In compliance with the
Canadian Privacy Legislation
some supporting forms
have been removed from
this dissertation.

While these forms may be included
in the document page count,
their removal does not represent
any loss of content from the dissertation.

SYNTHESIS OF SOME NEUTRAL AND ACIDIC ZONA PELLUCIDA OLIGOSACCHARIDES

by

John G. Riley

Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
January 2003

Dedicated to my parents John and Cheryl,
my sister Jennifer and my brother Patrick.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF SCHEMES

ABSTRACT

The glycoproteins constituting the Zona Pellucida (ZP) surrounds the mammalian oocyte and regulates critical steps in the fertilization process. This structure contains several target antigens that have potential use in the development of a contraceptive vaccine. Experimental evidence suggests that the initial interaction between sperm and oocyte is mediated by specific sperm proteins and ZP glycoproteins. This thesis describes the development of methods for the synthesis of neutral and acidic (6-*O*-sulfate) disaccharides that contain terminal β-*N*-acetylglucosamine (GlcNAc) and trisaccharides that contain terminal *N*-acetyllactosamine (LacNAc) units linked to O-3 of β-galactose (Gal) units. Efficient syntheses of these molecules with attached 5-azidopentyl linker arms have been developed and are ready for biological assays and conjugation to carrier proteins or peptides.

Several approaches to the formation of the various linkages were evaluated. In the first glycosylation approach, ethyl 6-(2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyloxy) hexanoate (**8**) was chosen as the glycosyl acceptor. In order to synthesise this molecule, a novel method for the de-*O*-benzylation of sterically hindered benzyl ethers, using light catalysed α-bromination in the presence of aqueous base, was employed. This method was further shown to be applicable to a number of substrates that bear functional groups traditionally used in carbohydrate synthesis. However, due to the unreactivity of **8**, a second glycosyl acceptor, 5-azidopentyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**48β**), was prepared. This molecule was found to condense with glycosyl donors in good yields to give disaccharide products.

In order to effect the second linkage and form an LacNAc glycosyl donor, the GlcNAc acceptor was protected with an O-6 2-naphthylmethyl ether to give phenyl 2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**74**). To form the β(1→4) linkage between Gal and GlcNAc, **74** was regioselectively galactosylated to form the LacNAc glycosyl donor phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**76**). It was found that compound **76** reacted sluggishly with acceptor **48β** giving a poor yield of the desired trisaccharide (22%), probably because of steric hindrance. Replacement of the benzoate esters of **76** by acetates gave phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**87**), which was found to react efficiently in a glycosylation reaction with **48β** to give a trisaccharide, 5-azidopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**88**) in 61% yield.

LIST OF ABBREVIATIONS AND SYMBOLS

Å	angstrom
Ac	acetyl
AgOTf	silver trifluoromethanesulfonate
All	allyl
amu	atomic mass units
APC	antigen presenting cell
Asn	asparagine
Ar	aromatic
Arg	arginine
Bn	benzyl
Bz	benzoyl
bp	boiling point
br d	broad doublet
br s	broad singlet
BSA	bovine serum albumin
Bu	butyl
c	concentration in grams per millilitre of solution
°C	degrees celsius
CAN	ammonium cerium(IV) nitrate

COSY	correlated spectroscopy
CSA	(±)-10-camphorsulfonic acid
d	doublet
DAST	diethylaminosulfur trifluoride
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	distortionless enhancement by polarization transfer
DDQ	1,2-dichloro-4,5-dicyanobenzoquinone
DMF	*N,N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribo nucleic acid
2,6-DTBMP	2,6-di-*tert*-butyl-4-methylpyridine
dt	doublet of triplets
EDG	electron donating group
EIMS	electron ionization mass spectrometry
ELISA	enzyme-linked immunosorbent assay
eq	equivalent
ESMS	electrospray mass spectrometry
Et	ethyl
EtOAc	ethyl acetate
EWG	electron withdrawing group
FABMS	fast atom bombardment mass spectrometry

Gal	D-galactopyranose
GalTase	β-(1→4)-galactosyltransferase
GalNAc	*N*-acetylgalactosamine
GlcNAc	*N*-acetylglucosamine
h	hour(s)
HETCOR	heteronuclear correlated spectroscopy
Hex	hexanes
His	histidine
HMQC	heteronuclear multiple quantum coherence
HRMS	high resolution mass spectrometry
Hz	hertz
IDCP	iodonium dicollidine perchlorate
J	coupling constant
lit.	literature
LacNAc	*N*-acetyllactosamine
Lys	lysine
m	multiplet
M	concentration unit: moles per liter
*m*CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
MHC	major histocompatability complex
MHz	megaHertz

min	minutes
mg	milligram(s)
mL	millilitre(s)
mmol	millimole(s)
mp	melting point
MPLC	medium pressure liquid chromatography
MS	mass spectroscopy
MW	molecular weight
NAP	2-naphthylmethyl
NBS	*N*-bromosuccinimide
NeuAc	*N*-acetylneuraminic acid
NeuGc	*N*-glycolylneuraminic acid
NIS	*N*-iodosuccinimide
NMR	nuclear magnetic resonance
P	protein
Ph	phenyl
Phth	phthalimido
Piv	pivaloate
ppm	parts per million
pr	propyl
pSIZP	porcine-soluble intact zona pellucida
q	quartet

R	rabbit
RCV	rabbit calcivirus
RHD	rabbit hemorrhagic disease
RHVD	rabbit hemorrhagic disease virus
R_f	fractional migration distance of a substance on TLC
s	singlet
Ser	serine
sp	sperm protein
t	triplet
TBDMS	*tert*-butyldimethylsilyl
TBDMSCl	*tert*-butyldimethylsilyl chloride
TBDPS	*tert*-butyldiphenylsilyl
TfOH	trifluoromethanesulfonic acid
THF	tetrahydrofuran
Thr	threonine
TLC	thin layer chromatography
TMS	tetramethylsilane
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Tol	toluene
Troc	2,2,2-trichloroethoxycarbonyl
δ	chemical shift in parts per million downfield from TMS
$[\alpha]_D$	specific rotation measured at temperature t 25 °C using the 589 nanometer

	sodium D line
ZBP	zona binding proteins
hZP	human zona pellucida
mZP	mouse (murine) zona pellucida
pZP	pig (porcine) zona pellucida
ZP	zona pellucida

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. T. B. Grindley for his help during the course of this study, Dr. R. G. Brown for his discussions about the biological aspects of this project and Dr. R. Carr and Dr. T. Lee for their discussions about the immunological aspects of this project. I also would like to thank Drs. J. A. Pincock and R. L. White for their discussions related to this thesis.

Thanks are extended to the Atlantic Region Magnetic Centre for NMR facilities, to Dr. M. D. Lumsden for running some 400 MHz NMR spectra, and to the Institute of Marine Biosciences (NRC) for access to their polarimeter. Thanks are also due to Dalhousie University for the graduate scholarship, NSERC, and to the Walter C. Sumner Foundation for financial support. I would also like to thank Dr. Kim and Dr. Micheal Potvin for obtaining some of the mass spectra provided in the thesis.

I would like to thank Hong Liang and Hussein Al-Mughaid for their help and friendship. Thanks are also extended to the current and former players of Forrest Hills 'Heartn Soul' Soccer Club, and especially my 'Uncle Coach' Pat Riley.

I would like to take this opportunity to deeply thank my family. My father John, mother Cheryl, sister Jennifer, brother Patrick and his wife Tena, my niece Talia, nephew Garrison, Grandmothers: Gladys Riley, and Arneita Meahan, and Grandfathers: Leo Riley and Frank Meahan. I would like to thank them for their continued love, compassion, help and support. Finally, I would like to thank God, through whom all things are possible.

1. Introduction and Background

Certain animal populations are considered to be out of control. When populations increase out of control, there is a need to find a safe, affordable, effective, and reversible method of influencing these changes. If these animal populations were managed, it would be possible to minimize environmental damage and prevent the spread of disease. However, the development of fertility control for wildlife populations is a process that must consider ethical, as well as scientific factors. Given the enormous impact of growing animal populations, contraception via vaccination is an attractive method.[1-5] An ideal vaccine would be: safe, effective, affordable, heat stable, long lasting, easy to administer, capable of inducing both humoral and cell-mediated immune responses, and multivalent.[2]

The problems associated with the overexpansion of animal populations is of concern to a number of nations including Canada. Rabbits (*Oryctolagus cuniculus*) in Australia,[6] wild horses (*Equas caballus*) in Nevada,[7] white-tailed deer (*Odocoileus virginianus*) in New York,[8] grey squirrels (*Sciurus carolinensis*) in Britain,[9] elephants (*Loxodonta africana*) in Africa,[10] and grey seals (*Halichoerus grypus*) in Atlantic Canada[11] are all examples of species considered to have excessive populations.

Expansions of the populations of a number of mammalian species in Australia are causing problems. These include rabbits (*Oryctolagus cuniculus*), which were imported by European settlers for the purpose of hunting. Rabbits are responsible for millions of dollars of damage annually in Australia to the environment and to the agriculture industry.[6,12,13] One method which has recently been employed for the control of this species is the rabbit

calcivirus (RCV),[6] also called the rabbit hemorrhagic disease virus (RHVD), which results in a fatal disease called rabbit hemorrhagic disease (RHD).[14] The disease causes blood clots in their lungs, hearts and kidneys.[6] Other Australian feral animals which cause significant economic and ecological damage include: pigs (*Sus scrofa*),[15] water buffalo (*Babalus bubalis*),[16] and donkeys (*Equus asinus*).[17] There is also a problem with large populations of goats *(Capra hircus)* in southern Australia.[18] One current method of population control under study involves shooting feral goats from a helicopter with or without the aid of ground spotters.[19] The study noted a 59% culling success when a spotter was used as opposed to 40% without a spotter. Overall, the average cost per goat was $61. This study further demonstrates the need for a more humane and cost effective method for controlling feral goat populations.

In Atlantic Canada, several populations of grey seals (*Halichoerus grypus*) have been identified as contributors to industrial, ecological and environmental problems.[11] Populations of Northeast Atlantic grey seals are geographically dispersed and are expanding rapidly. The population of seals that reproduces on Sable Island, Nova Scotia, Canada is growing by a rate of 12% per year meaning that existing populations are projected to double every seven years.[20] Similarly, grey seal populations that reproduce on ice packs in the Gulf of St. Lawrence are increasing by approximately 7.4% per year (for a map of these areas, see Figure 1). These populations are projected to double every ten years.[11] This situation has raised concerns that seals may have an impact on commercial fisheries through damage to fishing gear, transmission of parasites, and competition with fishers.[11]

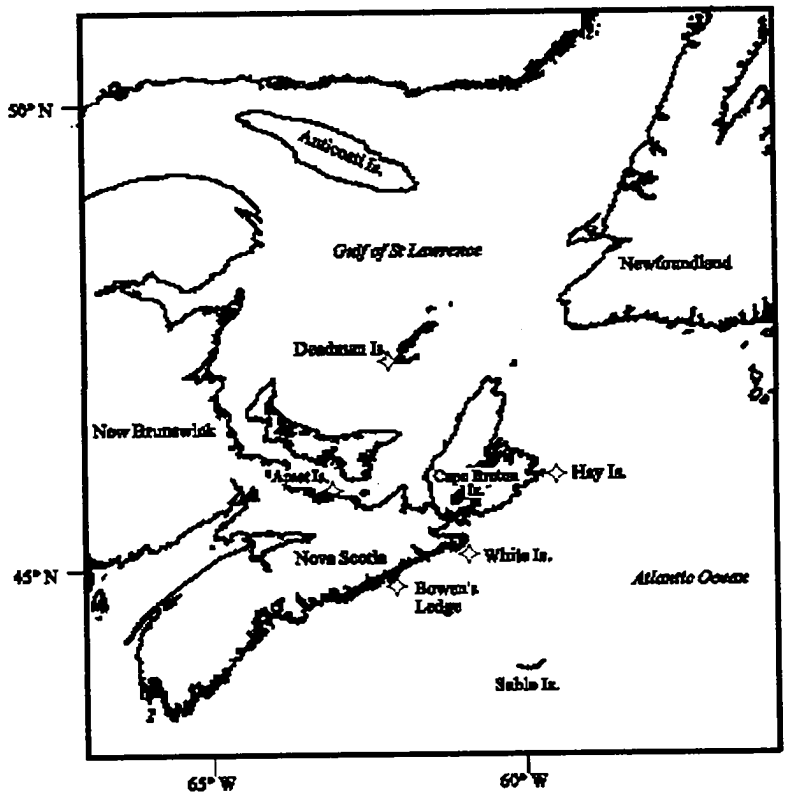


Figure 1 A map of the Atlantic region showing where grey seal populations reside[11]

Groundfish and grey seals are also in competition for smaller species such as capelin, which compounds the problem by reducing available food supplies. Sealworms spend part of their life cycle in seals and part in fish. Increases in the size of grey seal populations has coincided with an increase in sealworm levels in fish, which is also a problem of concern.[21] Therefore, there is a major need to devise a method to obtain a healthy seal population and an immunocontraceptive approach has significant advantages.

The use of pig zona pellucida for immunocontraception in certain populations of wildlife has potential because it has over 90% effectiveness for several species, the ability

for remote delivery by means of darts, and the lack of weakening side effects. It has been established that a single administration birth control vaccine based on liposome-encapsulated, porcine-soluble intact zona pellucida (SIZP), an extracellular matrix comprising the outer layer of an ovulated egg,[22-24] reduces the fertility of female grey seals by about 90% over four years.[25-27] A second approach has been to clone pig zona pellucida genes[28] and use the recombinant proteins produced using these genes without attached carbohydrates.[29]

1.1 General Concepts

Carbohydrates are polyhydroxy aldehydes, ketones, alcohols, or acids.[30] Monosaccharides are carbohydrate monomers which cannot be converted into smaller units by hydrolysis. Compounds containing two monosaccharides are termed disaccharides and those containing three to ten monosaccharides are called oligosaccharides. Polysaccharide is a term used to describe carbohydrates having more than ten monosaccharides. Carbohydrates can be linked to proteins to give glycoproteins, and similarly linked to lipids to give glycolipids. Such carbohydrate derivatives exist in all living species from viruses to mammals. In many cases, carbohydrates found on the surfaces of cells can act as receptors in a number of activities such as adhesion to other cells and surfaces, cell to cell recognition, and also may act as biochemical labels in molecular recognition, as exemplified by the human blood group antigens[31] and gamete interactions during mammalian fertilization.[32,33]

The following standard abbreviations will be used in this introduction: D-galactose

(Gal), D-glucose (Glc), 2-acetamino-2-deoxy-D-glucose (GlcNAc). All monosaccharides are in their pyranose forms. Galβ(1→3) indicates β-D-galactopyranose linked to O-3 of another monosaccharide.

Antigens are substances, usually biological in nature, such as proteins, glycoproteins, or polysaccharides, which are recognized by the vertebrate immune system as foreign to the body's normal structural make-up.[34] An antibody is a large multi-subunit protein complex that is produced by the B lymphocytes of the immune system and has specific sites for the binding of antigen.[34] Each complex is made up of two heavy chains and two light chains. Each chain contains constant regions (C) which are responsible for biological activity, and variable regions (V), which are responsible for antigen binding. Antigen-antibody interactions are the fundamental basis by which the immune system functions. These interactions are mediated by contacts between the foreign portions of an antigenic molecule, termed epitopes,[34] and the variable region of the antigen specific antibody.

Vaccines are substrates that can be related to substrates causing infections. A succesful anti-carbohydrate vaccine can be developed by conjugating target oligosaccharides to a carrier protein. Upon introduction of the synthetic materials (an antigen) to the immune system, several important cellular responses are initiated. The antigen is non-specifically phagocytosed (that is, brought into the cell) by antigen presenting cells (APC's), such as dendritic cells and macrophages.[35] After phagocytosis, the antigen is digested by lytic enzymes in low pH endosomes.[35] The resulting short peptide sequences are presented on the surface of the cell complexed with proteins called major histocompatability complexes (MHC's).[35] It is important to note that only the peptide portions of the conjugate vaccine are

presented by APC's. The peptide-MHC complex is then specifically recognized by naive T-cells which bear a complementary T-cell receptor.[36] This recognition results in the clonal expansion of the T-cell to form a population of activated T-cells capable of recognizing the peptide-MHC complex.

