, DCM, 74%; v. Ph_2SO, Tf_2O, -60^{o}C, acceptor, DCM, 1h, -60^{o}C to rt. then to -60^{o}C, Ph_2SO, Tf_2O, acceptor, 1h, -60^{o}C to rt. (one pot) 70%

disaccharide, the corresponding ortho-ester and a thiophenyl galactoside derived from *trans*-glycosylation[12]. The use of alternative anomeric leaving groups such as thioethyl, trichloroacetimidate and fluoride did not improve the outcome of the glycosylation. It is well known that the use of a C-2 benzoyl ester will suppress the formation of orthoesters[6]. Indeed,

employing 2,3,4,6-tetra-O-benzoyl galactopyranosyl bromide gave a clean formation of the expected product. However, benzoyl esters are not compatible with glycopeptide synthesis because the basic conditions required for their removal will lead to β-elimination of the threonine moiety[7]. Recently, it was shown that 2,5-di-fluoro-benzoyl esters (dFBz) [13] are efficient neighboring group participants in glycosylations, which suppress ortho-ester formation. This protecting group has as an additional advantage in that it can be removed under mild basic conditions without affecting threonine glycosides. However, an AgOTf mediated coupling of the 2,3,4,6-tetra-dFBz galactosyl bromide was very sluggish and, even after a reaction time of three days, only part of the glycosyl donor and acceptor had been converted into disaccharide. It is probable that the strongly electron withdrawing 2,5-di-fluoro-benzoyl esters deactivate the donor, rendering it less suitable for glycosylations. It is expected that the glycosyl donor containing a dFBz ester at C-2 and acetyl esters at C-3, C-4, and C-6 would have a higher reactivity, yet the favorable participating properties of the C-2 dFBz would be maintained[14]. Indeed, an AgOTf mediated coupling of the 2-*O*-(2,5-diflurorbenzoyl) galactosyl bromide with **3** gave within 1 hour, complete conversion of the glycosyl donor and the desired disaccharide was isolated in a yield of 63%. The new van Boom promoter system (Ph_2SO/Tf_2O) provided the possibility of a one-pot synthetic method by utilizing thioglycosides as donors to provide the desired core-2 building block **12**. Activation of thioglycosyl donor **2** with Ph_2SO and Tf_2O at -60^{o}C, followed by the addition of acceptor **3** provided the desired thiophenyl disaccharide which is immediately activated under the same conditions, and upon addition of the threonine acceptor **4**[15] provided compound **12** in a yield of 74% as only the α-anomer. Finally, glycosyl acceptor **19** was obtained by removal of the benzylidene acetal of **12** using aqueous acetic acid at 70^{o}C.

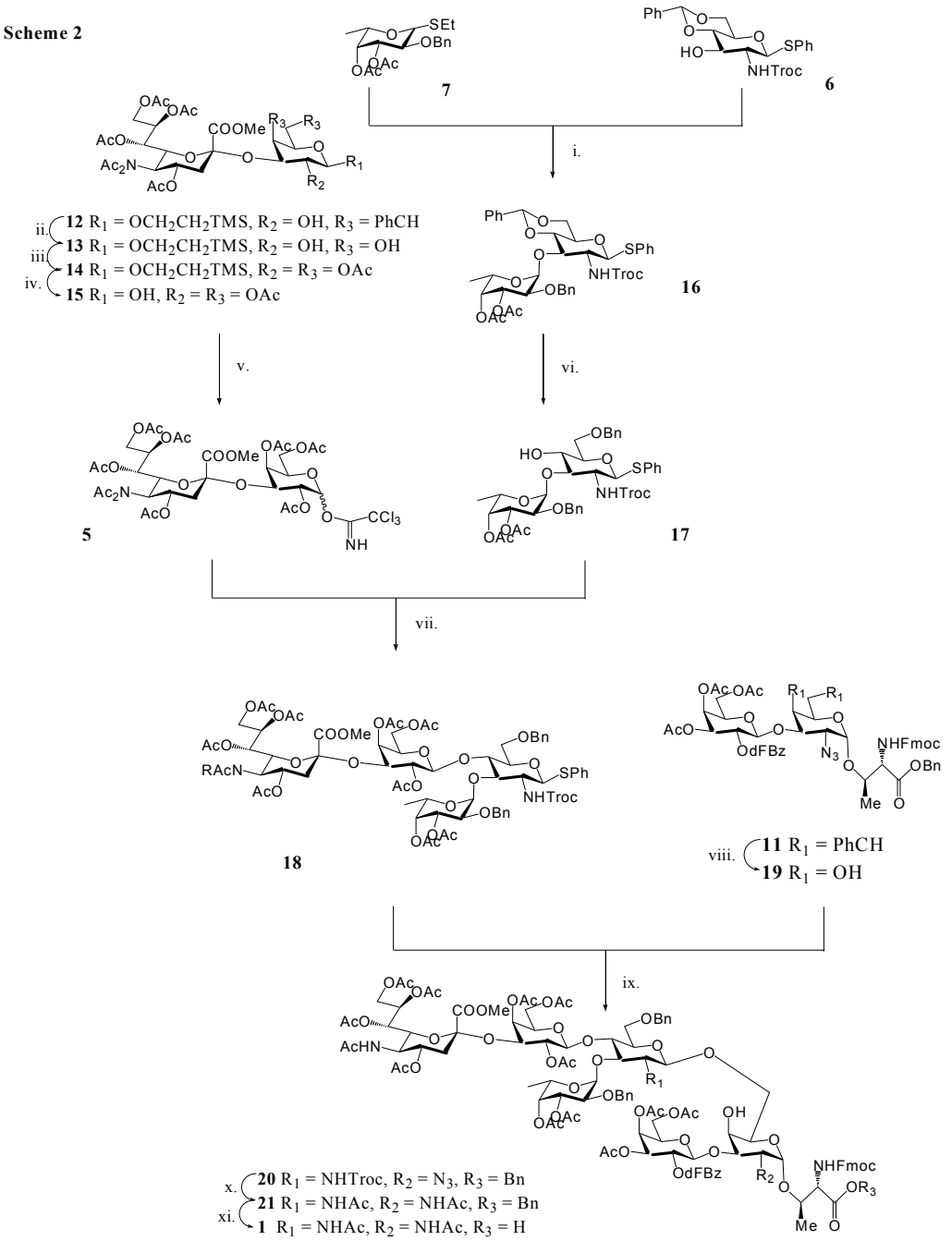
The next stage of the synthesis entailed the preparation of properly protected SLe^x glycosyl donor **18**, which will be coupled with glycosyl acceptor **19** to give hexasaccharide **20**. Compound **18** could be prepared from the readily available building blocks **5**, **6**, and **7** using a highly efficient armed-disarmed and chemoselective glycosylation sequence as shown in Scheme 2. Glycosyl donor **5**

could be obtained in a facile manner from the known disaccharide **12**[17] by a three-step reaction sequence. Thus, hydrogenation of **12** over Pd/C to remove the benzylidene acetal followed by acetylation of the resulting hydroxyls gave **14** in quantitative yield. Next, the anomeric trimethylsilylether (TMSOEt)[18] moiety was cleaved by treatment with trifluoroacetic acid in dichloromethane and the resulting lactol **15** was converted into trichloroacetimidate **5** by reaction with trichloroacetonitrile and DBU.