A second cellular response is the binding of B-cells to the antigen specifically between membrane-bound antibodies on the surface of the B-cell and the oligosaccharide component of the synthetic vaccine. This binding is specific for both the carbohydrate and peptide portions of the antigen. Upon binding, the antigen is phagocytosed, degraded and the peptides presented on the surface of the cell complexed with MHC's.[37] This complex is then recognized by activated T-cells. The recognition results in the clonal expansion of this B-cell which creates a population of plasma cells which secrete a soluble form of the membrane bound anti-carbohydrate antibody.[37]

Fertilization is the sum of the cellular mechanisms that pass the genome from one generation to the next and initiate the development of a new organism.[38] In mammalian species this is accomplished through the union of two haploid cells, the egg and the sperm, producing a new, unique, diploid cell, the zygote, which grows into a new individual. The egg is a large cell, that is enclosed by a layer of protein and carbohydrate called the zona pellucida.[38] It is generally believed that the sperm, a small cell with a head containing a sac of enzymes (the acrosome), binds to a class of *O*-linked oligosaccharides on the zona pellucida (however, see later).[39] This initial interaction results in the release of the enzymes contained within the acrosome (the acrosome reaction).[40,41] Following the acrosome reaction, the sperm further deploys enzymes, which remove the binding sites for other sperm

facilitating a block to polyspermic binding.[42] Then, with the aid of secreted enzymes, the sperm penetrates the ZP[43] and, having done so, binds to and fuses with the egg plasma membrane. These steps complete the cellular mechanisms that combine the haploid genome of the penetrating sperm with that of the egg, thus initiating the development of a new mammalian organism.

1.2 Potential candidates for immunocontraception

The function of a contraceptive vaccine is to induce circulating antibodies or cellular immune effector cells that can interrupt reproductive events without side effects.[2] Interference with the fertilization process through the use of the immune system is an attractive method to perform contraception and the zona pellucida, a female specific structure, represents a potentially ideal target.[1-3,23,44-48] The zona pellucida is an extracellular matrix of proteins that surrounds growing oocytes, ovulated eggs, and early embryos.[23,24,49] The interaction of the mammalian spermatozoa with the oocytes ZP is a critical first step leading to successful fertilization. This includes species-specific sperm recognition, induction of the acrosomal reaction, prevention of polyspermy and physical protection of the embryo during pre-implantation development. The ZP of different mammalian species are known to vary according to their morphological, physicochemical, immunochemical, and biochemical properties. Recent advances in the biosynthesis and molecular biology of the ZP have provided much information useful in the rational design of an immunocontraceptive vaccine. However, there remain major obstacles to using immunological reagents to prevent fertilization, including potential side effects, the lack of an adequate delivery system, the

need for multiple administrations, and the possibility of complete reversibility. A potential difficulty of immunocontraception is that a population of animals could be selected that had weaker immune systems. Those which respond well to immunocontraceptive methods will be unable to reproduce, thus those which respond poorly (due to weaker immune function) will retain their ability to reproduce, hence the overall population may become more susceptible to disease.[50]

The use of synthetic zona peptides, glycopeptides or carbohydrates as target immunogens provides a promising approach to safe and pure anti-fertility vaccines. The vaccine may prevent fertilization by interfering with the transport of spermatozoa or the sperm-zona binding.[2] Such a vaccine must evoke an immune response that blocks an essential step in the reproductive process. It works by developing antibodies against the synthetic materials presented to the immune system, which in turn act to immobilize, agglutinate, or coat either the sperm or the egg, thus preventing fertilization.

Immunocontraceptive antigens can either be used from the antigens of the ZP, sperm, or acrosome. They can be delivered by a variety of disseminating agents: bacteria,[51,52] viruses,[53] plants,[54] or by non-disseminating agents: direct injection,[25] oral bait,[55,56] biobullets,[57] or jab stick.[25]

1.3 Zona pellucida glycoproteins

The ZP of mammals is comprised of three major, charged glycoproteins characterized by size and charge heterogeneity: ZPA (largest), ZPB (intermediate) and ZPC (smallest), occurring in a ratio of 1:3:3 respectively.[28] It has been proposed that this system of

nomenclature (A,B,C) replace previous methods, which were based on numbers and/or Greek letters that varied from species to species. However this method has not been generally adopted. The A, B, C, method is based on the size of the gene encoding each glycoprotein, with ZPA having the largest DNA sequence. The ZP glycoproteins are glycosylated by both *N*- and *O*- linked oligosaccharides,[58] which differ in their mode of attachment. The *O*-linked carbohydrate chains are built onto the amino acids serine and threonine[22] sequentially, each monosaccharide addition being performed by a separate distinct enzyme.[59] Conversely, *N*-linked carbohydrates are added to asparagine[60] residues as a large oligosaccharide, which is later modified in several steps by enzymes located in the endoplasmic reticulum and golgi apparatus.[61]

In the sperm-zona interaction, it has been suggested that carbohydrate determinants of the ZP glycoproteins are involved in the sperm-egg interaction and thus serve as sperm receptors.[39,42,58,62-72] A variety of carbohydrates have been suggested to mediate fertilization in mouse, rat, pig, human, hamster, and rabbit. In the case of sperm, several candidate ZP-binding sperm proteins have been identified and can be grouped as primary or secondary receptors. Primary receptors are responsible for the initial interaction with the ZP and the initiation of the acrosome reaction; secondary receptors cause the sperm to adhere to the ZP following the acrosome reaction and during transit of the sperm through the ZP.

1.4 Mammalian zona and sperm antigens

1.4.1 Mouse zona and sperm antigens

In the mouse, the three major sulfated glycoproteins are as follows: ZPA (mZP2), ZPB (mZP1) and ZPC (mZP3),[28,49,73] where m indicates that material from the mouse is being described. MZP3 (44,000 MW) mediates the initial binding of sperm to the ZP and induces the acrosome reaction,[22] while mZP2 (120,000 MW) serves as a secondary sperm receptor[58,74] and likely mediates the adhesion of the ZP to the inner acrosomal membrane of acrosomal reacted sperm.[75] The fact that antibodies against the major zona glycoprotein, ZP3, block both the primary and secondary phases of sperm-zona binding suggests that this molecule might have potential use as a contraceptive vaccine.[44] MZP2 and mZP3 are present as hetero dimers, organized as filaments and inter-connected by mZP1 (200,000 MW).[22] MZP1 is a dimeric complex of two identical polypeptides, cross-linked by disulfide bonds.[22]

Early experimental reports indicate that *O*-linked oligosaccharides on mouse ZP3 are responsible for the initial interaction between sperm and egg.[39,76] This evidence was further substantiated through site-directed mutagenesis of the ZP3 protein.[77] These experiments demonstrated that serine-332 and serine-334 of the mZP3 amino acid sequence are essential for sperm binding. Mutagenesis at these sites results in total loss of sperm-ZP3 binding. A report made by Harris et al.[28] in 1995 also shows that these residues have been conserved in a number of species. This finding may indicate the evolutionary importance of these residues as mutations in this region would undoubtedly result in the production of eggs that

are incapable of binding sperm. Such animals would be negatively selected by evolution, based on their inability to reproduce.

The experimental reports, with respect to the oligosaccharide sequences attached to mZP3 that mediate sperm adhesion, are the source of considerable controversy. An early report by Bleil and Wasserman concluded that α-linked (α-Gal) at the non-reducing end of *O*-linked oligosaccharides of mZP3, is essential for binding of sperm to the ZP.[62] This was based on the observation that treatment of ZP3-derived *O*-linked oligosaccharides with α-galactosidase or galactose oxidase resulted in loss of sperm receptor activity. However, studies of knockout mice, which lack the enzyme α(1→3) galactosyl transferase and thus cannot have terminal Galα(1→3) units, retain their fertility and produce eggs that bind normal numbers of sperm.[78] Recent evidence reported by Avilés et al. demonstrates that α-Gal residues are differentially expressed during zona maturation.[72] These researchers state that α-Gal is present only during the later stages of zona secretion and, consequently, are confined to the inner portions of the mature zona pellucida.[79] Thus, due to location, α-Gal may be involved in interactions that occur after the initial fertilization step.

In 1992, Miller et al. reported that mouse sperm β-1,4-galactosyltransferase (Gal-transferase) specifically recognizes those oligosaccharides on ZP3 that have sperm-binding activity, but does not interact with other zona pellucida glycoproteins.[80] The Gal-transferase enzyme specifically recognizes oligosaccharide sequences with terminal *N*-acetylglucosamine residues.[80] Another report by Johnston *et al.* suggest that high affinity sperm binding requires two independent oligosaccharide binding sites. By testing a number of synthetic oligosaccharides it was found that the first (high affinity) site binds both an α3-

galactosyl-capped trisaccharide, Galα(1→3)Galβ(1→4)GlcNAc, and two fucosylated tetrasaccharides, Galβ(1→4)[Fucα(1→3)]GlcNAcβ(1→4)GlcNAc and Galα(1→3)Galβ(1→4)[Fucα(1→3)] GlcNAc. The second (low affinity site), binds a non-fucosylated β-galactosylated trisaccharide Galβ(1→4)GlcNAcβ(1→4)GlcNAc.[81] The part of this report suggesting the importance of fucose is contradicted by Avilés and co-workers who have observed that fucosyl residues linked to Lewis X glycosides are not detectable at any stage during zona pellucida development.[72] Johnston et al. also showed that sperm do not bind to a GlcNAcβ(1→4)GlcNAcβ(1→4)GlcNAc trisaccharide, indicating that the binding site specificity extends beyond the terminal GlcNAc residues, at least to the extent of the glycosyl linkage.

mZP3 oligosaccharides have been shown to be sialylated and sulfated, and the polypeptide unit (44,000 MW, 402 amino acids) is highly glycosylated. However, Wasserman et al. determined that these three factors (sialylation, sulfation, and the extent of glycosylation) are not directly involved in binding of sperm to mZP3 oligosaccharides.[76] These findings contrast with those reported for certain other glycoproteins, namely pro-acrosin, involved in cellular adhesion that require sulfate[82,83] and/or sialic acid[84] for bioactivity. A recent report by Howes and Jones indicates that poly-sulfated residues on mZP2 may be involved in secondary binding and hence may not contribute to the binding of mZP3 to sperm proteins.[74] The results of further experiments demonstrate that oligosaccharide constructs, terminating with *N*-acetyllactosamine (Galβ(1→4)GlcNAc) sequences, inhibited primary murine sperm-egg binding.[63] These units lack both sulfate and sialic acid, but carry terminal α or β galactose at their non-reducing end. Another report

indicated that the major *O*-linked oligosaccharide associated with murine ZP3 was a trisaccharide GlcNAc-Galβ(1→3)GalNAc but also showed the presence of *N*-linked poly-*N*-acetyllactosaminyl glycans on mZP2 and mZP3.[73] Furthermore, digestion of mZP3 oligosaccharides with endo-β-galactosidase released fragments containing GlcNAcβ(1→3)Gal and Galβ(1→4)GlcNAcβ(1→4)Gal as the major components.[85] Clark and co-workers,[58] found that a number of oligosaccharide structures were associated with mZP. These included Galβ(1→4)GlcNAcβ(1→6)[Gal β(1→3)]GalNAc structures, poly-*N*-acetyllactosamine repeats, and a variety of other structures.

Sp56, a 56,000 MW sperm protein located in the sperm plasma membrane, is a primary egg binding protein[22,66] that recognizes and binds to certain mZP *O*-linked oligosaccharides,[66] particularly to those with terminal galactose residues.[63] Recently, Cohen and Wassarman[86] have provided evidence that membrane vesicles prepared from acrosome-intact sperm contain sp56. These vesicles were found to inhibit binding of sperm to eggs in vitro. Furthermore, a monoclonal antibody directed against sp56 was found to relieve this inhibition. Finally, the results of immunoprecipitation studies using sperm extracts incubated with mZP3, and either a polyclonal antibody directed against mZP3 or a monoclonal antibody directed against sp56, suggest that mZP3 is specifically associated with sp56.[86]

Another sperm-head, plasma-membrane protein, β-(1→4)-galactosyltransferase (GalTase), binds to terminal *N*-acetylglucosamine residues (GlcNAc) and may play a vital role in their initial binding to the ZP.[63] However, sperm which lack the enzyme, display a 3 to 4 fold higher binding ability compared to normal sperm,[87] but do not undergo the

acrosome reaction as effeciently, *in vitro*, when exposed to mZP3. Mice lacking this enzyme are still fertile but are much less efficient (7%) than wild type mice in fertilizing eggs.[87] This suggests GalTase may play a more significant role in signal transduction invoking the acrosome reaction.[88] This is supported by recent evidence directly demonstrating that GalTase functions as a mZP3 receptor in a lectin-like capacity by binding to terminal *N*-acetylglucosamine residues on mZP3 oligosaccharides.[42] Shur and coworkers suggest that multivalent oligosaccharides on mZP3, as well as synthetic polymers terminating in *N*-acetylglucosamine aggregate GalTase, leading to activation of a heterotrimeric G protein cascade[43] that culminates in the acrosome reaction. Following fertilization, cortical granules release *N*-acetylglucosaminidase, which removes the binding site for sperm GalTase and facilitates the zona block to polyspermic binding.[42] These researchers showed that the overexpression of GalTase on sperm led to increased binding of ZP3, increased G protein activation and elevated acrosome reactions. Furthermore, sperm from mice made null for GalTase by homologous recombination are unable to bind soluble ZP3 and fail to undergo the acrosome reaction in response to zona glycoproteins. In contrast, GalTase null sperm were still capable of binding to the zona and achieved low rates of fertilization in vitro. This final result suggests that sperm-egg binding involves receptor-ligand interactions independent of GalT and ZP3, providing support for the existence of secondary binding receptors.

1.4.2 Pig zona and sperm antigens

The pig zona pellucida (pZP) is constructed of the following three glycoproteins:

ZPA (pZP1), ZPB (pZp3α), and ZPC (pZP3β).[28,89] PZP1 may be cleaved into two components, pZP2 and pZP4 by reduction of their disulfide bond linkage,[90] and is homologous to mZP2. pZp3α is the most biologically active component of the pig ZP.[90,91] As in the mouse, pZP glycoproteins contain both *N*-linked and *O*-linked oligosaccharides.

The *N*-linked carbohydrate chains are composed of neutral (28%) and acidic (72%) oligosaccharides[92] and also contain sulphated poly-lactosamine repeats.[67,93] Mori and co-workers demonstrated the existence of acidic *N*-glycans and that these glycans contained mono- to tetraantennary complex-type with and without *N*-acetyllactosamine repeating units. Sulfated residues were also located at the C-6 position of GlcNAc included in the *N*-acetyllactosamine repeating units, but also at the C-6 position of GlcNAc in the non-repeated antennae and at the C-3 position of reducing terminal GlcNAc residue.[94] Most recently, Dunbar et al. determined that lactosaminoglycans are associated with all three pZP glycoproteins.[33] Finally, Takasaki et al.[85] recently reported that approximately one fourth of the neutral oligosaccharides of pZP proteins contain linear *N*-acetyllactosamine repeats and that an additional 39% of neutral sequences terminated in non-reducing GlcNAc residues. This group also showed that acidic structures are sulphated primarily on C-6 of GlcNAc.

It has been suggested that the sperm-egg interaction is mainly mediated by neutral *N*-linked carbohydrate chains localized in the *N*-terminal region of the pZP3α protein.[93] Nakano and co-workers found that neutral, and not anionic *N*-linked glycans, inhibited sperm binding.[68] Further studies showed that the removal of *N*-linked, but not the *O*-linked, carbohydrates led to the loss in inhibitory effect.[80] This suggests that *N*-linked oligosaccharides are responsible for sperm-egg binding activity.[93,95] A recent study by

Nakano and Yonezawa has determined the location and structural make-up of *N*-linked sperm-ligand carbohydrate chains.[96] These researchers have shown that tri- and teta-antennary structures are located on Asn-220 of the pZPB protein, while di-antennary structures are located on Asn203, Asn220 and Asn 333. Conversely, in pZPC, triantennary and tetraantennary chains are found mainly at Asn271, whereas diantennary chains are present at all three N-glycosylation sites Asn124, Asn146 and Asn271.

The study of pig *O*-glycoslated residues showed that the major oligosaccharides have Gal β(1→4)-GlcNAc β(1→3) as the major terminal structures which in most cases, has a sulfate group at C-6 of the GlcNAc unit.[97] The primary structures of 32 *O*-linked oligosaccharides were assigned by NMR spectrometry and found to contain a complex combination of structural elements. Spijker *et al.* synthesized a hexasaccharide unit, [Galβ(1→4)-GlcNAc[6OSO_3^-]β-(1→3)-Galβ(1→4)-GlcNAcβ-(1→3)Galβ(1→3)GalNAcα-$O(CH_2)_3NH_2$] terminating in β–Gal, which was the smallest structure of those synthesized to inhibit porcine sperm binding.[98]

Proacrosin, the zymogen form of the serine protease acrosin,[99] is a protein that is localized within the acrosome of mammalian spermatozoa.[83] This protein has been shown to contain a series of basic amino acids, which make up a tertiary structure responsible for the non-enzymic binding of proacrosin to poly-sulfated oligosaccharide residues on porcine ZP2.[83,99-101] More specifically, two groups of basic amino acids (His47, Arg50, Arg51) and (Arg250, Lys252, Arg253) are involved in this interaction.[99] In this model, the initial binding of sperm to the ZP is mediated by pZP3 oligosaccharides and other sperm proteins. This initial binding induces the acrosome reaction, releasing proacrosin from the acrosome.

It is believed that proacrosin then mediates secondary sperm binding by complementary ionic interactions between basic amino acids on proacrosin and polysulfated oligosaccharides on pZP2.[102,103]

Very recently, it has been shown that aryl sulfatase A on the surface of porcine sperm binds to pZP3α.[104] It appears that this enzyme binds to the carbohydrate sulfates but does not cleave them.[105]

1.5 Other methods for Immunocontraception based on zona pellucida proteins

a. Vaccines using recombinant zona pellucida proteins ZPA,[29] ZP2,[51,106] and ZP3[51] have been investigated by several research groups. For example, Srivastava et al. used *E. coli* expressed recombinant dog ZP2 (dZP2) for immunization studies on female dogs. Three of four dogs immunized with the recombinant protein failed to conceive when mated. This apparent reduction in fertility was associated with elevated anti-dZP2 antibodies that were believed to be responsible for inhibiting the development of follicles resulting in anti-fertility.[51]

b. Short synthetic peptide sequences derived from ZP1,[52,107,108] and ZP3[107,109,110] have received considerable attention recently as potential candidates for contraceptive vaccines. For example, Paterson and co-workers induced active immunity to porcine ZP3 in a non-human primate, the marmoset (*Callithrix jacchus*). After observing the appearance of an associated ovarian pathology in response to ZP3,[111] these researchers analyzed potential B-cell epitopes on ZP3 to develop a successful peptide vaccine that would circumvent ovarian failure while maintaining antifertility properties.[110] Several candidate regions of ZP3 were

identified, synthesised, and incorporated into chimeric peptide vaccines. The results of these experiments show the production of antibodies capable of reducing sperm-egg binding by 60%, with no adverse side effects on ovarian function. In addition, Hasegawa et al.[108] immunized rabbits with a linear sequence of 18 amino acids from ZPA conjugated to a diptheria toxoid carrier protein. Sera produced against human and rabbit peptides conjugates were found to inhibit human sperm-egg binding, while sera against pig peptide did not.

c. DNA vaccines encoding zona pellucida proteins have also received recent attention.[112] Gupta and co-workers developed a plasmid DNA construct encoding bonnet monkey (*Macaca radiata*), ZPB (bmZPB). The cDNA corresponding to bmZPB was cloned into a mammalian expression vector and used to immunize male mice. Mice immunized with plasmid DNA were found to have responded by producing significant levels of antibodies that recognize *E. coli* expressed recombinant bmZPB. These antibodies were also found to recognize bonnet monkey as well as human ZP. The sera obtained from these immunized mice was also found to be capable of inhibiting the binding of sperm to the ZP.[112]

d. Anti-idiotypic antibodies have also been investigated as a potential anti-fertility vaccine candidate. Li et al. recently immunized rabbits with polyclonal antibodies generated against porcine zona pellucida.[113] The resulting rabbit anti-idiotypic antibodies were purified and used to immunize female mice. Mice immunized with anti-idiotypic antibodies were found to have a decreased pregnancy rate and a statistically significant reduction in fetal numbers. Histological analysis of ovaries demonstrated that exposure to anti-idiotypic antibodies interfered less with follicular development that did exposure to porcine ZP.[113]

1.6 Strategies for designing an immunocontraceptive vaccine based on synthetic glycoproteins

Developing a birth control vaccine has recently received much research attention and several approaches provide the promise of a potentially elegant and safe approach to contraception. The mouse ZP glycoproteins have been the most intensively investigated in the literature, but the carbohydrate chain structures have yet to be identified with certainty, mainly due to the limited availability of materials. Those of seals have not been explored as of yet. Most studies concerning structural determination of ZP oligosaccharides have been done using pZP because of the availability of larger amounts of pig ovaries from slaughterhouses than the ZP of other mammals.[92] However, oligosaccharides that are hydrolysed from ZP glycoproteins are obtained in heterogeneous mixtures that are difficult to purify and the extraction procedure is not feasible for larger scale application. Hence, the potential for their application in contraception is limited.

As mentioned above, a proposed and promising approach for a contraceptive vaccine is to synthesize glycoproteins. The close genetic relationship between ZP proteins of various mammalian species,[28] including those of mice and pig, has led us to target core oligosaccharides containing a repeating *N*-acetyllactosamine sequence (-Galβ(1→4)GlcNAcβ(1→3)-) as part of an ongoing project to test against anti-seal ZP antibodies that are available. Acidic oligosaccharides are those that contain sulfate at C-6 or a sialic acid residue. Oligosaccharides without these groups are termed neutral oligosaccharides. Due to the presence of the acidic oligosaccharides in a significant proportion of mammalian ZP glycoproteins, the aim of this project is to synthesize both

neutral and acidic (sulfate at C-6) glycoconjugates containing terminal β-*N*-acetylglucosamine linked to C-3 of a β-galactosyl residue. Each glycoconjugate will also be extendable from the reducing end via glycosylation with linker arms that can be further attached to available carrier proteins or synthetic peptide. As part of the attempt to design biologically active glycoconjugates, this synthetic effort may result in an effective immunocontraceptive vaccine.

1.7 Chemosynthetic method to develop a contraceptive vaccine

Methods for the synthesis of oligosaccharides have been widely studied. Their synthesis involves a suitable glycosyl leaving group for glycosylation, called the glycosyl donor, and a suitable nucleophile, called the glycosyl acceptor. Common nucleophiles bear hydroxyl, nitrogen and sulfur groups. A variety of glycosyl donors and methods for their activation is found in the literature.[114-121] These include glycosyl halides,[122] thioglycosides,[123] trichloroacetimidates,[124] glycals,[125-127] sulfoxides,[128] phosphates,[129-131] selenoglycosides[132] and 4-pentenyl glycosides.[133]

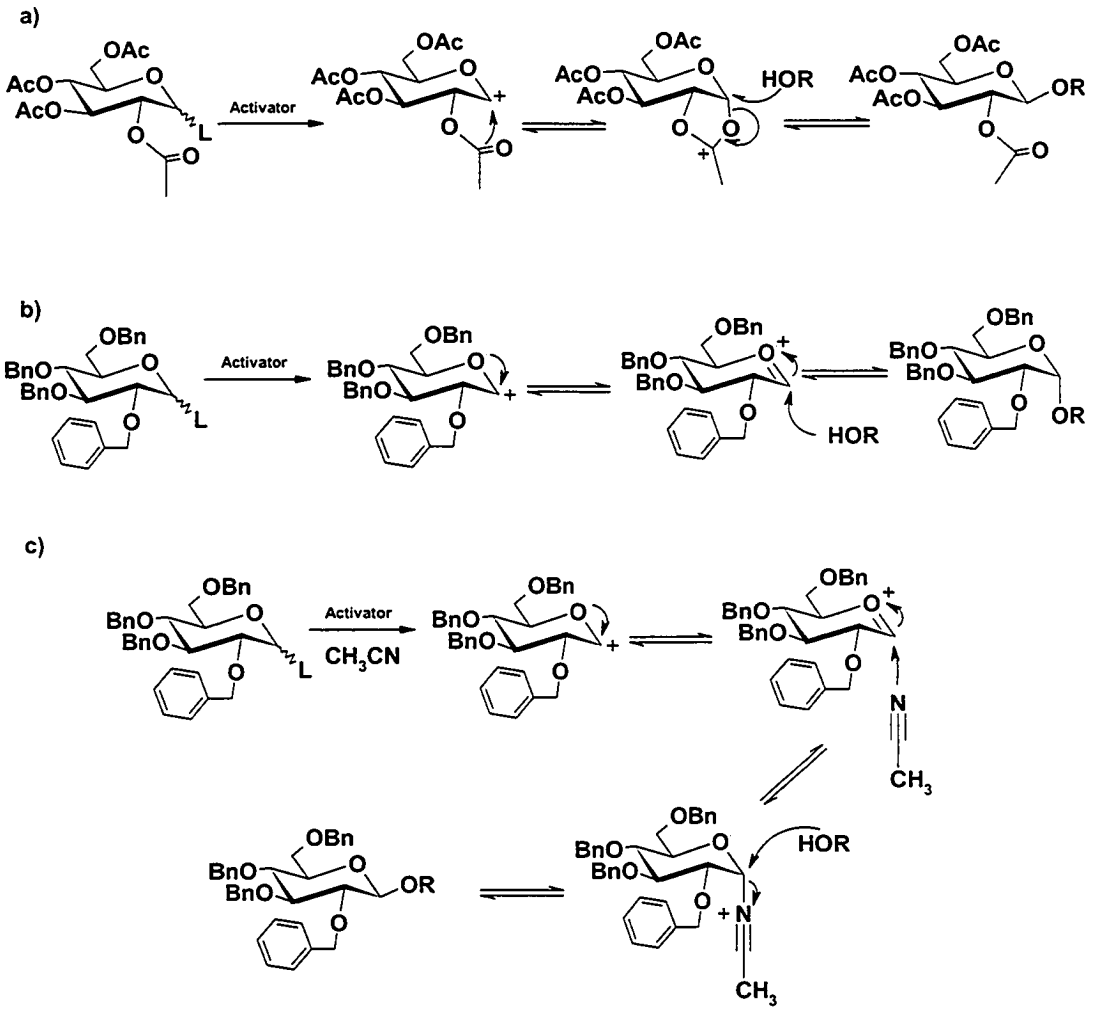
Glycosylation reactions are often done in the presence of an activator whose role is to aid in the departure of the leaving group. Formation of the oxocarbenium ion can lead to either α or β selectivity, depending on which type of protecting group is present. If a participating group, such as an ester, is present on O-2 of the glycosyl donor, 1,2-*trans* glycosylation is achieved preferentially (Scheme 1.7.1a). If non-participating groups are present, such as an ether, an α-linkage is favored (Scheme 1.7.1b). This latter preference arises from a phenomenon known as the anomeric effect and occurs at carbons which have

geminal electronegative groups, such as C-1 of an aldopyranose ring.[134] A third method of glycosylation involves solvent participation to effect the formation of β-linkages when non-participating groups reside on O-2 of the glycosyl donor (Scheme 1.7.1c). One such method involves performing glycosylation reactions in acetonitrile.[118] In the first step, activation of the glycosyl leaving group generates the oxocarbenium ion, which then reacts with the lone pair on the nitrogen of acetonitrile. Due to the anomeric effect, an α-nitrilium ion is preferentially formed, a phenomenon known as the nitrile effect. In the second step the α-nitrilium ion intermediate reacts with a suitable glycosyl acceptor at the β-face, generating the desired β-linkage.

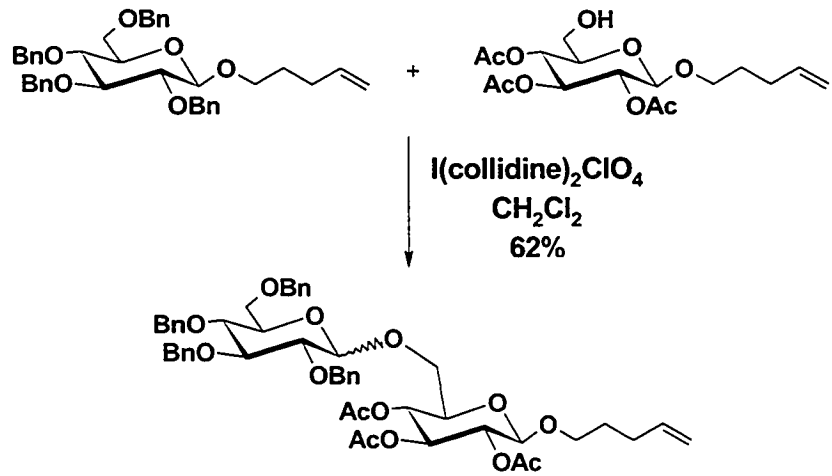
Another problem, which generally arises in the synthesis of carbohydrates, is that pertaining to regioselective glycosylation. Carbohydrates have several positions at which glycosylation can occur. Hence, in order to achieve the correct connectivity, protecting groups must be used to block groups which would otherwise react. This leaves only the desired hydroxyl available for glycosylation. Common hydroxyl protecting groups employed in the synthesis of carbohydrates include acetals (benzylidene acetals,[135] isopropylidene acetals[136]), alkyl ethers (allyl,[137] benzyl,[138] p-methoxybenzyl,[139] 2-naphthylmethyl[140]), silyl ethers (*tert*-butyldimethylsilyl ethers,[141] *tert*-butyldiphenylsilyl ethers[142]) or esters (acetates,

benzoates,[143] pivalates[144]). The most common amino protecting group is the imide (phthalimides,[145] or tetrachlorophthalimides[146]). In some cases, however, the 2,2,2-trichloroethoxycarbonyl[147] (N-Troc) has been employed. An amino group can also be introduced via the azido group (N_3^-),[148,149] a non-particpating group, which can be later

transformed into an amino functional group by reduction.



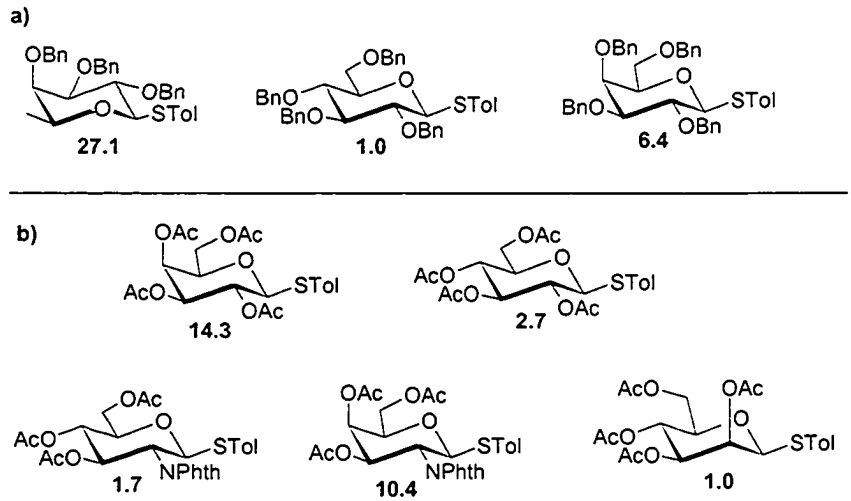
Scheme 1.7.1 **Stereoselective glycosylation methods**



Scheme 1.7.2 Chemoselective synthesis of a disaccharide using the armed-disarmed glycosylation strategy.[140]

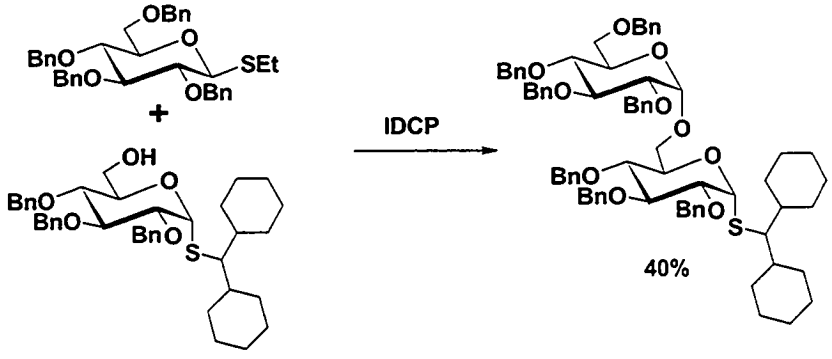
Another topic that has received considerable attention of late is that pertaining to armed and disarmed glycosyl donors.[150-153] This concept was introduced in 1988, by Fraser-Reid and coworkers who studied the phenomenon using *O*-pentenyl glycosides. This group stated that the pentenyl group can be "armed" or "disarmed" by the type of protecting group placed on the C-2 oxygen.[150] The approach relies on the fact that C-2 ethers activate (arm) and C-2 esters deactivate (disarm) the anomeric centre. Thus, coupling of a glycosyl donor having a C-2 ether protecting group with a glycosyl acceptor bearing a C-2 ester protecting group proceeds highly chemoselectively (Scheme 1.7.2) to give cross coupling products.[154] This effect has been further elaborated to include the entire protecting group complement of a given glycosyl donor by Ley and coworkers.[155,156] Later, Wong and coworkers used *p*-methylphenyl thioglycosides to quantify the effect of various protecting groups on the relative reactivity of glycosyl donors.[157,158] These researchers also found that a number of

factors play a role in glycosyl donor reactivity including the position and characteristics of each protecting group, as well as the reactivity of different sugar cores (Figure 1.7.3).[157] Based on this information, the reactivity order of monosaccharides appears to be fucose > galactose > *N*-acetylgalactosamine > glucose > *N*-acetylglucosamine > mannose.