Ethyl thio-fucosyl donor **7**[19] was coupled with the less reactive phenyl thioglycosyl acceptor **6** using Ph_2SO, Tf_2O as the promoter to give disaccharide **16** in a yield of 82%. The difference in reactivity of thioethyl- compared to thiophenyl glycosides and the deactivating effect of the benzylidene acetal[20] and *N*-trichloroethyloxycarbonyl (Troc) [21] group makes the fucose more reactive than the galactosamine. In addition, 6-deoxysaccharide derivatives are generally more reactive than their hydroxyl counterpart. The regioselective ring opening of the benzylidene acetal of **16** using $NaCNBH_3$ and HCl[22] in diethyl ether gave glycosyl acceptor **17** in an almost quantitative yield. Next, a TMSOTf mediated coupling[23] of trichloroacetimidate **5** with **17** gave the protected SLe^x tetrasaccharide **18** in a good yield. The thiophenyl moiety of **18** has functioned as an effective anomeric protecting group. However, in the next glycosylation it was activated with Ph_2SO, Tf_2O and coupling[24] with **19** gave formation of the targeted hexasaccharide **20** in a yield of 69%. As expected, no glycosylation of the less reactive C-4

hydroxyl of **19** was observed. Furthermore, due to effective neighboring group participation of the *N*-Troc group of **18** only the β-glycoside was formed.

Scheme 2



i. Ph_2SO, Tf_2O, -60°C, Acceptor, DCM, 85%; ii. Pd on C, H_2, MeOH:H_2O (30:1), 96%; iii. Ac_2O, pyr. 99%; iv. 15%TFA in DCM, 0°C, 95%; v. trichloroacetonitrile, DBU, DCM, 0°C, 95%; vi. NaCNBH3, HCl in Et_2O, THF, 96%; vii. TMSOTf, DCM, -30°C, 70%; viii. 80% AcOH aq., 70°C, 96%; ix. Ph_2SO, Tf_2O, -60°C, Acceptor, DCM, 76%; x. Zn dust, sat. aq. $CuSO_4$, (3:2:1) THF:Ac_2O:AcOH, 73%; xi. Pd on C, H_2, (1:1) isopropanol:pyr, 83%.

In order to obtain a derivative that can be used in glycopeptide synthesis, the Troc and the azido moiety of **20** were converted into acetamido functions by reduction with $Zn/CuSO_4$ in a mixture of THF, acetic anhydride and acetic acid. Finally, the benzyl ester of the threonine moiety was selectively removed by catalytic hydrogenation over Pd/C in a mixture of pyridine and *i*-propanol to give the target compound **1**.

In conclusion, this chapter describes the first synthesis of a properly protected PSGL-1 oligosaccahride linked to a threonine that can be used for glycosulfopeptide synthesis. A highly convergent strategy combined with armed-disarmed, chemoselective and regioselective glycosylations was employed to minimize the number of protecting group manipulations during oligosaccharide assembly. It is to be expected that the strategic principles employed in the synthesis of **1** are relevant to the preparation of many other complex oligosaccharides of biological and medicinal importance.

EXPERIMENTAL

General: NIS was purchased from Fluka and re-crystallized from dioxane/CCl_4. All other chemicals were purchased from Aldrich, Acros, and Fluka and used without further purification. Molecular sieves were activated at 145 °C for 10 h. All solvents employed were of reagent grade and dried by refluxing over appropriate drying agents. TLC was performed using Kieselgel 60 F_{254} (Merck) plates, with detection by UV light (254 nm) and/or by charring with 8% sulfuric acid in ethanol. Column chromatography was performed on silica gel (Merck, mesh 70-230). Extracts were concentrated under reduced pressure at ≤ 40 °C (water bath). ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova300 spectrometer, and a Varian Inova500 spectrometer equipped with Sun workstations. ^{1}H spectra recorded in $CDCl_3$ were referenced to

residue $CHCl_3$ at 7.26 ppm or TMS, and ^{13}C spectra to the central peak of $CDCl_3$ at 77.0 ppm. Assignments were made using standard 1D and gCOSY, gHSQC and TOCSY 2D experiments. Positive ion matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were recorded using an HP-MALDI instrument using gentisic acid as a matrix.

1,3,4,6-tetra-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-α-D-galactopyranose (9). To a solution of 1,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose **8** (700 mg, 2.01mmol) in dry pyridine (8 mL) was added 4-(dimethylamino)pyridine (49 mg, 0.402 mmol) and the solution was stirred at rt for 30 min. 2,5-difluorobenzoyl chloride (0.5 mL, 4.02 mmol) was added drop-wise over a 10 min period and the stirring was continued for 18 h. The reaction was quenched by addition of methanol (4 mL) and after stirring for 1 h, the solution was diluted with CH_2Cl_2 (120 mL) and washed with water (150 mL). The aqueous phase was extracted with CH_2Cl_2 (50 mL) and the combined organic phases were dried ($MgSO_4$), and concentrated to dryness. After purification by silica gel chromatography (Hexane/EtOAc 8:1), compound **9** (942 mg, 96%) was obtained as a white solid; TLC (Hexane/EtOAc 1:1), $R_f = 0.68$; $[\alpha]_D + 20.0$ (c 2 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^1H, δ 7.55-7.05 (m, 3H, dFBz), 6.53 (d, 1H, $J_{1,2}$ 3.3 Hz, H-1), 5.60-5.41 (m, 3H, H-2, H-3, H-4), 4.39 (dt, 1H, $J_{5,6}$ 6.6 Hz, H-5), 4.16-4.07 (m, 2H, H-6a, H-6b), 2.18, 2.14, 2.04, 2.03 (4 x s, 12H, 4xCH_3CO); ^{13}C, δ 20.7, 20.8, 20.9, 21.0 (4xCH_3CO), 61.4 (C-6), 67.6 (C-5), 67.7 (C-3), 68.0 (C-4), 69.1 (C-2), 89.7 (C-1), 118.3-122.2 (aromatic C), 170.6, 170.3, 170.2, 170.1 (4xCH_3CO); HR MALDI-TOF MS: m/z: calc for $C_{21}H_{22}F_2O_{11}$: 488.1130; found 511.1029 $[M+Na]^+$.

3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-D-galactopyranose (10). Hydrazine acetate (192 mg, 2.08 mmol) was added to a solution of **9** (925 mg, 1.89 mmol) in DMF (9 mL) and the resulting reaction mixture was heated at 60°C for 3 h. After cooling the reaction mixture, it was

diluted with EtOAc (75 mL) and washed with 20% aqueous NaCl (75 mL). The aqueous phase was extracted with EtOAc (40 mL) and the combined organic layers were dried ($MgSO_4$), and concentrated under reduced pressure. Silica gel chromatography purification (Hexane/EtOAc 2:1) gave **10** (776 mg, 92%); TLC (Hexane/EtOAc 2:1), R_f = 0.22; $[\alpha]_D$ + 7.4 (c 2.0 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^{1}H, δ 7.60-7.10 (m, 3H, dFBz), 5.68 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.59 (dd, 1H, $J_{2,3}$ 10.5 Hz, $J_{3,4}$ 3.0 Hz, H-3), 5.52 (d, 1H, $J_{4,5}$ 2.5Hz, H-4), 5.36 (dd, 1H, $J_{1,2}$ 10.7 Hz, $J_{2,3}$ 3.6 Hz, H-2), 4.52 (t, 1H, $J_{5,6}$ 6.6 Hz, H-5), 4.20-4.10 (m, 2H, H-6), 3.10 (bs, 1H, OH), 2.18, 2.17, 2.06 (3 x s, 9H, 3xCH_3CO); ^{13}C, δ 20.8, 20.9, 21.0 (3xCH_3CO), 62.0 (C-6), 66.7 (C-5), 68.5 (C-3), 69.9 (C-4), 71.5 (C-2), 90.8 (C-1), 118.2-122.2 (aromatic C), 170.2, 170.4, 170.7 (3xCH_3CO); HR MALDI-TOF MS: m/z: calc for $C_{19}H_{20}F_2O_{10}$: 446.1025; found 469.0920 $[M+Na]^+$.

3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-α-D-galactopyranosyl trichloroacetimidate (11). Compound **10** (250 mg, 560 μmol) was dissolved in dry CH_2Cl_2 (5 mL) at 0°C and trichloroacetonitrile (1.2 mL, 8.40 mmol) was added followed by 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU, 8 μL, 53.5 μmol). The mixture was stirred for 2 h at 0°C, concentrated under reduced pressure, and purified by silica gel chromatography (Hexane/EtOAc/TEA 5:1:0.01) to yield **11** (317 mg, 96%); TLC (Hexane/EtOAc 2:1), R_f = 0.39; $[\alpha]_D$ + 26.25 (c 2.0 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^{1}H, δ 7.57-7.06 (m, 3H, dFBz), 6.74 (d, 1H, $J_{1,2}$ 2.2 Hz, H-1), 5.55-5.70 (m, 3H, H-2, H-3, H-4) 4.51 (t, 1H, $J_{5,6}$ 6.6 Hz, H-5), 4.24-4.08 (m, 2H, H-6), 2.20, 2.03, 1.99 (3 x s, 9H, 3xCH_3CO); ^{13}C, δ 20.8, 20.9, 21.0 (3xCH_3CO), 61.5 (C-6), 67.7 (C-3), 67.8 (C-4), 68.2 (C-2), 69.4 (C-5), 93.6 (C-1), 118.2-122.5 (aromatic C), 161.0 (C=NH) 170.1, 170.3, 170.5 (3xCH_3CO); HR MALDI-TOF MS: m/z: calc for $C_{21}H_{20}Cl_3F_2NO_{10}$: 589.0121; found 612.0019 $[M+Na]^+$.

Ethyl 3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-1-thio–β-D-galactopyranose (2).

To a solution of trichloroacetoimidate **11** (300 mg, 508 μmol) and ethane thiol (60 μL, 813 μmol) in dichloromethane (7 mL) was stirred at 0°C. BF_3*Et_2O (83 mL, 660 mmol) was added dropwise to the stirring solution and the mixture was stirred for 2 h at 0°C, concentrated to dryness and purified by silica gel chromatography (Hexane/EtOAc 5:1) to yield **2** (185 mg, 74 %); TLC (Hexane/EtOAc 2:1), R_f = 0.39; $[\alpha]_D$ + 16.25 (c 2.0 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^{1}H, δ 7.57-7.10 (m, 3H, dFBz), 5.86 (d, 1H, H-1, $J_{1,2}$= 5.5 Hz), 5.52-5.46 (m, 2H, H-2, H-4), 5.36 (dd, 1H, H-3), 4.65 (t, 1H, H-5), 4.13 (m, 2H, H-6), 2.56 (m, 2H, CH_2), 2.16, 2.04, 1.96 (3 x s, 9H, 3 x OAc), 1.24 (t, 3H, CH_3); ^{13}C, δ 14.9 (CH_3), 20.7, 20.8, 20.9 (3 x $COCH_3$), 24.4 (CH_2), 62.0 (C-6), 66.9 (C-5), 68.3 (C-3), 68.3 (C-4), 69.4 (C-2), 82.2 (C-1), 118.3-122.3 (aromatic-C), 170.1, 170.4, 170.7 (3 x C=O); HR MALDI-TOF MS: m/z: calc for $C_{21}H_{24}F_2O_9S$: 490.1109; found 513.1007 $[M+Na]^+$.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-[2-Azido-4,6-O-benzylidene-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine Benzylester (12).** To a solution of compound **2** (52 mg, 106 μmol), Ph_2SO (60 mg, 297 μmol) in dry CH_2Cl_2 (6 mL) was added trifluoromethanesulfonic anhydride (19 μL, 115 μmol) at -60°C. The mixture was stirred for 10 min, after which a solution of acceptor **3** (34 mg, 88 μmol) in CH_2Cl_2 (1.5 mL) was added. The reaction was stirred at -60°C for 1 h and then it was slowly warmed to rt and it is then once again cooled to -60°C. Ph_2SO (50 mg, 246 μmol) then trifluoromethanesulfonic anhydride (19 μL, 115 μmol) were added to the stirring solution and the mixture was stirred for 10 min. for activation, then **4** (76 mg, 176 μmol) in DCM (2.5 mL) was added to the reaction slowly. After 1 hr at -60°C the reaction returned to rt. slowly then

it was quenched by the addition of saturated aqueous $NaHCO_3$ (2 mL). The organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Purification by silica gel chromatography (Hexane/EtOAc 3:1) gave **12** (82 mg, 82%); TLC (Hexane/EtOAc 1:1) R_f =0.67; $[\alpha]_D$ + 21.0 (c 2 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^1H, δ 7.60-6.97 (m, 21H, dFBz, Ph, Bn, Fmoc), 5.71 (d, 1H, J 9.3 Hz, NH), 5.54 (t, 1H, $J_{1,2,3}$ = 9.8Hz, H-2'), 5.45 (d, 1H, $J_{4,5}$ 2.5 Hz H-4'), 5.18 (dd, 1H, $J_{2,3}$ 10.3 Hz, $J_{3,4}$ 2.9 Hz, H-3'), 5.13 (s, 1H, PhC*H*), 4.93 (d, 1H, $J_{1,2}$ 7.8 Hz H-1'), 4.89 (d, 1H, $J_{1,2}$ 2.9 Hz, H-1), 4.50-4.13 (m, 10 H, Fmoc 3H, H_α, H_β, H-4, H-5, H-6_{ax}, H-6'), 4.02-3.97 (m, 2H, H-3, H-5'), 3.73 (dd, 1H, $J_{1,2}$ 2.9 Hz, $J_{2,3}$ 10.3 Hz, H-2), 3.63 (m, 1H, H-6_{eq}), 2.18, 2.03, 1.93 (3 x s, 9H, 3xC*H*$_3$CO), 1.27 (d, 3H, CH_3); ^{13}C, δ 19.2 (CH_3), 20.8, 20.9, 21.0 (3x*C*H_3CO), 59.3 (Cα), 59.8 (C-2), 63.6 (C-6), 69.2-66.2 (C-4', CH_2-Fmoc), 70.1 (C-2'), 71.3 (C-5', C-3'), 75.8 (C-3), 75.6 (C-4), 76.3 (Cβ), 99.2 (C-1), 101.0 (*C*HPh), 102.4 (C-1'), 117.9-130.2 (aromatic C), 169.2-171.1 (5xC=O); HR MALDI-TOF MS: m/z: calc for $C_{58}H_{56}F_2N_4O_{18}$: 1134.3558; found 1157.3450 $[M+Na]^+$.