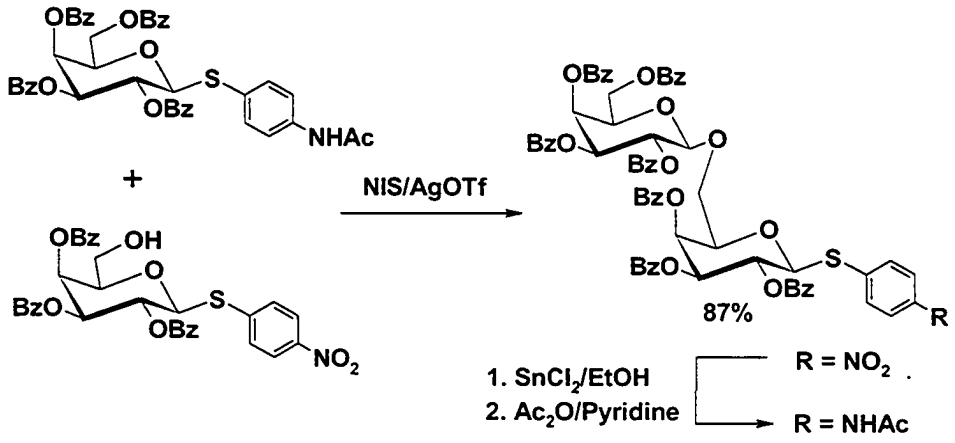
Scheme 1.7.3 Relative reactivity of different sugar cores[154]

Other studies using thioglycosides have shown that glycosyl donors bearing functionally similar glycosyl leaving groups can be selectively activated. This selectivity is based on both the steric[154,159] and electronic[160-162] nature of the glycosyl leaving group. For example, ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside can be selectively activated by IDCP in the presence of lone pair on sulfur for iodination in the first step of glycosyl leaving group activation (Scheme 1.7.4). The lone pair on the sulfur is hindered in the case of the dicyclohexylmethylthio group[157] while the ethylthio moiety is readily iodinated.



Scheme 1.7.4 Selective activation of thioglycosides based on steric factors[154]

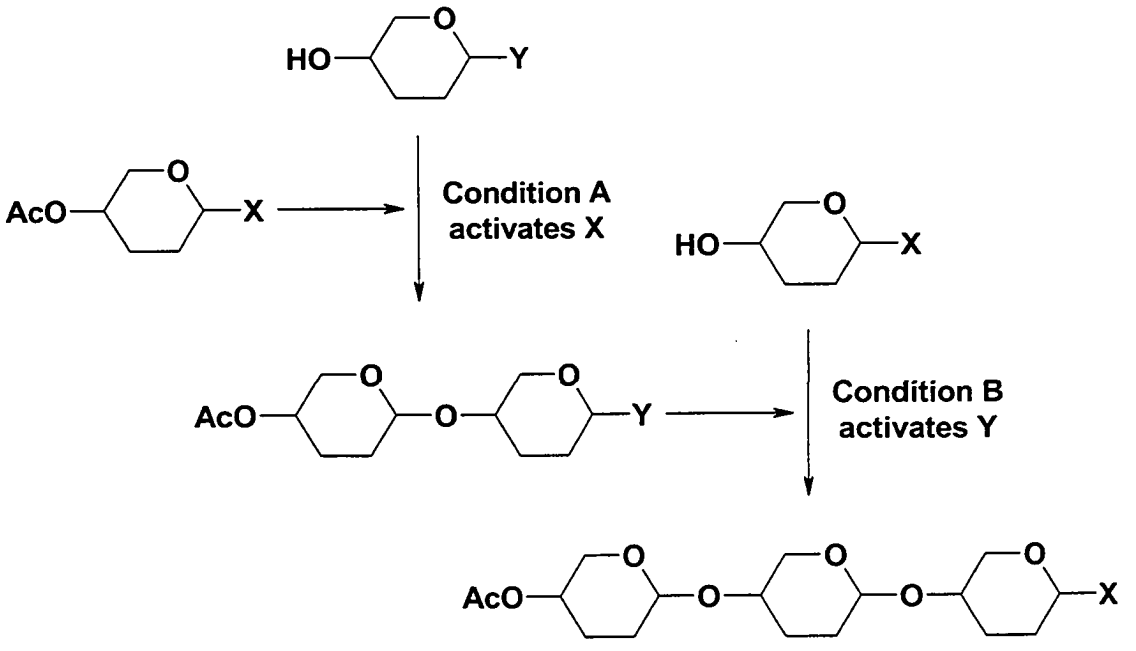
Finally, Roy and co-workers introduced the concept of "active" and latent" thioglycosyl donors.[160] This strategy is based on the use of single arylthio glycosides, the reactivity of which can be determined by the electronic characteristics substitutents on the arylthio leaving group. These researchers found that arylthio groups bearing electron donating groups (EDG), termed "active" can be selectively activated in the presence of arylthio groups bearing electron withdrawing substituents (EWG), termed "latent". Furthermore, the reactivities of "latent" arylthio glycosides could be turned on by converting the EWG to an EDG. For example *p*-acetamidophenyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-galactopyranoside can be selectively activated using the NIS/TfOH promtor system in the presence of *p*-nitrophenyl 2,3,4-tri-O-benzoyl-β-D-galactopyranoside to give the cross coupled product in excellent yield (Scheme 1.7.5).[162] The resulting "latent" thioglycoside can then be converted into its "active" counterpart by reducing the *p*-nitro group using tin(II) dichloride/ethanol, followed by *N*-acylation.



Scheme 1.7.5 The "active" and "latent" glycosyl donor concept[162]

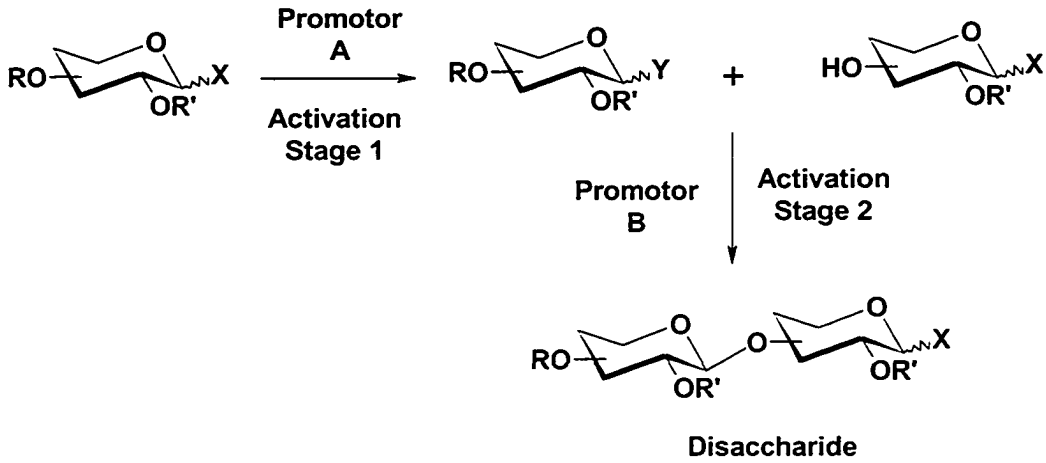
1.8 The Orthogonal Glycosylation Strategy

The orthogonal glycosylation strategy was first introduced by Ogawa and coworkers in 1992.[163] These researchers investigated the possibility of using two sets of chemically distinct (orthogonal) glycosyl donors and activation conditions (Scheme 1.8.1). For this concept to be practical **X** should be unaffected by condition **B**, required to activate the other donor (**Y**) and vice-versa. Also, both **X** and **Y** should remain compatible with subsequent manipulations of temporary protecting groups.[163] Several groups have developed this strategy to employ the chemistry of various glycosyl leaving groups including: thioglycosides and glycosyl fluorides,[163-165] thioglycosides and trichloroacetimidates,[166] selenoglycosides and glycosyl fluorides,[156] thioglycosides and glycosyl bromides,[167] to name a few.



Scheme 1.8.1 The orthogonal glycosylation strategy[168]

This method has been described by Nicolaou[168] and follows a two stage activation procedure combining the chemistry of two or more glycosyl leaving groups, Scheme 1.8.2. In the first activation stage, an orthogonally protected carbohydrate monomer with glycosyl leaving group **X** is converted into an new glycosyl leaving group **Y** using promotor **A**. The resulting glycosyl donor can undergo subsequent condensation with a suitable acceptor alcohol to produce non-reducing disaccharide product. Nicolaou described this procedure using thioglycosides and glycosyl fluorides[168] (entry 1, Figure 1.8.4), although other glycosyl leaving groups and promotor systems may be employed (Figure 1.8.4). The resulting disaccharide can be used in several ways to generate larger oligosaccharides. The inherent glycosyl leaving group **X** can be selectively activated using a third promotor system



Scheme 1.8.2 The two stage activation procedure used in orthogonal glycosylation

X	Promotor A	Y	Promotor B
SPh	DAST or HF/Pyridine	F	$AgClO_4$ / $SnCl_2$
SPh	Br_2	Br	AgOTf
SPh	1. NBS/Acetone 2. CCl_3CN/K_2CO_3	imidate	TMSOTf
SPh	m-CPBA	SOPh	Tf_2O

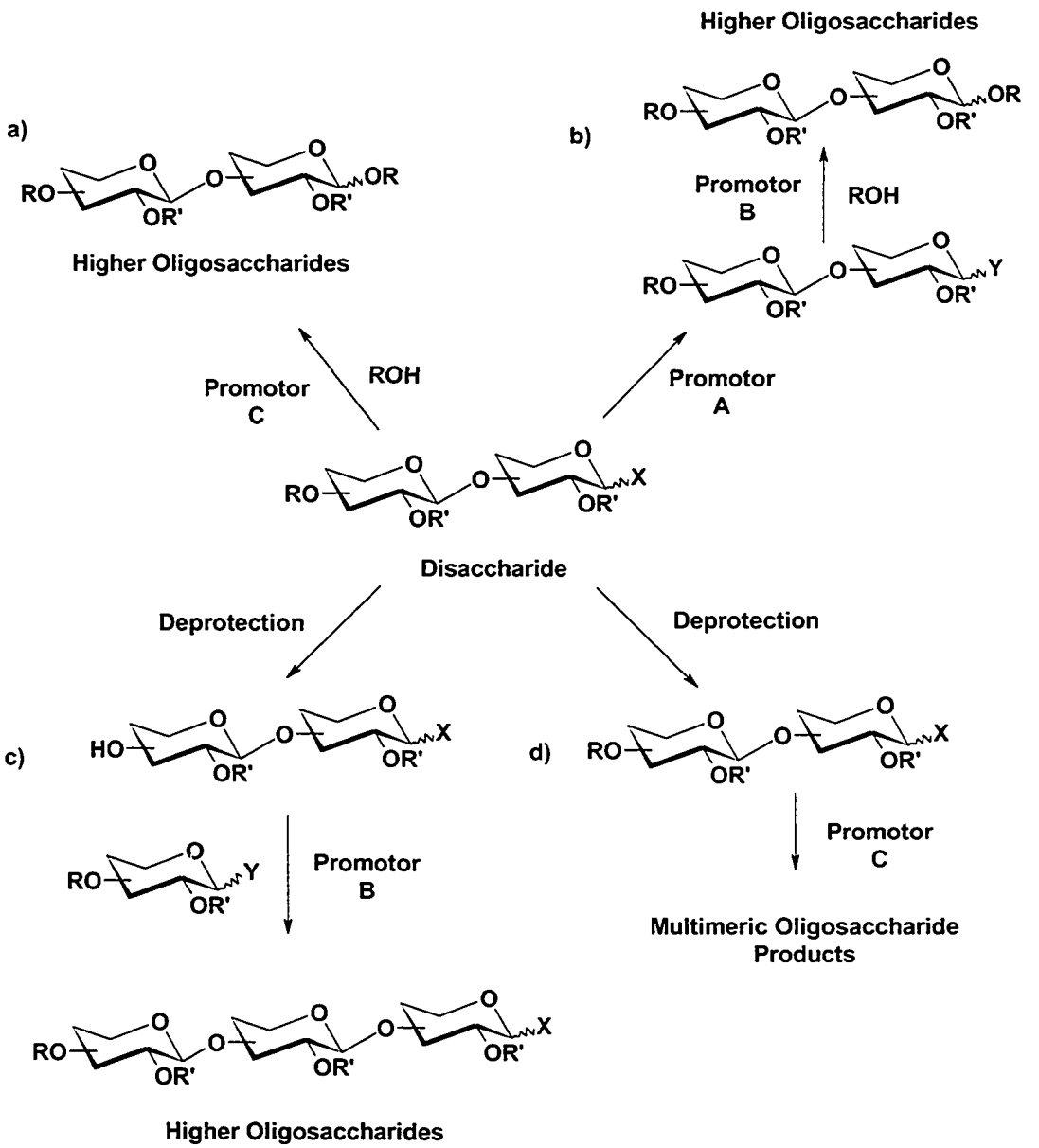
Figure 1.8.4 Alternative leaving groups and promotors for the two step activation method

(promoter C) and condensed with an appropriate acceptor alcohol (Scheme 1.8.3a), which follows the methods reported by Ogawa and coworkers.[163] Glycosyl leaving group **X** can also be converted into an alternative leaving group, **Y**, using promotor **A**. The resulting

product can also be condensed with an acceptor alcohol to generate higher oligosaccharides (Scheme 1.8.3b). In both cases, the disaccharide acts as a glycosyl donor in reactions that extend the oligosaccharide chain at the reducing end of the molecule.

Scheme 1.8.3c demonstrates that the disaccharide can be selectively deprotected exposing one or more hydroxyl groups. In this manner, the subsequent product can act as a glycosyl acceptor in a reaction with an appropriate glycosyl donor. This methodology allows the oligosaccharide chain to be extended at the non-reducing end to produce higher oligosaccharides.

Finally, in Scheme 1.8.3d, the disaccharide can be deprotected to reveal a single hydroxyl group. Subsequent activation of the glycosyl leaving group allows for a self-condensation reaction in which the disaccharide can act both as a glycosyl donor and a glycosyl acceptor. In this procedure, the oligosaccharide chain can be extended at both the reducing and non-reducing end to produce multimeric products.



Scheme 1.8.3 Various methods for generating higher oligosaccharides from disaccharide core

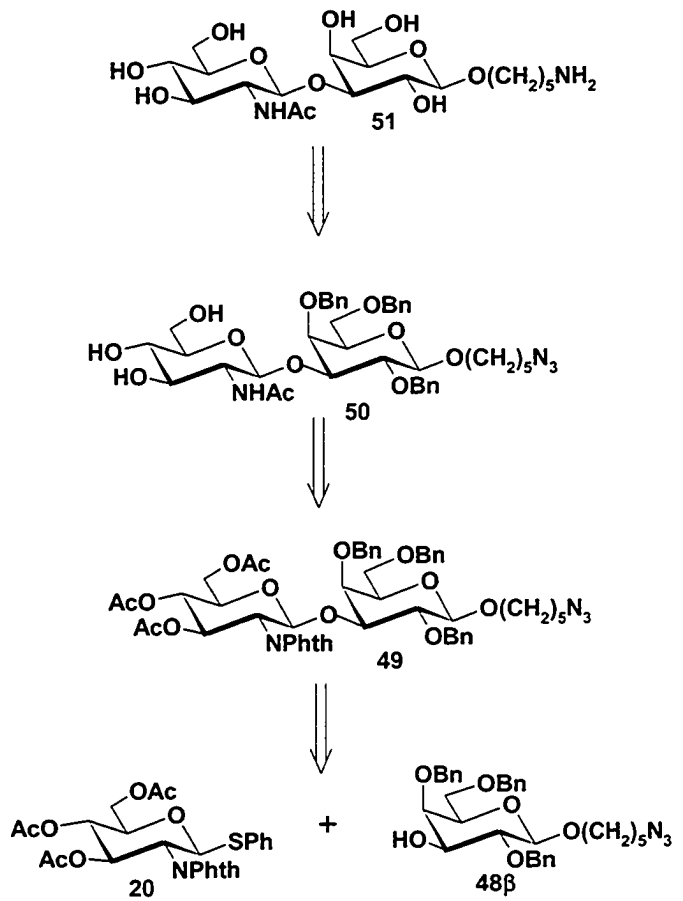
1.9 Retrosynthetic analysis

In the carbohydrate synthetic strategies developed here, several points of concern are addressed: the regioselective protection and deprotection of several hydroxyl groups, the stereochemistry of the glycosidic bonds, the relative reactivity of the glycosyl donor during glycosylation reactions, and the steric effects governing the availability of both the glycosyl leaving group and the nucleophilic hydroxyl group of the glycosyl acceptor. In proposing a strategy to form multimeric products from a 2-amino-2-deoxy-D-glucopyranose derivitive, it is important that this monomer be extendable not only at the non-reducing end with mono, di, and trisaccharides, but also at the reducing end with oligosaccharides or linker arms that can be used for attachment to proteins, peptides, or clustering agents. The synthetic pathways chosen to the various targets will be discussed in turn.

Scheme 1.9.1 shows the final retrosynthetic analysis of the neutral disaccharide glycoconjugate that arose after examining the information gathered during earlier studies and preliminary experiments.[169] Aspects of alternative plans originally evaluated will be discussed in the context of the overall synthetic strategy.

In the final synthetic scheme for the linker-arm-connected neutral disaccharide, phenyl 3,4,6-tri-*O*-acetyl-2-deoxyl-2-phthalimido-1-thio-β-D-glucopyranoside (**20**) was chosen as the glycosyl donor and 5-azidopentyl 2,4,6-tri-*O* benzyl-β-D-galactopyranoside (**48β**) as the glycosyl acceptor. A neighboring protecting group in the glycosyl donor must be selected such that the 2-*N*-substituent can be involved in neighboring group participation thus directing the glycosylation to give the 1,2-*trans*-configuration with high stereoselectivity. It was decided to use the *N*-phthalimido group, a divalent protecting group,

at C-2 of the glycosyl donor.



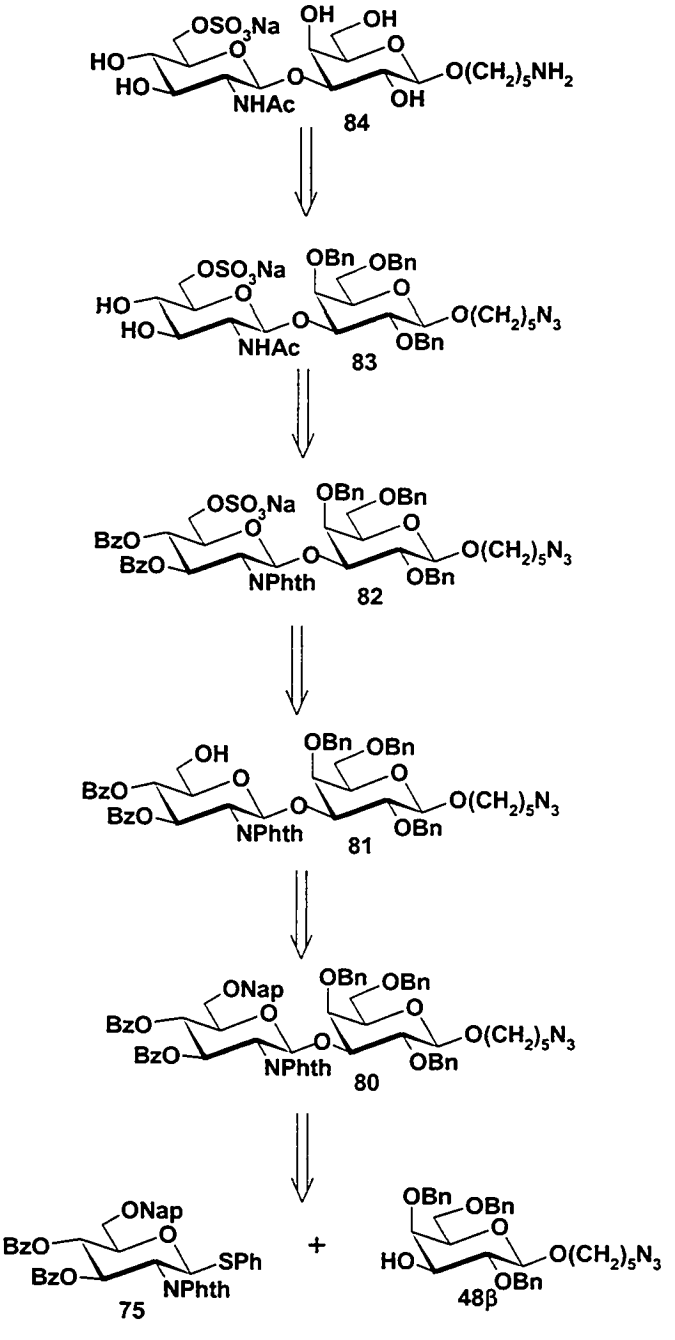
Scheme 1.9.1 Retrosynthetic analysis for the synthesis of the linker-arm-attached neutral disaccharide (51)

This was chosen in preference to the *N*-acetyl group, which, under basic conditions, can result in the formation of oxazoline derivatives during glycosylations reactions. The linker, 5-azido-1-pentanol, was synthesized separately and attached in acetonitrile to a glycosyl acceptor with a non-participating benzyl group at C-2. This reaction successfully

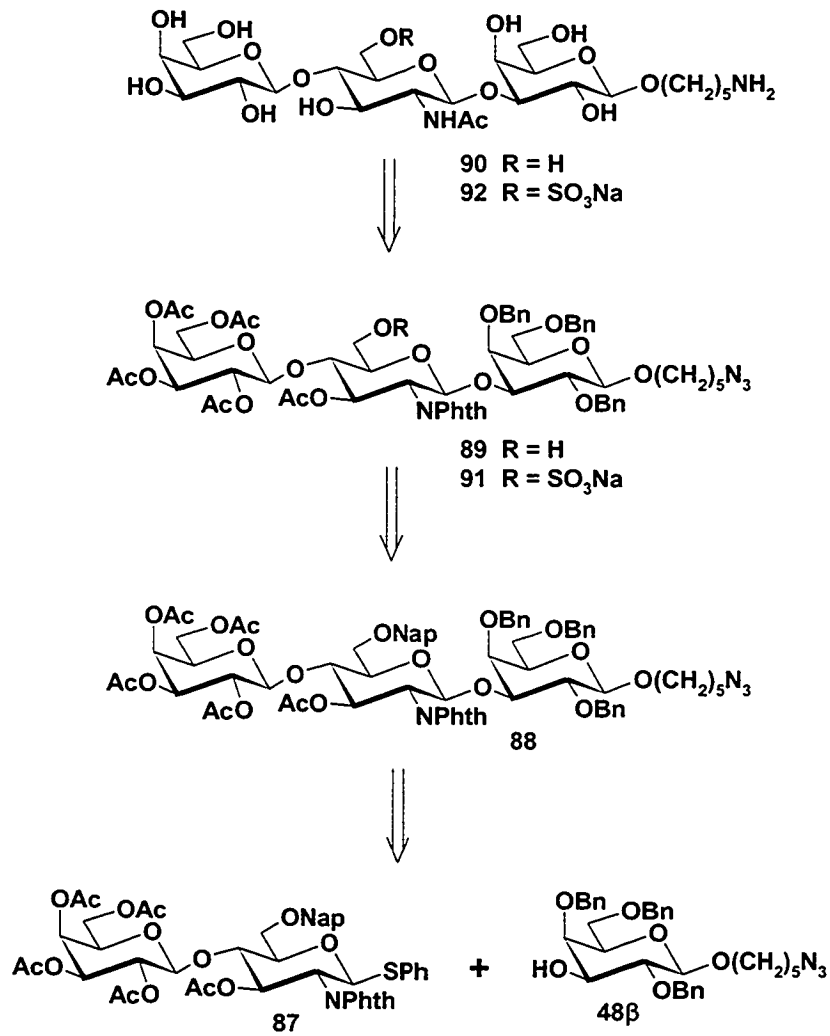
employed the nitrile effect to give the 1,2-*trans*-configuration with high stereoselectivity. The glycosyl donor and acceptor were synthesized separately and then coupled together at the anomeric center to form the desired disaccharide derivative. De-*O*-acetylation and de-*N*-phthalimidation was performed by refluxing **49** with 1-aminobutane in methanol followed by *N*-acetylation with acetic anhydride in methanol to give disaccharide **50**. In the final step, de-*O*-benzylation and azide reduction afforded the disaccharide product.

Scheme 1.9.2 shows the final retrosynthetic analysis for the synthesis of linker-arm-attached acidic disaccharide (**84**). In this scheme, phenyl 3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-6-*O*-(2-naphthylmethyl)-1-thio-β-D-glucopyranoside was chosen as the glycosyl donor. The C-2 phthalimide protecting group was also employed in this scheme, as well as selective protection of O-6 using a 2-naphthylmethyl ether. The remaining oxygens at positions 3 and 4 were subsequently protected with benzoate esters to give the final, orthogonally protected, glycosyl donor (**75**). The linker arm compound discussed previously participated as the glycosyl acceptor in a glycosylation reaction with **75** to form acidic disaccharide derivative **80**. Removal of the 2-naphthylmethyl group of **80** was performed using DDQ in dichloromethane:methanol to give 6-*O*-deprotected derivative **81**. Subsequent sulfation of the free hydroxyl group of **81** using SO_3/NMe_3 complex in DMF resulted in the formation of acidic disaccharide **82**. De-*O*-acetylation, de-*O*-benzoylation, and de-*N*-phthalimidation was performed by refluxing **82** with 1-aminobutane in methanol followed by *N*-acetylation in acetic anhydride:methanol to give disaccharide **83**. In the final step, de-*O*-benzylation and azide reduction afforded the final disaccharide product, **84**.

Scheme 1.9.3 shows the final retrosynthetic analysis for the synthesis of neutral and



Scheme 1.9.2 Retrosynthetic analysis for the synthesis of acidic disaccharide (84)



Scheme 1.9.3 Retrosynthetic analysis for the synthesis of neutral (90) and acidic (92) trisaccharides

acidic trisaccharides (**90**, **92**). In this scheme, disaccharide 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-2-phthalimido-6-*O*-(2-naphthylmethyl)-1-thio-β-D-glucopyranoside (**87**) was chosen as the glycosyl donor. The preparation of this

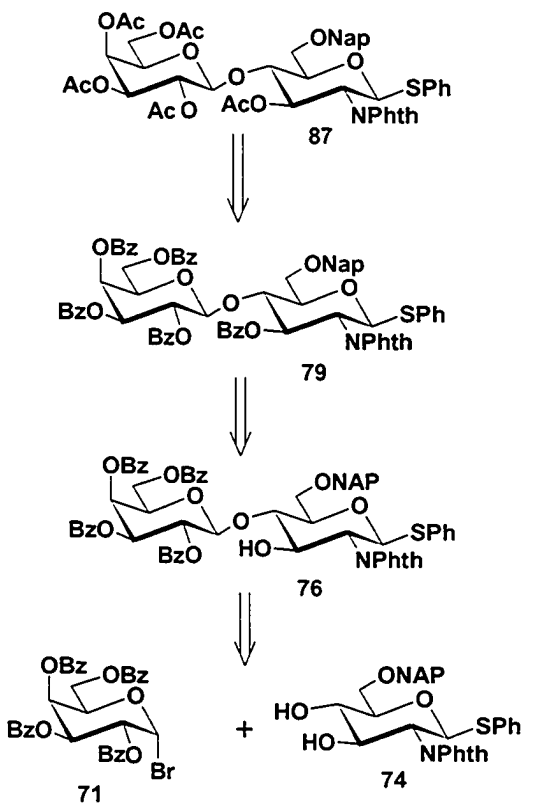
compound is described in Scheme 1.9.4. The linker arm compound discussed previously participated as the glycosyl acceptor in a glycosylation reaction with glycosyl donor **87** to form orthogonally protected trisaccharide **88**. Selective de-*O*-naphthylmethylation of **88** was performed using DDQ in dichloromethane-methanol to give the 6-*O*-deprotected trisaccharide derivative **89**.

To complete the synthesis of the neutral trisaccharide, compound **89** was de-*O*-acetylated, de-*O*-benzoylated, and de-*N*-phthalimidated by refluxing with 1-aminobutane in methanol followed by *N*-acetylation with acetic anhydride in methanol. This intermediate was immediately de-*O*-benzylated and the azide reduced to afford the final neutral trisaccharide product **90**.

To complete the synthesis of the acidic trisaccharide, compound **89** was subsequently sulfated using SO_3/NMe_3 complex in DMF, which resulted in the formation of acidic trisaccharide **91**. De-*O*-acetylation, de-*O*-benzoylation, and de-*N*-phthalimidation was performed by refluxing **91** with 1-aminobutane in methanol followed by *N*-acetylation with acetic anhydride in methanol followed by de-*O*-benzylation and azide reduction to afford the final acidic trisaccharide product **92**.

Scheme 1.9.4 shows the retrosynthetic analysis for disaccharide **87** required for the synthesis of neutral and acidic trisaccharides (**90**, **92**). In this scheme, 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl bromide (**71**) was chosen as the glycosyl donor. The C-2 benzoate was implemented here to induce the 1,2-trans-relationship during glycosylation. The glycosyl acceptor chosen in this scheme is 2-deoxy-2-phthalimido-6-*O*-(2-naphthylmethyl)-1-thio-β-D-glucopyranoside (**74**), an intermediate in the synthesis of

glycosyl donor **75**. Diol **74** is protected at C-2 by phthalimido group, a requirement for stereoselective chain extension at the reducing end, while O-6 is protected by a 2-naphthylmethyl ether. Condensation of glycosyl donor **71** and diol **74** employs a regioselective glycosylation strategy using the Koenigs-Knorr method. Subsequent benzoylation of the remaining hydroxyl group using benzoyl chloride in pyridine affords the desired disaccharide, **76**. It was found that glycosyl donor **76** did not couple with acceptor **48β** in good yield. Therefore, **76** was subsequently de-*O*-benzoylated using sodium methoxide in methanol and *O*-acetylated with acetic anhydride in pyridine to give donor **87**, which gave the desired trisaccharide in good yield.



Scheme 1.9.4 Retrosynthetic analysis for the synthesis of disaccharide 87

2. Results and Discussion

2.1 General

Our current understanding of immune function indicates that acquired immunity mechanisms have evolved to defend the body against the onslaught of invading pathogenic organisms. However, the immune system needs to be "taught" to recognize harmful agents before it can identify and eliminate them. This is normally done through the process of an infection which, of course, has the risk of a fatal outcome. Vaccination with attenuated or synthetic materials allows the immune system to generate the appropriate cells and molecules to fight infection without the potential for harmful effects.

Oligosaccharides located on the cell surface glycoproteins and glycolipids serve as biological recognition markers for the immune system. The glycoproteins constituting the Zona Pellucida (ZP), which surround the mammalian oocyte, regulate critical steps in the fertilization process and contain several target antigens that have potential for an immunocontraceptive vaccine. Experimental evidence suggests that the initial interaction between sperm and oocyte is mediated by specific sperm proteins and the carbohydrate moieties of ZP glycoproteins. Further evidence indicates that β-*N*-acetylglucosamine and β-galactose residues may be crucial for fertilization to occur. With this in mind the focus of this research is to develop an appropriate means by which the mammalian immune system can be "taught" to recognize the components required for fertilization as harmful and to generate the necessary cells and antibody molecules required to eliminate them.

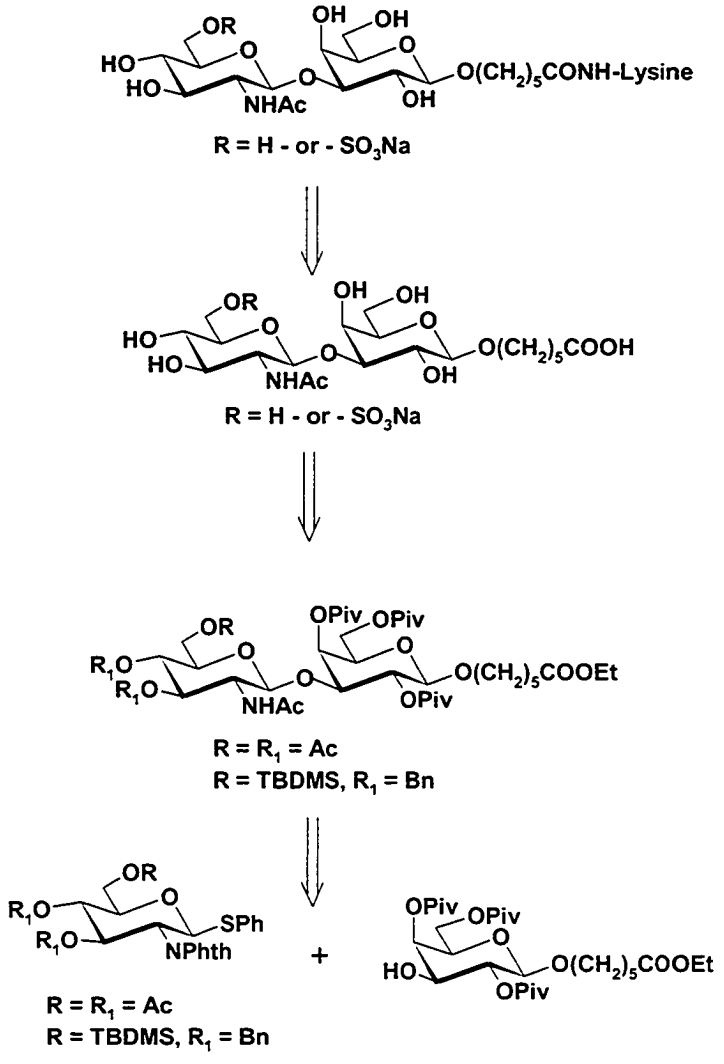
In order to further understand the substrate specificity of the carbohydrates of the ZP

glycoproteins essential in fertilization, preparations and properties of various glycosyl donors and acceptors will be discussed in this chapter. The glycosyl precursors that led to the syntheses of disaccharide derivatives containing terminal β-*N*-acetylglucosamine linked β(1→3) to galactose residues will be discussed. The application and expansion of a novel method for the removal of sterically hindered benzyl ethers is also presented. Several novel approaches investigated in the synthesis of galactose β(1→4) linked to *N*-acetylglucosamine will then be discussed with their eventual application in the synthesis of trisaccharide derivatives. Each target structure can ultimately be conjugated to an appropriate carrier protein or peptide through the linker arm. Such a conjugate vaccine may generate a class of cells and antibodies that are capable of mounting an immune response against the ZP oligosaccharide structures responsible for sperm egg binding. If such a response is elicited, this series of compounds may lead to the development of an effective immunocontraceptive vaccine.