***N*-(9-Fluorenylmethyloxycarbonyl)-*O*-[2-Azido-2-deoxy-3-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonine Benzylester (19).** Compound **12** (70 mg, 61.7 μmol) in 80% aqueous acetic acid (5 mL) was heated at 70°C for 3 h. After cooling to rt, the solvent was evaporated by coconcentration with toluene (20 mL). Silica gel column chromatography (Hexane/EtOAc 2:5) afforded compound **19** (62 mg, 59.2 mmol, 96%); TLC (Hexane:EtOAc 1:3) R_f =0.44; $[\alpha]_D$ - 0.7 (c 2 mg/mL, $CHCl_3$); NMR data ($CDCl_3$): ^1H, δ 7.77-6.76 (m, 16H, dFBz, Bn, Fmoc), 5.63 (d, 1H, NH), 5.52 (dd, 1H, H-2', $J_{2,3}$ = 10.2 Hz, $J_{1,2}$ = 8.3 Hz), 5.45 (d, 1H, H-4', $J_{3,4,5}$ = 2.9 Hz), 5.20 (dd, 1H, H-3', $J_{2,3}$ = 10.7 Hz, $J_{3,4}$ = 3.4 Hz), 5.12 (dd, 2H, CH_2Bn), 4.86 (d, 1H, H-1, $J_{1,2}$ = 8.30 Hz), 4.80 (d, 1H, H-1', $J_{1,2}$ = 3.4 Hz), 4.46-3.39 (m, 2H, H-6', Hα-Thr), 4.29 (dd, 1H, Hβ-Thr), 4.22-4.10 (m, 4H, H-4, H-6', CH_2-

Fmoc), 3.98 (t, 1H, H-5'), 3.92-3.84 (m, 3H, H-3, H-6, CH-Fmoc), 3.77 (dd, 1H, H-5), 3.63 (m, 1H, H-6), 3.50 (dd, 1H, H-2, $J_{1,2}$ = 3.4 Hz, $J_{2,3}$ = 10.7 Hz), 2.19, 2.05, 1.95 (3 x s, 9H, 3 x OAc), 1.27 (d, 3H, CH_3); ^{13}C, δ 18.8 (CH_3), 20.7, 20.8, 20.9 (s, 9H, 3 x $COCH_3$), 47.4 (C-4), 58.9 (C-2), 61.8 (CH_2-Fmoc), 62.8 (C-4'), 67.6 (Cβ-Thr), 67.9 (CH_2Bn), 69.4 (C-5), 69.8 (CH-Fmoc), 69.8 (C-2'), 70.8 (C-3'), 71.7 (C-5'), 76.3 (Cα-Thr), 78.3 (C-3), 99.2 (C-1'), 102.0 (C-1), 118.2-128.9 (aromatic C), 170.2-170.7 (5xC=O); HR MALDI-TOF MS: m/z: calc for $C_{51}H_{52}F_2N_4O_{18}$: 1046.3245; found 1069.3123 $[M+Na]^+$.

Phenyl (3,4-Di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1->3)-[4,6-O-benzylidene-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside] (16).

Compound **7** (91 mg, 238 μmol) was dissolved in dichloromethane (7 mL) and cooled to -60°C Phenyl sulfoxide (135 mg, 666 μmol) was added followed by triflic anhydride (44 μL, 262 μmol). The mixture was stirred for 10 min at -60°C, and then the acceptor **6** (106 mg, 198 μmol) in dichloromethane (2 mL) was slowly dripped into the stirring reaction mixture. The reaction was allowed to stir at -60°C for an hour, and then slowly returned to room temperature. The reaction was diluted with dichloromethane (50 mL) and filtered through celite. The organic solution was washed with saturated aqueous sodium bicarbonate (50 mL) and then with brine (50 mL) then dried ($MgSO_4$), filtered, and concentrated in vacuuo. Silica gel column chromatography (5:1 Tol:EtOAc) of the residue gave compound **16** (146 mg, 86%) as a white solid. TLC (2:1 Hex:EtOAc), R_f = 0.55; $[\alpha]_D$ -5.49 (c 2 mg/mL, $CHCl_3$) NMR data: $CDCl_3$, ^1H, δ 7.45-7.24 (m, 15H, 2 x Ph, Bn), 5.49 (s, 1H, PhCH), 5.40 (d, 1H, $J_{1,2}$=7.33 Hz, H-1), 5.34 (d, 1H, NH), 5.25 (d, 1H, $J_{2,3,4}$=2.93 Hz, H-3'), 5.09 (m, 2H, H-1', H-4'), 4.86 (d, 1H, $PhCH_2$), 4.66 (dd, 2H, Troc), 4.57 (d, 1H, $PhCH_2$), 4.35 (dd, 1H, J=10.7 Hz, 3.9 Hz, H-6_{eq}), 4.30 (t, 1H, J=8.8 Hz,

H-3), 4.24 (q, 1H, H-5'), 3.81 (dd, 1H, $J_{1,2}$=10.7 Hz, $J_{2,3}$=3.4 Hz, H-2'), 3.74 (t, 1H, J=9.77 Hz, H-6_{ax}), 3.58-3.51 (m, 2H, H-4, H-5), 3.11 (bt, 1H, H-2), 2.06, 1.93 (2 x s, 6H, 2xOAc), 0.52 (d, 3H, $J_{5,6}$=6.35 Hz, H-6'); ^{13}C δ 15.3 (C-6'), 20.9, 21.1 (2 x CH_3CO), 58.2 (C-2), 65.0 (C-5'), 68.9 (C-6), 70.4 (C-3'), 70.8 (C-5), 71.9 (C-4'), 73.6 (Troc-CH_2), 74.3 (C-2'), 74.6 (Bn-CH_2), 76.4 (C-3), 80.3 (C-4), 85.9 (C-1), 98.4 (C-1'), 102.3 (CHPh), 125.0-145.8 (aromatic C), 145.8 (CCl3), 153.9 (Troc-C=O), 170.3, 170.7 (2 x CH_3CO); HR MALDI-TOF MS: m/z: calc for $C_{39}H_{42}Cl_3NO_{12}S$: 855.1744; found 878.1643 $[M+Na]^+$.

Phenyl (3,4-Di-*O*-acetyl-2-*O*-benzyl-α-L-fucopyranosyl)-(1->3)-[6-O-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside] (17).