2.2 Structural assignments

In most cases, structures were assigned by analysis of the ^{1}H and ^{13}C NMR spectra of the products and by comparison with those of known compounds if available. There are characteristic features for both the ^{1}H and ^{13}C NMR spectra of carbohydrates.[170,171] For most carbohydrate derivatives, the signal for H-1 is a sharp doublet that is less shielded than the other skeletal protons in the carbohydrate molecule. The chemical shift of H-1 is highly dependent on the protecting group complement of the carbohydrate molecule. Electron withdrawing groups deshield the anomeric hydrogen signal, while electron donating groups

shield H-1.[172] The signal for C-1 is always between 90-110 ppm, if the substituent on C-1 bears an oxygen atom, the most deshielded of those of the skeletal carbons in the carbohydrate structure. In many derivatives synthesized in this thesis, the substituent is a sulfur atom, in which case the chemical shift range is 83-88 ppm, still more deshielded than almost all secondary carbons bearing oxygen only. The signals of methylene carbons such as benzyl carbons (OCH_2Ph), allyl carbons (CH_2=$CHCH_2O$), 2-naphthylmethyl carbons (OCH_2NAP), linker arm OCH_2, and C-6 are easily recognized by the DEPT technique and from their chemical shifts, in the range δ 60 to δ 76. Carbonyls of the *O*-acetyl and phthalimido groups usually resonate around 170 and 167 ppm, respectively. The signal for the methyl carbon of the *O*-acetyl group is usually around 20 ppm. Most ^1H and ^{13}C NMR spectral assignments were confirmed by 2D-COSY and 2D-HETCOR experiments.



Scheme 2.2.1 Retrosynthetic analysis for the first glycosylation approach in the synthesis of neutral and acidic disaccharides

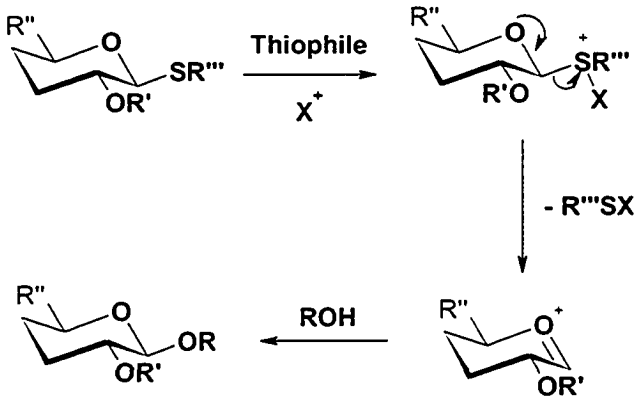
2.3.1 Synthesis of ethyl 6-(2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyloxy)hexanoate, glycosyl acceptor 8

2.3.1.1 General

Thioglycosides were first introduced by Ferrier in 1973.[173] These glycosyl leaving groups have been extensively studied as glycosyl donors due to their high stability to many organic operations, ease of preparation, and rich chemistry. Several derivatives of alkyl- and arylthio groups have been developed with their appropriate activating reagents.[119] Currently, the most commonly used aglycons in this class of compounds are the phenylthio and ethylthio derivatives. The most widely used activators in thioglycoside chemistry include NIS-TfOH,[174,175] NIS-AgOTf,[175] MeOTf,[176-178], iodonium di-*sym*-collidine perchlorate,[154,179] NIS-TMSOTf,[154] DMTST,[180,181] and $NOBF_4$.[182] The accepted mechanism (Scheme 2.3.1) for thioglycoside-based glycosylation is similar to that of the Koenigs-Knorr method.[183] The thiophilic reagent used as an activator ($\mathbf{X}^+$) initiates displacement of the sulfur by the lone pair of electrons on the ring oxygen to form the oxocarbenium species. This species may undergo neighboring group participation with C-2 esters to form β-glycosides (Scheme 1.7.1a) or formation of highly reactive anomeric triflates,[184] depending on the promotor system. The final step involves condensation with an appropriate alcohol acceptor.

Thioglycosides are usually prepared from the anomeric acetates by using the appropriate thiol with $BF_3.OEt_2$[185] or with zinc chloride[186] or Bu_3SnSPh with $SnCl_4$.[187]

Thioglycosides are also useful precursors for the synthesis of other glycosyl leaving groups. Glycosyl fluorides are easily prepared from thioglycosides using DAST/NBS



Scheme 2.3.1 Mechanism for the activation of thioglycosides.[168]

reagents.[168] Glycosyl bromides are prepared using bromine in dichloromethane,[167] while trichloroacetimidates can be synthesised in a two-step procedure involving sulfide hydrolysis using *N*-bromosuccinimide/acetone to give the corresponding lactol,[188] followed by base-catalysed glycosyl imidate formation using trichloroacetonitrile.[149] The more active glycosyl sulfoxides can be formed by oxidation with hydrogen peroxide,[189] or *meta*-chloroperoxybenzoic acid,[190] or peroxyacetic acid at 25 °C.[191,192] For these reasons, thioglycosides are excellent choices as intermediates in orthogonal glycosylation methods.

The regioselective manipulation of hydroxyl groups in carbohydrates, via dibutylstannylene acetals, was an important advance in carbohydrate chemistry due to the high selectivity that is usually observed. Dibutylstannylene acetals are usually prepared by the reaction of diols with dibutyltin oxide in methanol, or in benzene or toluene with azeotropic removal of water using a Dean-Stark apparatus.[193] A recent review observed that in non-polar solvents with added nucleophiles, the dibutylstannylene acetals of *cis*-diols on

pyranose rings react much faster than those of *trans*-diols and give product mixtures that are normally dominated by substitution on the equatorial oxygen atom.[193] It has been found that the regioselectivity of the reaction depends on the steric disposition of the hydroxyl groups involved. Five-membered cyclic stannylene acetals are quite stable species and their formation is favoured particularly when the five-membered ring is *cis*-fused rather than *trans*-fused to a pyranose ring.[193-196] After a stannylene acetal is formed, electrophiles react at equatorial oxygen atoms adjacent to axial oxygen atoms in preference to those adjacent only to other equatorial oxygen atoms. When a primary oxygen atom is free and more than two free secondary hydroxyl groups are present, it is possible to selectively substitute a 1,2-axial-equatorial pair without affecting the usually more reactive 6-primary hydroxyl group. The stannylene acetal method offers a simple one-pot reaction and it is not necessary to separate or purify the intermediate. The efficiency of an alkylation step can be greatly improved by using added nucleophiles, such as tetrabutylammonium iodide, cesium fluoride, or tertiary amines.

Benzyl ethers are common protecting groups. They are stable over a broad range of conditions, including both acidic and basic media.[197] In addition, they are easily and selectively removed. These ethers are traditionally prepared by several methods including with NaH-BnCl,[198] NaH-BnBr in DMF,[199] and KOH-BnCl.[138] Benzyl ethers are most commonly removed by a catalytic hydrogenolysis using Pd/C or $Pd(OH)_2/C$ and a hydrogen source.[197,198] The O-3 oxygen atom of thiogalactoside **4** can be selectively benzylated using the chemistry of stannylene acetals and reacting the intermediate with benzyl bromide in DMF with a cesium fluoride catalyst.

Esters are also commonly used for the protection of hydroxyl functional groups. They are stable over a broad range of synthetic transformations, including oxidation, reduction, and conditions requiring mildly acidic media.[197] Acylation is usually performed using the appropriate acid chloride or acid anhydride in a weakly basic, non-nucleophilic solvent such as pyridine. Esters are traditionally removed under basic conditions with the most common reagent being the Zemplen de-*O*-acylation reagent, sodium methoxide in methanol.[197] In terms of the overall synthetic strategy, esters are capable of inducing 1,2-*trans* glycosylation when chosen as the group to protect O-2 of galactose derivatives (Scheme 1.7.1a). Positions O-2, O-4, and O-6 of 3-O-benzyl thiogalactoside derivative **5** can be pivaloated using excess pivaloyl chloride in pyridine to give orthogonally protected galactose derivative **6**.

The use of activated esters is a traditional method for the formation of amide bonds in peptide synthesis. In this system, the terminal carboxyl group of the first amino acid is activated using a carbodiimide.[200] The resulting activated acid is then allowed to react with nucleophiles such as *N*-hydroxysuccinimide producing the activated ester product. In the second step, the activated ester is stirred with a compound containing a free amino group, usually the next amino acid in the sequence, resulting in the formation of the desired peptide bond.[201] This method has been expanded to include the coupling of carbohydrates to proteins using linker arms. For these purposes a linker arm that can react with carbohydrate residues at one end and with the free amine of lysine on proteins at the other is required. Ethyl 6-hydroxyhexanoate is such a bifunctional linker arm. This compound offers a primary hydroxyl group for glycosylation reactions at one end and an acid group, protected as its ethyl ester, at the other. In a later step, the ethyl ester of 6-hydroxyhexanoate can be transformed

into an acid for conjugation to a protein using activated ester chemistry.[201] Furthermore, this compound has a five-carbon spacer between each functional group. This allows the attached carbohydrates to be extended into the aqueous media surrounding the carrier protein to maximize potential oligosaccharide-antibody interactions. In the formation of orthogonally protected linker arm derivative (**7**), ethyl 6-hydroxyhexanoate will act as the glycosyl acceptor in a glycosylation reaction with glycosyl donor (**20**).

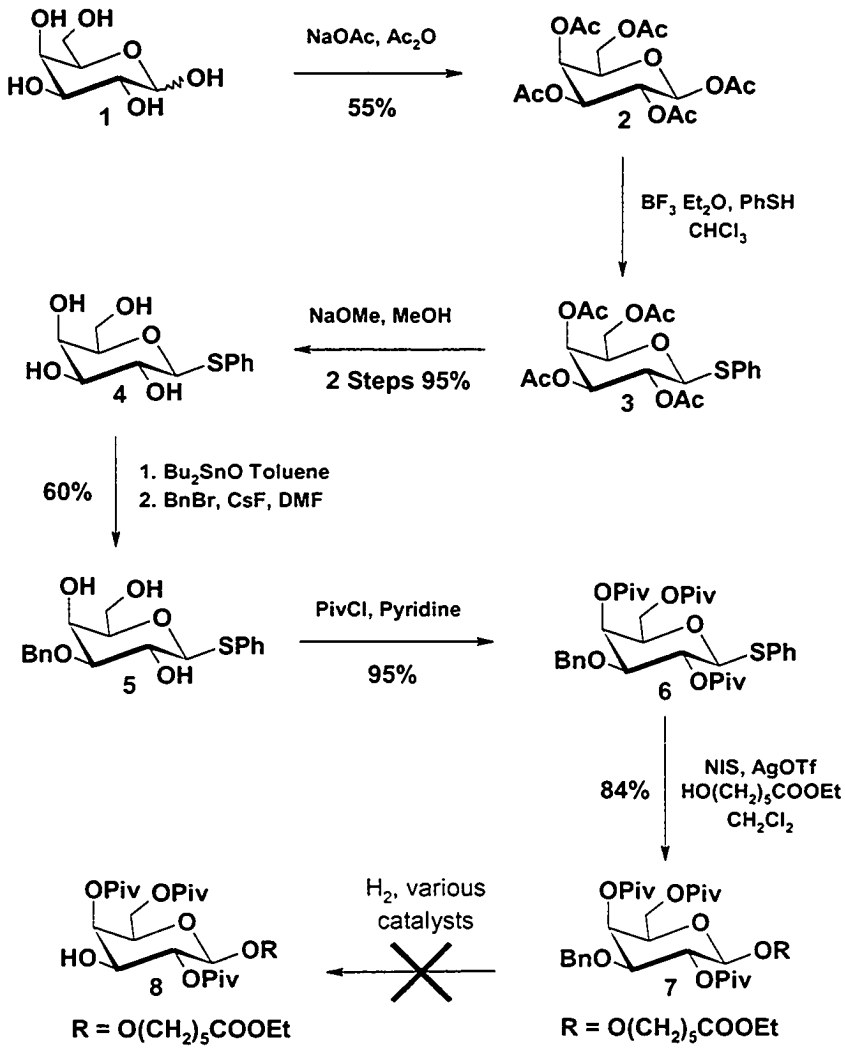
2.3.1.2 Preparation of glycosyl acceptor 8

Ethyl 6-(3-*O*-benzyl-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyloxy) hexanoate, an intermediate in the synthesis of glycosyl acceptor **8**, was prepared in six steps from D-galactose (**1**) (Scheme 2.3.1.2) following literature procedures. The first reaction performed was the preparation of β-D-galactopyranose pentaacetate (**2**) from free sugar starting material **1** using sodium acetate and acetic anhydride.[202] The anomeric acetate of compound **2** was then activated with the Lewis acid boron trifluoride diethyl etherate and coupled to thiophenol in the formation of thioglycoside **3**, as described by Ferrier *et al.*[185] Crude compound **3** was immediately de-*O*-acylated using a catalytic amount of sodium methoxide in methanol to give fully deprotected thioglycoside **4**, in an overall yield of 52%.[203] Stannylenation of phenyl 1-thio-β-D-galactopyranoside (**4**) with dibutyltin oxide was performed in refluxing toluene overnight with the azeotropic removal of water using a Dean-Stark trap. After solvent removal, treatment of the resulting 3,4-*O*-dibutylstannylene acetal with benzyl bromide and a cesium fluoride catalyst in *N*,*N*-dimethylformamide at room temperature gave the 3-*O*-benzylated galactose derivative (**5**) in 60% yield.[204] In the ^{1}H NMR spectrum of compound

5, the appearance of an AB quartet centred at 4.74 ppm indicates the presence of the methylene (OCH_2Ph) hydrogens of the benzyl group. The presence of the benzyl group on O-3 is indicated by a change in the chemical shift for the doublet of doublets representing H-3. In the starting material (**4**), H-3 appears at 3.49 ppm, while in the product (**5**), H-3 is shifted to 3.36 ppm. Furthermore, the ^{13}C NMR spectrum shows the deshielding β-effect on C-3 due to alkylation.[170] In the spectrum of compound **4**, C-3 appears at 70.4 ppm, while in the alkylated product, C-3 has shifted to 78.2 ppm. The chemical shifts of the remaining carbons remain relatively unchanged, verifying that alkylation has taken place on O-3. The identity of compound **5** was confirmed by comparison of its melting point and optical rotation values with those of the known compound.[205]

Pivaloylation of **5** using pivaloyl chloride in pyridine[206] at 75 °C for 12 hours afforded the tri-*O*-pivaloated product, compound **6** in 85% yield. The ^1H NMR spectrum shows the presence of three singlets at 1.14, 1.21, and 1.21 ppm representing the 27 (3 x 9) hydrogens associated with all of the *tert*-butyl esters ($(CH_3)_3CCO$). The deshielding effect of acylation is also reflected in the chemical shifts of H-2, H-4, and H-6, which appeared at 5.16, 5.54, and 4.16 ppm, respectively. The appearance of three singlets in the ^{13}C NMR spectrum at 26.8-27.0 ppm ($(CH_3)_3CCO$) in combination with three singlets at 38.3-38.8 ppm ($(CH_3)_3CCO$) and three singlets at 176.4-177.8 ppm ($(CH_3)_3CCO$) are indicative of success in synthesis of a tri-*O*-pivalated galactoside. The molecular formula of **6** was confirmed by elemental analysis.

Ethyl 6-(3-*O*-benzyl-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyloxy)hexanoate (**7**) was obtained in 84% yield by reacting thioglycoside **6**, the glycosyl donor, with the linker



Scheme 2.3.1.2 Synthesis of glycosyl acceptor 8

compound, ethyl 6-hydroxyhexanoate, using NIS and AgOTf, conditions reported by Fraser-Reid and coworkers.[175] Since the pivaloate ester protecting group at O-2 is a neighboring participating group, the reaction was stereoselective, favoring the formation of the desired β product. In the ^{1}H NMR spectrum of compound **7**, the coupling constant ($J_{1,2}$ = 8.2 Hz) of the doublet at 4.36 ppm is characteristic of β-glycosides. All of the protons of the linker arm are

present in the spectrum. The appearance of C-1 at 101.3 in the ^{13}C NMR spectrum is indicative of glycoside formation, while the appearance of four methylene carbons in the alkyl region, 24.6-34.2 ppm, as well as an additional carbonyl signal at 173.7 ppm reaffirm the successful condensation of the glycosyl donor with ethyl 6-hydroxyhexanoate. The molecular formula of **7** was confirmed by HRMS.

Several attempts at removing the benzyl ether at position O-3 of compound **7** using conventional reductive methods failed to generate the desired product, glycosyl acceptor (**8**). Hydrogenation of compound **7** in methanol using Pd/C for 24 hours at room temperature and atmospheric pressure had no effect. Increasing the pressure to 4 atm failed to solve this problem. Other, more active, catalysts were enlisted to complete the transformation including $Pd(OH)_2$ and $Pd(OAc)_2$, both of which were employed at one and at four atm pressure of hydrogen. Each attempt failed to produce the target compound. After exhaustive efforts to reduce the benzyl group, alternative procedures were investigated.

After deliberation it was decided to try a seldom used method[207-209] discovered by Binkley,[210] which uses light-catalysed α-bromination of benzyl ethers in a two-phase system of carbon tetrachloride and water using NBS and $CaCO_3$ reagents. To our delight, the reaction afforded the desired compound, ethyl 6-(2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyloxy)hexanoate (**8**), quickly (15 min) and in excellent yield (95%) with no observable by-products on TLC. The structure of the synthetic target was confirmed by ^1H NMR spectroscopy. In the spectrum of **8**, the AB quartet characteristic of the methylene group (OCH_2Ph) had disappeared, as well as the signals for aryl protons. The chemical shift of the doublet of doublets assigned to H-3 also changed from 3.56 to 4.11 ppm. The changes

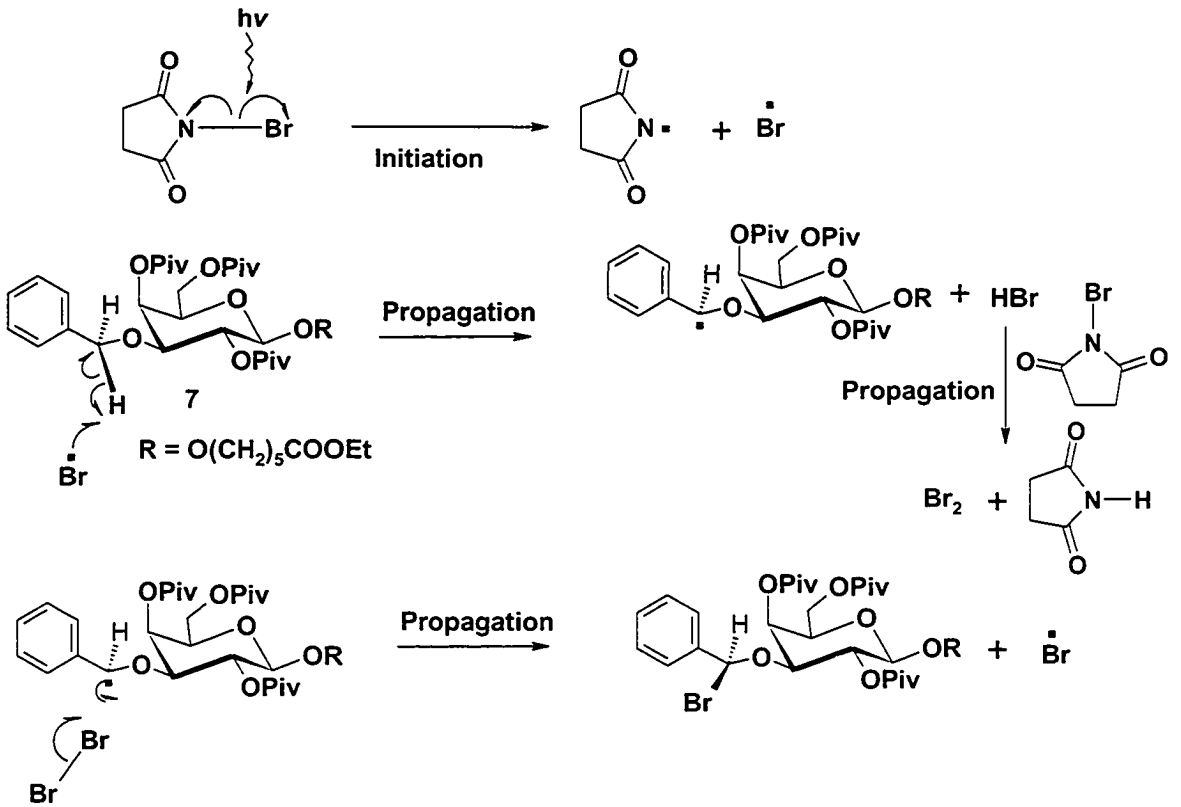
in the ^{13}C NMR spectrum were consistent with the conclusions drawn from the proton NMR spectrum. The absence of aromatic carbons, as well as the disappearance of one methylene (OCH_2Ph) group implies that the de-*O*-benzylation was successful.

A proposed mechanism involving for the de-*O*-benzylation of compound (**7**) is depicted in Scheme 2.3.1.3. This mechanism involves two stages. The first stage is the bromination of the benzyl ether at the α position using radical intermediates. The second step results in cleavage of the α-brominated benzyl ether requiring aqueous base.

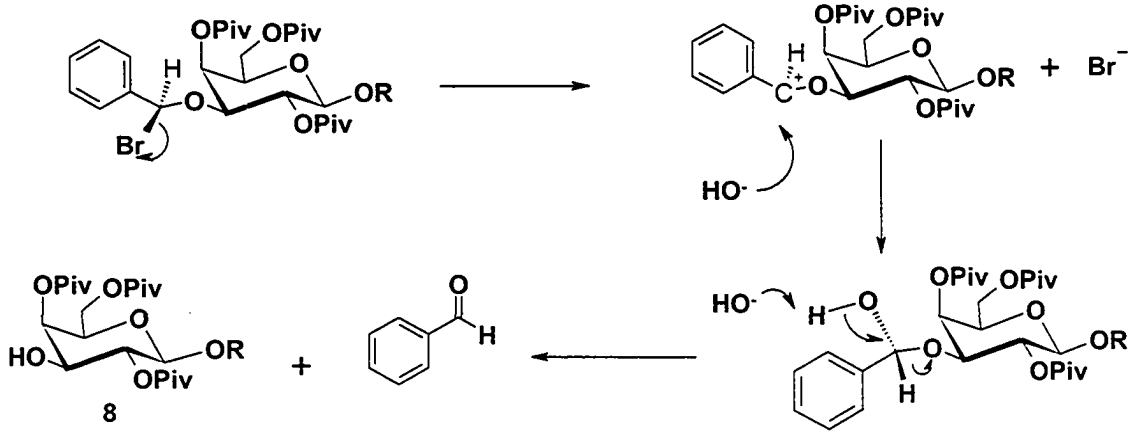
In the initiation step, light of the appropriate wavelength homolytically cleaves the nitrogen-bromine bond of NBS. This generates a bromine atom that then propagates the reaction by abstracting one of the benzylic hydrogens. Due to the various resonance contributors associated with a benzylic radical, hydrogen abstraction is most favorable at this site. Propagation continues by a reaction with the benzylic radical and a second molecule of NBS. For this reason, two equivalents of NBS are required for complete α-bromination. In the second step, the bromine of the α-brominated intermediate spontaneously decomposes into a stable benzylic cation (S_N1 mechanism) which then reacts with an aqueous hydroxide anion generated by $CaCO_3$. This forms a hemi-acetal, which readily falls apart under basic conditions to give the deprotected galactose derivative **8**, and benzaldehyde.

An explanation for the inability to remove the benzyl group on O-3 of compound (**7**) comes from understanding the current mechanistic model for hydrogenation. It is known that hydrogen is adsorbed onto the metal surface of the catalyst, presumably forming metal-hydrogen bonds like those in transition-metal hydride complexes. Groups containing π electrons, including alkenes and arenes, are similarly adsorbed onto the metal surface.

Stage 1


hv
Initiation
Propagation
Propagation
Propagation
7
R = O(CH2)5COOEt
HBr
Br2

Stage 2



Scheme 2.3.1.3 Proposed mechanism for the de-*O*-benzylation of compound 7

Hydrogen is then added to the double bond co ordinated to the metal catalyst.[211] Surface scientists have determined that, in the case of arenes, the aromatic ring lies flat on the metal surface for optimal coordination during the hydrogenation process.[212,213] Spencer et al. recently reported that substitution on an aromatic ring could have an adverse steric effect that would interfere with the planar geometry required for effective coordination and thus reduce its affinity for the metal surface. This reduction in affinity is responsible for the apparent sluggish reactivity observed for substituted benzyl groups as opposed to unsubstituted ones.[214]

These steric factors may extend to groups surrounding the aromatic ring of the benzyl group. In our case, it is believed that the bulky pivaloate esters on adjacent oxygens O-2 and O-4 of compound **7** prevent the necessary coordination of the aromatic ring with the surface of the catalyst. This results in a substrate that resists hydrogenation. In the proposed mechanism for de-*O*-benzylation using light catalysed α-bromination, coordination of the

aromatic ring with a metal surface is not essential. This method requires that the methylene of the substrate benzyl group is accessible by bromine atoms and nucleophilic hydroxide. For these reasons this methodology provides a viable alternative for de-*O*-benzylation. An investigation of this reaction and its application to other substrates is reported in the following section.

2.4 Removal of sterically hindered benzyl ethers[215]

2.4.1 General

Benzyl ethers are probably the most commonly used protecting groups for alcohols. The normal technique for their removal is hydrogenolysis, either catalytic or by transfer of hydrogen.[197,216-218] Palladium on charcoal is the most commonly used catalyst, but some benzyl groups resist hydrogenolysis with this catalyst, therefore other catalysts, such as Pearlman's catalyst ($Pd(OH)_2$), are often tried. Many other methods have been utilized, such as dissolving metal reductions,[219-221] various oxidative methods that convert benzyl ethers to benzoyl esters,[222-225] or cleavage with Lewis acids.[226] However, virtually all of these alternative methods are not compatible with esters and/or thioglycosides or other sensitive functional groups.

The method discovered by Binkley and Hehemann,[210] which uses light-catalysed α-bromination of benzyl ethers in the presence of water, has only been used preparatively three times.[207-209] In the following section it is shown that this method is very effective for the removal of hindered benzyl ethers and is especially compatible with many sensitive functional groups, such as thioglycosides and esters.

A key step in the synthesis of galactose derivative **8** required the removal of a benzyl group having pivaloyl esters on the two adjacent carbons. Despite repeated attempts, this benzyl group could not be removed by hydrogenation at 4 atm using Pd/C, $Pd(OH)_2$ or $Pd(OAc)_2$ as catalysts. Because most alternatives were not compatible with ester protecting groups, photochemical α-bromination[210,227] was attempted and this method proved to work extremely well.

2.4.2 Preparation of substrates used in de-*O*-benzylation reactions

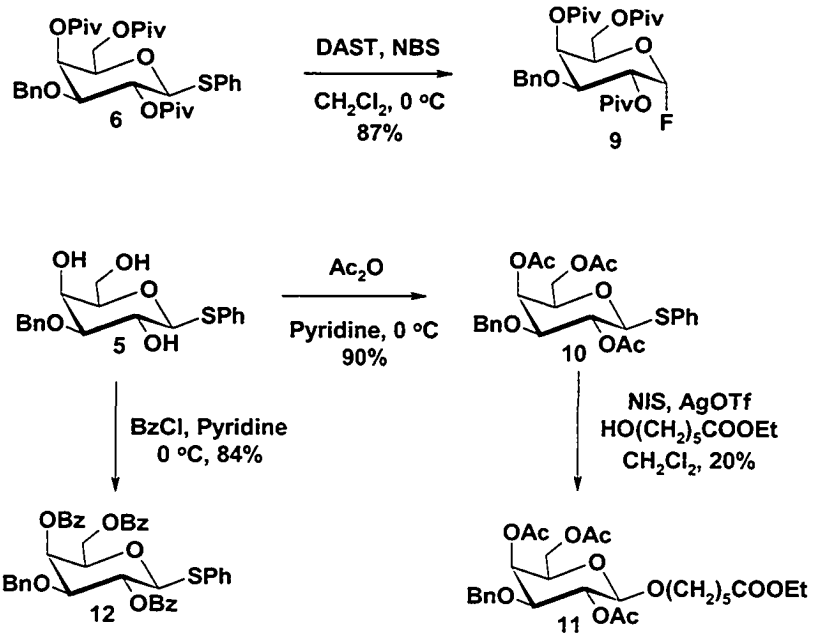
Glycosyl fluorides were first introduced as glycosyl donors by Mukaiyama and co-workers in 1981.[228] This group demonstrated the activation and coupling of anomeric fluorides using AgOTf-$SnCl_2$. Since their introduction a number of other promotors have been employed in their activation including: BF_3-Et_2O,[229] TMSOTf,[230] and AgOTf-Cp_2HfCl_2.[231] The chemistry of glycosyl fluorides is well established in the literature due to their ease of formation under mild conditions, stability towards silica gel chromatography, and a wide selection of activating conditions. Glycosyl fluorides are also used in conjunction with thioglycosides during orthogonal glycosylation procedures.[163-165] For these reasons, the first additional target substrate for de-*O*-benzylation was glycosyl fluoride **9**.

Thioglycoside **6** was treated with DAST and NBS at O °C as outlined by Nicolaou and Ueno,[168] to afford α-glycosyl fluoride **9** in 87% yield (Scheme 2.4.2.1). Due to the electronegativity of the fluoride atom the α anomer was obtained preferentially. This is a result of the anomeric effect, which increases with the increased electronegativity of the atom bonded to C-1. In this case, the effect dominates over other factors as fluorine is the most

electronegative atom.[232] Conversion to the glycosyl fluoride was confirmed by ^{1}H NMR spectroscopy as H-1 is more deshielded (5.75 ppm) in the spectrum due to the electron-withdrawing characteristics of the fluorine atom. The assignment of the α configuration is supported by the small coupling constant ($J_{1,2}$ = 2.6 Hz) between H-1 and H-2. The signal for H-1 also appears as a doublet of doublets. This is a result of the large geminal coupling constant ($J_{1,F}$ = 53.9 Hz) between H-1 and the ^{19}F atom, which has a nucleus with a spin of one half. The ^{13}C NMR spectrum supports the conclusions drawn from the proton NMR. Mainly, C-1 appears as a doublet centred at 104.7 ppm. The chemical shift is due to the large α-effect of fluorine, while the multiplicity is due to the coupling of C-1 with the fluorine atom. The molecular formula of **9** was confirmed by EIMS and elemental analysis.

Phenyl 2,4,6-tri-*O*-acetyl-3-*O*-benzyl-1-thio-β-D-galactopyranoside (**10**) was prepared by the acetylation of **5** using acetic anhydride in pyridine at 0 °C (Scheme 2.4.2.1). The reaction afforded the tri-*O*-acetylated product, compound **10**, in 90% yield. The structure of **10** was established by ^{1}H and ^{13}C NMR spectroscopy and confirmed by HRMS and elemental analysis.

Ethyl 6-(3-*O*-benzyl-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyloxy) hexanoate (**11**) was obtained in low yield, 20%, by reacting thioglycoside **10**, the glycosyl donor, with the linker compound, ethyl 6-hydroxyhexanoate, using NIS and AgOTf. This reaction was performed by Dawn Johnston, a summer research assistant, as part of a previous synthetic scheme. Since the acetate ester protecting group at O-2 is a neighboring group participating group, the reaction was stereoselective, favoring the formation of the desired β product.