To a solution of **16** (135 mg, 158 μmol) in THF (6 mL) was added $NaCNBH_3$ (99 mg, 1.58 mmol) and the mixture was allowed to stir with molecular sieves under an argon atmosphere. 2M HCl in ether (3 mL) was added dropwise with ventilation of the flask for gas release. The reaction stirred for 2 h, and the solution was diluted with DCM (50 mL), filtered through celite, and concentrated in vacuuo. Silica gel column chromatography (5:2 Hex:EtOAc) gave compound **17** (130 mg, 152 μmol, 96%) as a white solid. TLC (2:1 Hex:EtOAc), R_f = 0.23; $[\alpha]_D$ + 0.52 (c 2 mg/mL, $CHCl_3$); NMR data: $CDCl_3$, ^{1}H, δ 7.48-7.20 (m, 15H, 2 x Bn, Ph), 5.41 (d, 1H, NH), 5.28-5.25 (m, 2H, H-3', H-4'), 5.06 (d, 2H, H-1, H-1'), 4.67-4.49 (m, 6H, CH_2-Troc, 2 x CH_2Bn), 4.38 (q, 1H, H-5', $J_{5,6}$=6.3 Hz), 3.87-3.84 (m, 2H, H-3, H-5), 3.81 (dd, 1H, H-2', $J_{2,3}$=5.3 Hz, $J_{1,2}$=2.5 Hz), 3.73 (dd, 1H, H-6), 3.64 (bs, 1H, OH), 3.56-3.52 (m, 2H, H-4, H-6), 3.28 (bt, 1H, H-2), 2.10, 1.95 (2 x s, 6H, 2xOAc), 1.09 (d, 3H, H-6'); ^{13}C δ 16.2 (C-6'), 20.9, 21.0 (2 x OAc), 56.0 (C-2), 66.1 (C-5'), 70.2 (C-6), 70.3 (C-2'), 70.9 (C-3'), 71.6 (C-4'), 73.8 (2 x CH_2Bn), 73.9 (C-5), 74.5 (CH_2-Troc), 78.9 (C-4), 83.9 (C-3), 86.0 (C-1), 98.5 (C-1'), 125.1-

138.3 (Aromatic-C, CCl_3-Troc), 154.1 (C=O Troc), 170.2, 170.6 (2 x OAc); HR MALDI-TOF MS: m/z: calc for $C_{39}H_{44}Cl_3NO_{12}S$: 857.1903; found 880.1797 $[M+Na]^+$.

2-(Trimethylsilyl)ethyl [methyl 5-(N-acetylacetamido)-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-a-D-galacto-2-non-ulopyranosylonate]-(2->3)-O-b-D-galactopyranoside (13).

To a solution of **12** (250 mg, 283 μmol) in DCM:MeOH (30:1; 15 mL) under an argon atmosphere was added Pd on Charcoal (150 mg) and the mixture stirred for 20 min. at room temperature. The argon was replaced with H_2, and the reaction stirred for 1 h. The solution was diluted with DCM (50 mL), filtered through celite, and concentrated. Silica gel column chromatography (5:2 Tol:Acetone) afforded compound **13** (220 mg, 277 μmol, 98%) as a white solid. TLC (1:1 Tol:Acetone), R_f =0.48 $[\alpha]_D$ + 4.54 (c 2 mg/mL, $CHCl_3$) NMR data: $CDCl_3$, ^1H, δ 5.52 (ddd, 1H, H-4'), 5.34 (dd, 1H, H-8'), 5.15 (d, 1H, H-7' J=8.0 Hz), 4.94 (d, 1H, H-6' J=10.3 Hz), 4.42 (d, 1H, H-1 J=7.6 Hz), 4.32 (dd, 1H, H-9'), 4.21 (t, 1H, H-5' J=10.2 Hz), 4.12 (dd, 1H, H-3, $J_{2,3}$=9.4 Hz, $J_{3,4}$=3.1 Hz), 4.08 (dd, 1H, H-9'), 4.02 (m, 1H, OCH_2), 3.91 (dd, 1H, H-6), 3.86 (s, 3H, $COOCH_3$), 3.83 (dd, 1H, H-6), 3.72 (d, 1H, H-4), 3.69-3.64 (m, 2H, H-2, OCH_2), 3.55 (t, 1H, H-5), 2.84 (dd, 1H, H-3'$_{eq}$), 2.37, 2.30 (2 x s, 6H, $N(COCH_3)_2$), 2.11, 2.10, 2.02, 1.98 (4 x s, 12H, 4 x $COCH_3$), 1.95 (dd, 1H, H-3'$_{ax}$), 1.25-0.97 (m, 2H, CH_2SiMe_3), 0.01 (s, 9H, $SiMe_3$);); ^{13}C δ -1.17 (3 C, $Si(CH_3)_3$), 18.4 (CH_2Si), 20.96, 20.97, 21.08, 21.37 ($COCH_3$), 26.5, 28.2 ($N(COCH_3)_2$), 38.6 (C-3'), 53.6 ($COOCH_3$), 56.9 (C-5'), 62.3 (C-9'), 62.7 (C-6), 66.9 (C-4'), 67.0 (C-7'), 67.3 (OCH_2), 68.8 (C-8'), 68.9 (C-2), 69.6 (C-4), 70.3 (C-6'), 73.8 (C-5), 97.8 (C-2'), 102.8 (C-1), 128.4, 129.3 ($N(C=O)_2$), 168.2, 169.9, 170.3, 170.4, 170.9 (4 x COOMe, COMe); HR MALDI-TOF MS: m/z: calc for $C_{33}H_{53}NO_{19}Si$: 795.2981; found 818.2880 $[M+Na]^+$.

2-(Trimethylsilyl)ethyl [methyl 5-(N-acetylacetamido)-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-a-D-galacto-2-non-ulopyranosylonate]-(2->3)-O-(2,4,6-tri-O-acetyl-b-D-galactopyranoside) (14).

Compound **13** (215 mg, 270 μmol) was dissolved in pyridine (10 mL) and acetic anhydride (5 mL) and the reaction stirred for 14 h. at room temperature. The solvent was evaporated by coconcentration with toluene (3 x 50 mL). Silica gel column chromatography (1:1 Hex:EtOAc) afforded compound **14** (246 mg, 267 μmol, 99%) as a white solid. TLC (1:3 Hex:EtOAc), R_f =0.58 $[\alpha]_D$ +1.2 (c 2 mg/mL, $CHCl_3$) NMR data: $CDCl_3$, ^1H, δ 5.54-5.50 (m, 2H, H-4', H-8'), 5.15 (dd, 1H, H-7', J=9.3 Hz, 2.4 Hz), 4.61-4.56 (m, 3H, H-2, H-6', H-1, $J_{1,2}$=8.3 Hz), 4.31-4.27 (m, 2H, H-5', H-9') 4.09-4.01 (m, 3H, H-6, H-9') 3.97 (dt, 1H, OCH_2), 3.88 (s, 3H, $COOCH_3$), 3.85 (t, 1H, H-5), 3.59 (dt, 1H, OCH_2), 2.64 (dd, 1H, H-3'$_{eq}$), 2.35, 2.28 (2 x s, 6H, $N(COCH_3)_2$), 2.19, 2.18, 2.07, 2.03, 2.02, 1.94 (7 x s, 21H, 7 x $COCH_3$), 1.62 (m, 1H, H-3'$_{ax}$), 0.99 (m, 2H, CH_2Si), 0.00 (s, 9H, $Si(CH_3)_3$); ^{13}C, δ -1.17 (3C, $Si(CH_3)_3$), 18.2 (CH_2Si), 21.7-20.8 (7 x $COCH_3$), 26.9, 28.3 (2 x $NCOCH_3$), 38.6 (C-3'), 53.3 ($COOCH_3$), 56.2 (C-5'), 62.3 (C-9'), 62.4 (C-6), 67.2 (C-2', C-7'), 67.5 (C-4), 67.9 (OCH_2), 68.0 (C-2), 69.6 (C-6'), 70.3 (C-4), 70.6 (C-5), 72.0 (C-3), 96.9 (C-1'), 100.7 (C-1), 128.4, 129.2 (2 x NC=O), 168.1-174.3 (8C, C=O); HR MALDI-TOF MS: m/z: calc for $C_{39}H_{59}NO_{22}Si$: 921.3298; found 944.3184 $[M+Na]^+$.

[methyl 5-(N-acetylacetamido)-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-a-D-galacto-2-non-ulopyranosylonate]-(2->3)-O-(2,4,6-tri-O-acetyl-b-D-galactopyranoside) (15)

To a solution of compound **14** (240 mg, 260 μmol) in DCM (10 mL) at 0°C was added TFA (2 mL). The reaction stirred for 4 h. at 0°C. The solvent was evaporated by coconcentration with toluene (2 x 20 mL). Silica gel column chromatography (2:5 Hex:EtOAc) of the residue afforded compound **15** (205 mg, 250 μmol, 96%, 1:1 α:β) as a white solid. TLC (1:2

Hex:EtOAc), R_f =0.10 $[\alpha]_D$ +0.65 (c 2 mg/mL, $CHCl_3$) NMR data: $CDCl_3$, ^1H, δ 5.96-5.05 (m, 2H, H-8', H-4'), 5.32 (d, 1H, H-1α, $J_{1,2}$=3.8 Hz), 5.18-5.10 (m, 2H, H-7', H-2), 5.01 (t, 2H, H-4, H-1β, $J_{1,2}$=7.6 Hz), 4.74 (dd, 1H, H-3), 4.64 (m, 1H, H-6'), 4.39-4.22 (m, 2H, H-5', H-5), 4.15-4.39 (m, 4H, H-6, H-9'), 3.90 (s, 3H, $COOCH_3$), 2.69-2.63 (m, 1H, H-3'), 2.36, 2.30 (2 x s, 6H, $NCOCH_3$), 2.24, 2.09, 2.06, 2.05, 2.04, 2.03, 1.95 (7 x s, 21H, 7 x $COCH_3$), 1.70-1.60 (m, 1H, H-3'); ^{13}C, δ 20.8-27.0 (7 x $COCH_3$), 28.3, 29.9 (2 x $NCOCH_3$), 53.4 ($COOCH_3$), 56.0 (C-5), 62.6 (C-9'), 63.0 (C-5'), 66.7 (C-6), 67.0 (C-4'), 67.5 (C-7'), 68.7 (C-3), 68.9 (C-4), 69.6 (C-6'), 91.9 (C-1β), 95.7 (C-1α), 97.0 (C-1'), 168.0-174.3 (10 x C=O); HR MALDI-TOF MS: m/z: calc for $C_{34}H_{47}NO_{22}$: 821.2590; found 844.2488 $[M+Na]^+$.

[methyl 5-(N-acetylacetamido)-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-a-D-galacto-2-non-ulopyranosylonate]-(2->3)-O-(2,4,6-tri-O-acetyl-b-D-galactopyranosyl) trichloroacetimidate (5).

Compound **15** (200 mg, 207 μmol), in DCM (10 mL), stirred at 0°C under an argon atmosphere. Trichloroacetonitrile (3 mL, 20.7 mmol) was added to the solution followed by DBU (14 μL, 94.8 μmol). The reaction stirred for 2 h. at 0oC, then the solvent is evaporated at 28°C. Silica gel column chromatography (1:2 Hex:EtOAc) of the syrup afforded compound **5** (190 mg, 197 μmol, 95% 3:1 β:α) as a white solid. TLC (1:2 Hex:EtOAc), R_f =0.28 $[\alpha]_D$ +8.95 (c 2 mg/mL, $CHCl_3$) NMR data: $CDCl_3$, ^1H, δ 7.26 (s, 1H, NH), 6.47 (d, 1H, H-1, $J_{1,2}$=3.8 Hz), 5.92 (d, 1H, H-1, $J_{1,2}$=8.2 Hz), 5.53-5.49 (m, 2H, H-4', H-8'), 5.25 (dd, 1H, H-2), 5.14 (dd, 1H, H-7'), 5.04 (d, 1H, H-4), 4.76 (dd, 1H, H-3), 4.60 (dd, 1H, H-6'), 4.34-4.26 (m, 2H, H-5', H-9'), 4.17-3.99 (m, 3H, H-5, H-6), 3.92 (m, 1H, H-9'), 3.88 (s, 3H, $COOCH_3$), 2.66 (dd, 1H, H-3'), 2.27, 2.23 (2 x s, 6H, $NCOCH_3$), 2.17, 2.15, 2.09, 2.08, 2.02, 1.98, 1.94 (7 x s, 21H, 7 x $COCH_3$), 1.61 (dd, 1H, H-3'); ^{13}C, δ 14.4 (c-3'), 20.9-21.8 (7 x $COCH_3$), 28.3, 29.9 (2 x $NCOCH_3$), 53.4

($COOCH_3$), 56.1 (C-5), 61.9 (C-9'), 62.6 (C-5'), 67.1 (C-6), 67.3 (C-8'), 67.6 (C-4'), 68.0 (C-7'), 68.7 (C-3), 69.1 (C-4), 71.4 (C-6'), 96.3 (C-1), 96.9 (C-1'), 161.3 (CCl_3), 168.0-174.3 (10 x C=O);

Phenyl [O-methyl 5-(N-acetylacetamido)-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-a-D-galacto-2-non-ulopyranosylonate]-(2->3)-O-(2,4,6-tri-O-acetyl-b-D-galactopyranosyl)-(1->4)-O-[(3,4-di-O-acetyl-2-O-benzyl-a-L-fucopyranosyl)-(1->3)]-O-[6-O-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside] (18).

Compound **5** (108 mg, 112 μmol) and compound **17** (60 mg, 70 μmol) were dissolved in DCM (6 mL). The solution was cooled to -30°C and TMS Triflate (mL, mmol) was added and the reaction stirred for 30 min at -30°C. The reaction was diluted with DCM (30 mL) and filtered through celite. The organic solution was washed with water (mL), $NaHCO_3$ (mL), dried ($MgSO_4$), filter and concentrated. Silica gel column chromatography (1:3 Hexane:EtOAc) afforded compound **18** (mg, mmol, %) as a white solid. TLC (1:3 Hex:EtOAc), R_f =0.23 $[\alpha]_D$ +0.35 (c 2 mg/mL, $CHCl_3$) NMR data: $CDCl_3$, ^1H, δ 7.42-7.13 (m, 15H, 2 x Bn, Ph), 5.56-5.51 (m, 2H, H-4''', H-8'''), 5.30 (d, 1H, NH), 5.27 (d, 1H, H-4'), 5.20 (dd, 1H, H-3'), 5.15 (dd, 1H, H-7'''), 5.00 (d, 1H, H-1', $J_{1,2}$=3.5 Hz), 4.90-4.86 (m, 2H, H-5', H-2''), 4.83 (d, 1H, H-4''), 4.82 (d, 1H, H-1'', $J_{1,2}$=8.2 Hz), 4.78 (d, 1H, CH_2Bn), 4.68-4.64 (m, 4H, CH_2Bn, CH_2Troc), 4.61 (dd, 1H, H-2'), 4.56 (dd, 1H, H-6'''), 4.50 (d, 1H, CH_2Bn), 4.29 (t, 1H, H-5'''), 4.23-4.14 (m, 5H, H-4, H-6, H-5'', H-9'''), 4.01 (dd, 1H, H-9'''), 3.95 (t, 1H, H-3), 3.87 (s, 3H, $COOCH_3$), 3.87-3.80 (m, 3H, H-6'', H-1, $J_{1,2}$=7.4 Hz), 3.77 (t, 1H, H-5), 3.51 (m, 1H, H-2), 2.60 (dd, 1H, H-3'''), 2.34, 2.27 (2 x s, 6H, $N(COOCH_3)_2$), 2.15, 2.15, 2.11, 2.09, 2.06, 2.00, 1.94, 1.93, 1.92 (9 x s, 27H, 9 x $COOCH_3$), 1.60 (dd, 1H, H-3'''), 1.19 (d, 3H, H-6'); HR MALDI-TOF MS: m/z: calc for $C_{73}H_{89}Cl_3N_2O_{33}S$: 1658.4134; found 1681.4021 $[M+Na]^+$.

N-(9-Fluorenylmethoxycarbonyl)-O-[2-azido-2-deoxy-3-O-(3,4,6-tri-O-acetyl-2-O-2,5-diflurorbenzoyl-β-D-galactorpyranosyl)-6-O-(O-methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-a-D-galacto-2-non-ulopyranosylonate)-(2->3)-O-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1->4)-O-[(3,4-di-O-acetyl-2-O-benzyl-α-L-fucopyranosyl)-(1->3)]-O-[6-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl]-a-galactopyranosyl]-L-threonine benzyl ester (20).

Compound **18** (35 mg, 21 μmol) in DCM (4 mL) was cooled to -60°C, and phenyl sulfoxide (12 mg, 59 μmol) followed by triflic anhydride (5 μL, 30 μmol) were added to the stirring reaction mixture. The reaction stirred for 10 min at -60°C, then compound **19** (24 mg, 23 μmol) in DCM (1 mL) was added slowly, and the reaction stirred for 1 h at -60°C. It was then allowed to return to room temperature and was diluted with DCM (15 mL). The organic solution was filtered through celite, washed with $NaHCO_3$ (25 mL), with brine (25 mL), dried ($MgSO_4$), filtered, and concentrated in vacuuo. Silica gel column chromatography (3:1 Tol:Acetone) afforded compound **20** (41 mg, 76%) as a white solid. TLC (2:1 Tol:Acetone), R_f =0.51 $[\alpha]_D$ +0.24 (c 2 mg/mL, $CHCl_3$) NMR data: $CDCl_3$,^{1}H, δ 7.77-6.76 (m, 26H, 3 x Bn, Fmoc, dFBz), 5.63 (d, 1H, NH), 5.56-5.51 (m, 3H, H-2', H-4''''', H-8'''''), 5.45 (d, 1H, H-4', $J_{3,4,5}$ = 2.93 Hz), 5.30 (d, 1H, NH), 5.27 (d, 1H, H-4'''), 5.20 (m, 2H, H-3''', H-3'), 5.15 (dd, 1H, H-7'''''), 5.12 (dd, 2H, CH_2Bn), 5.00 (d, 1H, H-1''', $J_{1,2}$=3.5 Hz), 4.90-4.86 (m, 2H, H-5''', H-2''''), 4.86 (d, 1H, H-1, $J_{1,2}$ = 8.30 Hz), 4.83 (d, 1H, H-4''''), 4.82 (d, 1H, H-1'''', $J_{1,2}$=8.2 Hz), 4.80 (d, 1H, H-1', $J_{1,2}$ = 3.42 Hz), 4.78 (d, 1H, CH_2Bn), 4.68-4.64 (m, 4H, CH_2Bn, CH_2Troc), 4.61 (dd, 1H, H-2'''), 4.56 (dd, 1H, H-6'''''), 4.50 (d, 1H, CH_2Bn), 4.46-4.39 (m, 2H, H-6', Hα-Thr), 4.29 (m, 2H, H-5''''', Hβ-Thr), 4.23-4.14 (m, 5H, H-4'', H-6'', H-5'''', H-9'''''), 4.22-4.10 (m, 4H, H-4, H-6', CH_2-Fmoc), 4.01 (dd, 1H, H-9'''''), 3.98 (t, 1H, H-5'), 3.95 (t, 1H, H-3''), 3.92-3.84 (m, 3H, H-3, H-

6, CH-Fmoc), 3.87 (s, 3H, $COOCH_3$), 3.87-3.80 (m, 3H, H-6’’’’, H-1’’, $J_{1,2}$=7.4 Hz), 3.77 (t, 1H, H-5’’), 3.51 (m, 1H, H-2’’), 2.60 (dd, 1H, H-3’’’’’), 2.34, 2.27 (2 x s, 6H, $N(COOCH_3)_2$), 2.19, 2.15, 2.15, 2.11, 2.09, 2.06, 2.05, 2.00, 1.95, 1.94, 1.93, 1.92 (12 x s, 36H, 12 x $COOCH_3$), 1.60 (dd, 1H, H-3’’’’’), 1.27 (d, 3H, CH_3),1.19 (d, 3H, H-6’’’); HR MALDI-TOF MS: m/z: calc for $C_{118}H_{135}Cl_3F_2N_6O_{51}$: 2594.7189; found 2617.7086 $[M+Na]^+$.

N-(9-Fluorenylmethoxycarbonyl)-O-[2-acetamido-2-deoxy-3-O-(3,4,6-tri-O-acetyl-2-O-2,5-diflurorbenzoyl-b-D-galactorpyranosyl)-6-O-(O-methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-a-D-galacto-2-non-ulopyranosylonate)-(2->3)-O-(2,4,6-tri-O-acetyl-b-D-galactopyranosyl)-(1->4)-O-[(3,4-di-O-acetyl-2-O-benzyl-a-L-fucopyranosyl)-(1->3)]-O-[6-O-benzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl]-a-galactopyranosyl]-L-threonine benzyl ester (21).

Compound **20** (25 mg, 9.6 μmol) was dissolved in a solution of (3:2:1) THF:Ac_2O:AcOH (mL). Zinc dust (30 mg) and sat. aq. $CuSO_4$ (0.4 mL) was added and the reaction stirred for 4 h at rt. The reaction was diluted with DCM (mL), filtered through celite, and the solvent was evaporated by coconcentrated with toluene (3 x 25 mL). Silica gel column chromatography (25:1 DCM:MeOH) afforded compound **21** (17 mg, 73%) as a white solid. TLC (25:1 DCM:MeOH), R_f =0.30 $[\alpha]_D$ +0.19 (c 2 mg/mL, $CHCl_3$) NMR data: $CDCl_3$, ^1H, δ 7.77-6.76 (m, 26H, 3 x Bn, Fmoc, dFBz), 5.63 (d, 1H, NH), 5.56-5.51 (m, 3H, H-2’, H-4’’’’’, H-8’’’’’), 5.45 (d, 1H, H-4’, $J_{3,4,5}$ = 2.93 Hz), 5.30 (d, 1H, NH), 5.27 (d, 1H, H-4’’’), 5.20 (m, 2H, H-3’’’, H-3’), 5.15 (dd, 1H, H-7’’’’’), 5.12 (dd, 2H, CH_2Bn), 5.00 (d, 1H, H-1’’’, $J_{1,2}$=3.5 Hz), 4.90-4.86 (m, 2H, H-5’’’, H-2’’’’), 4.86 (d, 1H, H-1, $J_{1,2}$ = 8.30 Hz), 4.83 (d, 1H, H-4’’’’), 4.82 (d, 1H, H-1’’’’, $J_{1,2}$=8.2 Hz), 4.80 (d, 1H, H-1’, $J_{1,2}$ = 3.42 Hz), 4.78 (d, 1H, CH_2Bn), 4.68-4.64 (m, 2H, CH_2Bn), 4.61 (dd,

1H, H-2’’’), 4.56 (dd, 1H, H-6’’’’’), 4.50 (d, 1H, CH_2Bn), 4.46-4.39 (m, 2H, H-6’, Hα-Thr), 4.29 (m, 2H, H-5’’’’’, Hβ-Thr), 4.23-4.14 (m, 5H, H-4’’, H-6’’, H-5’’’’, H-9’’’’’), 4.22-4.10 (m, 4H, H-4, H-6’, CH_2-Fmoc), 4.01 (dd, 1H, H-9’’’’’), 3.98 (t, 1H, H-5’), 3.95 (t, 1H, H-3’’), 3.92-3.84 (m, 3H, H-3, H-6, CH-Fmoc), 3.87 (s, 3H, $COOCH_3$), 3.87-3.80 (m, 3H, H-6’’’’, H-1’’, $J_{1,2}$=7.4 Hz), 3.77 (t, 1H, H-5’’), 3.51 (m, 1H, H-2’’), 2.60 (dd, 1H, H-3’’’’’), 2.37. 2.34, 2.30, 2.27 (4 x s, 12H, $N(COOCH_3)_2$), 2.19, 2.15, 2.15, 2.11, 2.09, 2.06, 2.05, 2.00, 1.95, 1.94, 1.93, 1.92 (12 x s, 36H, 12 x $COOCH_3$), 1.60 (dd, 1H, H-3’’’’’), 1.27 (d, 3H, CH_3),1.19 (d, 3H, H-6’’’); HR MALDI-TOF MS: m/z: calc for $C_{119}H_{140}F_2N_4O_{51}$: 2478.8453; found 2501.8350 $[M+Na]^+$.

N-(9-Fluorenylmethoxycarbonyl)-O-[2-acetamido-2-deoxy-3-O-(3,4,6-tri-O-acetyl-2-O-2,5-diflurorbenzoyl-b-D-galactorpyranosyl)-6-O-(O-methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-a-D-galacto-2-non-ulopyranosylonate)-(2->3)-O-(2,4,6-tri-O-acetyl-b-D-galactopyranosyl)-(1->4)-O-[(3,4-di-O-acetyl-2-O-benzyl-a-L-fucopyranosyl)-(1->3)]-O-[6-O-benzyl-2-deoxy-2-acetamido-β-D-glucopyranosyl]-a-galactopyranosyl]-L-threonine (1).

Compound **21** (15 mg, 6 μmol) was dissolved in a solution of (1:1) pyridine:isopropanol (4 mL). Under an argon atmosphere, Pd on Charcoal (35 mg) was added to the solution and the argon was then replaced with hydrogen. The reaction stirred for 30 min under hydrogen, then the atmosphere was replaced with argon. The solution was diluted with DCM (20 mL), filtered through celite, and the solvent was coconcentrated with toluene (2 x 20 mL). Silica gel column chromatography (15:1 DCM:MeOH) afforded compound **1** (12 mg, 83%) as a white solid. TLC (15:1 DCM:MeOH), R_f =0.27 $[\alpha]_D$ +0.24 (c 2 mg/mL, $CHCl_3$) NMR data: $CDCl_3$, ^{1}H, δ 7.77-

6.76 (m, 21H, 2 x Bn, Fmoc, dFBz), 5.63 (d, 1H, NH), 5.56-5.51 (m, 3H, H-2', H-4''''', H-8'''''), 5.45 (d, 1H, H-4', $J_{3,4,5}$ = 2.93 Hz), 5.30 (d, 1H, NH), 5.27 (d, 1H, H-4'''), 5.20 (m, 2H, H-3''', H-3'), 5.15 (dd, 1H, H-7'''''), 5.12 (dd, 2H, CH_2Bn), 5.00 (d, 1H, H-1''', $J_{1,2}$=3.5 Hz), 4.90-4.86 (m, 2H, H-5''', H-2''''), 4.86 (d, 1H, H-1, $J_{1,2}$ = 8.30 Hz), 4.83 (d, 1H, H-4''''), 4.82 (d, 1H, H-1'''', $J_{1,2}$=8.2 Hz), 4.80 (d, 1H, H-1', $J_{1,2}$ = 3.42 Hz), 4.78 (d, 1H, CH_2Bn), 4.68-4.64 (m, 2H, CH_2Bn), 4.61 (dd, 1H, H-2'''), 4.56 (dd, 1H, H-6'''''), 4.50 (d, 1H, CH_2Bn), 4.46-4.39 (m, 2H, H-6', Hα-Thr), 4.29 (m, 2H, H-5''''', Hβ-Thr), 4.23-4.14 (m, 5H, H-4'', H-6'', H-5'''', H-9'''''), 4.22-4.10 (m, 4H, H-4, H-6', CH_2-Fmoc), 4.01 (dd, 1H, H-9'''''), 3.98 (t, 1H, H-5'), 3.95 (t, 1H, H-3''), 3.92-3.84 (m, 3H, H-3, H-6, CH-Fmoc), 3.87 (s, 3H, $COOCH_3$), 3.87-3.80 (m, 3H, H-6'''', H-1'', $J_{1,2}$=7.4 Hz), 3.77 (t, 1H, H-5''), 3.51 (m, 1H, H-2''), 2.60 (dd, 1H, H-3'''''), 2.37. 2.34, 2.30, 2.27 (4 x s, 12H, $N(COOCH_3)_2$), 2.19, 2.15, 2.15, 2.11, 2.09, 2.06, 2.05, 2.00, 1.95, 1.94, 1.93, 1.92 (12 x s, 36H, 12 x $COOCH_3$), 1.60 (dd, 1H, H-3'''''), 1.27 (d, 3H, CH_3),1.19 (d, 3H, H-6'''); HR MALDI-TOF MS: m/z: calc for $C_{112}H_{134}F_2N_4O_{51}$: 2388.7983; found 2411.7881 $[M+Na]^+$.

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