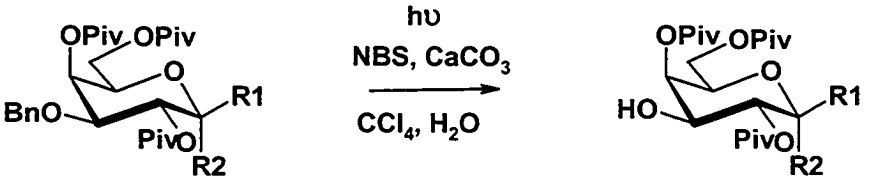


Scheme 2.4.2.1 Synthesis of galactose derivatives 9, 10, 11, and 12

In the ^{1}H NMR spectrum of **11** the coupling constant ($J_{1,2}$ = 8.2 Hz) of the doublet at 4.35 ppm is characteristic of β-glycosides. All of the protons of the linker arm moiety are present in the spectrum. The appearance of C-1 at 101.4 ppm in the ^{13}C NMR spectrum is indicative of glycoside formation, while the appearance of four methylene carbons in the alkyl region, 24.6-34.2 ppm, as well an additional carbonyl signal at 173.6 ppm reaffirm the successful condensation of the glycosyl donor with ethyl 6-hydroxyhexanoate. The molecular formula of **11** was confirmed by HRMS.

Phenyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-galactopyranoside (**12**) was prepared by the benzoylation of compound **5** using benzoyl chloride in pyridine at 0 °C (Scheme 2.4.2.1). The reaction afforded the tri-*O*-benzoylated product, compound **12**, in 84% yield. The structure of **12** was established by ^{1}H and ^{13}C NMR spectroscopy and confirmed

by HRMS.



6 R1 = SPh, R2 = H

7 R1 = $O(CH_2)_5CO_2Et$, R2 = H

9 R1 = H, R2 = F

13 R1 = SPh, R2 = H

8 R1 = $O(CH_2)_5CO_2Et$, R2 = H

14 R1 = H, R2 = F

Scheme 2.4.3.1 De-*O*-benzylation of Pivaloated Galactose Derivatives

2.4.3 De-*O*-benzylation reactions

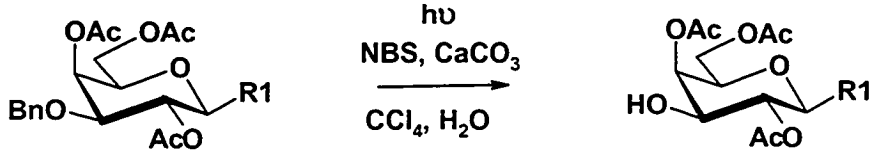
De-*O*-benzylation reactions were performed for a variety of galactose derivatives as well as one glucose derivative. Each compound was dissolved, along with NBS, in carbon tetrachloride and a suspension of calcium carbonate in water was added. The solutions were then purged with nitrogen for one hour to remove molecular oxygen, whereupon they were irradiated with a 350 Watt incandescent lamp. Changes in the polarity of de-*O*-benzylated compounds, were easily monitored by TLC analysis and supported by ^{1}H NMR spectroscopy. When comparing products with starting materials, the signals in the region of 7.1-7.4 ppm (*Ph*), and the characteristic doublet of doublets centred at approximately 4.5 ppm (C*H*$_2$Ph) were absent. Furthermore, the position of the doublet of doublets (H-3) in galactose derivatives has been shown to be more deshielded when the ^{1}H NMR spectra of the de-*O*-benzylated products are compared with those of the starting materials.

De-*O*-benzylation reactions were carried out on compound **7** which was required to complete Scheme 2.3.1.2. A second reaction was performed on the thioglycoside precursor to compound **7**, compound **6**. These reactions demonstrated the utility of this method. Thiols generally poison palladium catalysts. In the case of **6**, however, the reaction proceeded efficiently in a 95% yield. Thus, this method presents a means of removing benzyl ethers in the presence of thioglycosides. The reaction of **9** expands the method to include glycosyl fluorides, which are often used in orthogonal glycosylation strategies in conjunction with thioglycosides. The synthesis of glycosyl fluoride **9** from compound **6** is shown in Scheme 2.4.2.1.

Reactions of compounds **10** and **11** expand the utility of the reaction. The good yields of compounds **15** (87%) and **16** (82%) reinforce the efficiency of this method. Acetates are considerably more sensitive to base than pivaloates and the good yields obtained show that exposure to base in this refluxing two-phase system is not a problem in the short time required for this reaction. Both acetates and thioglycosides can be sensitive to reductive conditions, which are normally followed for the removal of benzyl ethers. In both cases, this method gave good yields of deprotected products leaving the acetate esters intact. The synthesis of compound **17** from **12** gave the lowest yield (55%) of all the galactose derivatives tested for reasons that are not obvious.

De-*O*-benzylation of a glucose derivative, compound **23**, the synthesis of which is shown in Scheme 2.5.2.2, was performed and gave a 50% yield of deprotected product (**22**). This reaction demonstrates the use of this method as an alternative for the complete de-*O*-benzylation of glucose derivatives. Also of note is the presence of the thiophenyl group at

the anomeric position, which in most cases, prevents the removal of benzyl groups under reductive conditions. The reaction of this compound indicates that other groups such as silyl ethers and phthalimides are also stable under these conditions.



29 R1 = $O(CH_2)_5CO_2Et$ **30** R1 = $O(CH_2)_5CO_2Et$

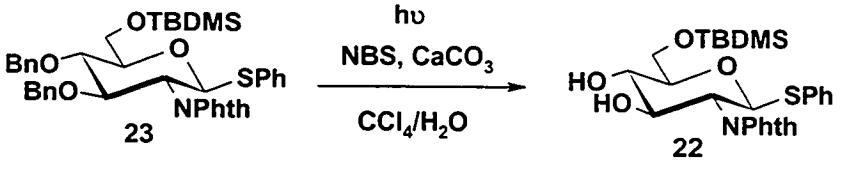
31 R1 = SPh **32** R1 = SPh

Scheme 2.4.3.2 De-*O*-benzylation of Acetylated and Benzoylated Galactose Derivatives.

2.4.4 Conclusions

De-*O*-benzylation reactions have been performed on a variety of compounds using a literature method which was seldom used. Galactose derivatives **6**, **7**, and **9** all have benzyl groups on position O-3 of the sugar with bulky pivaloate groups on adjacent hydroxyls, positions O-2 and O-4. Repeated attempts at removal of this benzyl group using conventional methods proved unfruitful due to steric effects. Support for the idea that steric effects are responsible for the original difficulty comes from the successful removal of the benzyl group on position-3 of compound **10**, which has smaller acetate esters on positions O-2 and O-4 of the galactose sugar, by hydrogenation using Pd/C as a catalyst. The method using light-catalysed α-bromination of benzyl ethers in the presence of water was used successfully in the synthesis of compound **8**, which will allow the present synthetic strategy to continue.

The utility of this reaction was also demonstrated on other galactose derivatives having acetates (compounds **10** and **11**) or benzoates (compound **12**) on the positions flanking the benzyl group as well as a di-*O*-benzylated glucose derivative (compound **23**). In nearly all cases the reaction proceeded in greater than 80% yield, a very useful result. The reaction has proven to be applicable to compounds containing sterically hindered benzyl groups, thiols, esters, silyl ethers, phthalimides, and fluorides.



Scheme 2.4.3.3 De-*O*-benzylation of a glucose derivative, compound 23

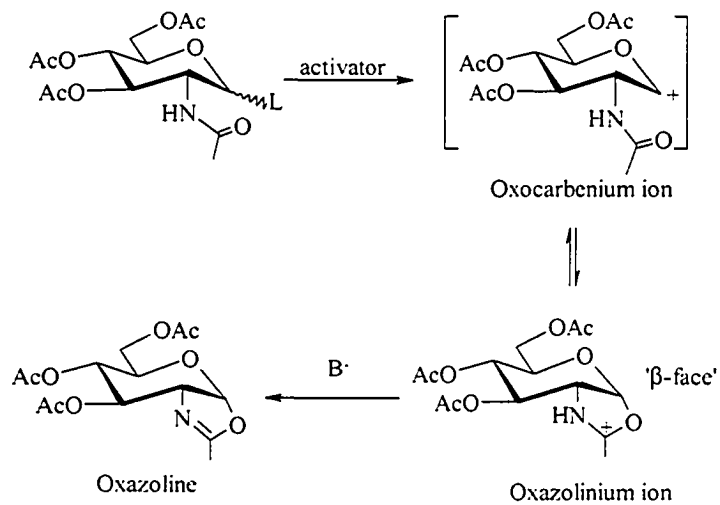
2.5.1 Glycosyl donors 20, 23, and 25.

2.5.1.1 Glycosyl donor 20

In continuance of the orthogonal glycosylation approach outlined in Scheme 1.8.1, thioglycosides were chosen as the glycosyl leaving group for reasons outlined previously. In order to obtain 1,2-*trans* -glycosylation and to prevent the formation of an oxazoline ring from basic conditions that result from glycosylation (Scheme 2.5.1.1), a divalently protected 2-amino group, the 2-*N*-phthalimido group, was chosen. Baker et al. introduced this group in 1954 as a protecting group for C-2 of glucosamine derivatives.[234] These protecting groups are usually prepared with phthalic anhydride and base and are stable to a variety of

conditions.[197] For these reasons the *N*-phthalimido group was chosen to protect the C-2 amino group of glycosyl donor **20**.

1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (**19**) was prepared in 76% yield from D-glucosamine hydrochloride (**18**) using literature procedures.[145,178,235] Treatment of compound **19** with the Lewis acid boron trifluoride diethyl etherate, and thiophenol gave phenyl 2,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**20**) in 69% yield (Scheme 2.5.1.2). The physical data and spectral properties of the compound obtained matched those found in the literature.[236]

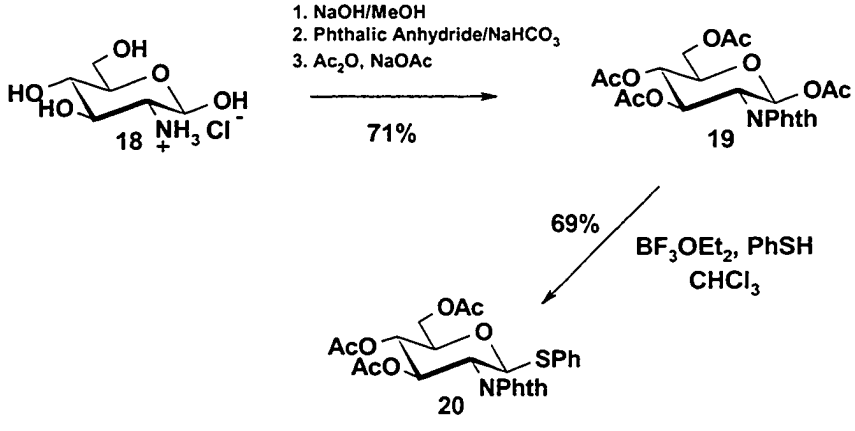


Scheme 2.5.1.1 1,2-trans glycosylation resulting in oxazoline formation

2.5.2.1 Glycosyl donor 23

Silyl ethers are among the most frequently used protective groups for the hydroxyl functional group. This is due largely from the fact that their reactivity (both formation and cleavage) can be modulated by a suitable choice of substituents on the silicon atom. The most

common substituted silyl groups found in the literature include: trimethylsilyl ethers (TMS), triisopropylsilyl ethers (TIPS), *tert*-butyldimethylsilyl ethers (TBDMS), and *tert*-butyldiphenylsilyl ethers (TBDPS). The controlling mechanisms for silyl ether cleavage depend on both the steric and electronic characteristics of the groups bonded to the silicon atom. For example, acid hydrolysis of the aforementioned silyl ethers occurs in the order of TMS (1) < TBDMS (20,000) < TIPS (700,000) < TBDPS (5,000,000).[197]

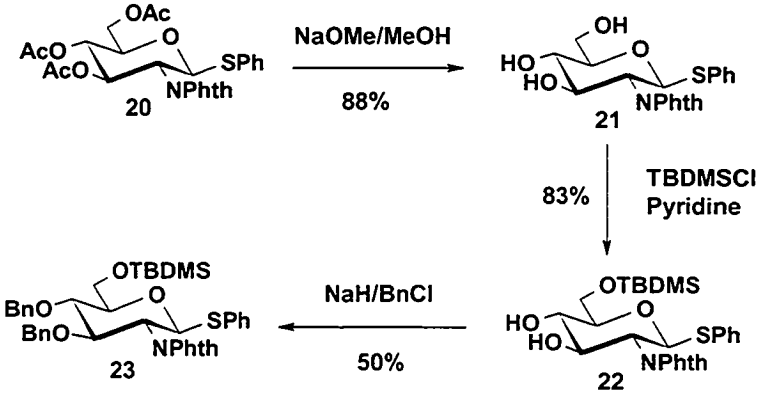


Scheme 2.5.1.2 Preparation of glycosyl donor phenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (20)

The TBDMS group was first introduced by Corey and Venkateswarlu in 1972.[237] Since its introduction this group has been used in a variety of synthetic sequences including the synthesis of complex oligosaccharides.[154,162,238-241] The TBDPS ether was later developed by Hanessian and Lavallee[142] as a more stable alternative to the TBDMS group. Formation of both TBDMS and TBDPS ethers occurs on primary hydroxyl groups in preference to secondary hydroxyl groups.

The most common method for preparing TBDMS ethers is TBDMSCl in DMF using a catalytic amount of base,[237] although other methods exist. Removal of the TBDMS group takes advantage of the strength of the silicon-fluorine bond, which is 30 kcal/mol stronger than the silicon-oxygen bond. Thus, silyl ether cleavage is most readily achieved using fluoride salts, with the most common reagent being tetrabutylammonium fluoride.[237]

Accordingly, it was decided to investigate the TBDMS ether as a protecting group for the primary alcohol of *N*-acetylglucosamine derivatives because of: its selectivity for the protection of primary hydroxyl groups; the potential for selective removal to allow sulfation at a later stage; and the lower cost of reagents (in comparison with the *tert*-butyldiphenylsilyl chloride) for its introduction.



Scheme 2.5.2.2 Synthesis of glycosyl donor 23

2.5.2.2 Preparation of phenyl 3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (23)

Phenyl 3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**23**) was prepared in three steps from compound **20** (Scheme 2.5.2.2). De-*O*-acylation of compound **20** using a solution of sodium methoxide in methanol afforded compound **21** in 88% yield. Physical data and spectral characteristics of compound **21** were in good agreement with those reported by Ogawa et al.[187]

Phenyl 2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**22**) was prepared from compound **21** in 83% yield, using TBDMSCl in pyridine.[215] In the ^{1}H NMR spectrum the appearance of two singlets, one with a relative integral of six hydrogens at 0.11 ppm ((C*H*$_3$)$_2$Si) and one with a relative integral of nine hydrogens at 0.91 ppm ((C*H*$_3$)$_3$CSi), show that silylation has been achieved. The change in the chemical shift of the signals representing H-6a,b indicated that silylation has occurred at this site. These protons resonate at 4.26 ppm in the starting material and at 3.93 ppm in the product. The ^{13}C NMR spectrum of **22** also establishes the presence of the silyl group. The appearance of two highly shielded signals at -5.4 ppm is representative of the methyl groups of the 6-*O*-*tert*-butyldimethylsilyl ether ((*C*H$_3$)$_2$Si). The signal at 18.2 ppm identifies the quaternary carbon associated with the *tert*-butyl portion ((CH$_3$)$_3$*C*Si) of the silyl ether, while the signal at 25.8 ppm belongs to the three *tert*-butyl methyl groups ((*C*H$_3$)$_3$CSi). The molecular formula of compound **22** was confirmed by HREIMS and elemental analysis.

The target glycosyl donor, phenyl 3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**23**), was then prepared in 50% yield by

treating compound **22** with sodium hydride in a solution of benzyl chloride. In the ^{1}H NMR spectrum, the appearance of four sets of doublets (4.43, 4.78 ppm and 4.77, 4.86 ppm) representing two AB quartets demonstrated the presence of two benzylic methylene groups. The ^{13}C NMR spectrum of compound **23** confirmed the presence of two benzyl groups. Signals at 72.1 ppm and 75.0 ppm represent the benzylic methylene carbons, while additional signals in the aromatic region are indicative of di-*O*-benzylation.

The low yield of compound **23** may be due to several factors. Crich and Ritchie reported that the basic conditions required for benzylation, benzyl chloride/NaH, are capable of inducing silyl ether migration.[242] This group noted that a 3-O-substituted TBMDS group migrated to the 4 position of a glucal derivative under these conditions. Shekani and co-workers also noted that the TBDMS group can be removed using NaH in HMPA.[243] Although each of these examples offers a potential explanation for the low yield of compound **23**, the predicted products resulting from silyl ether migration or deprotection were not observed.

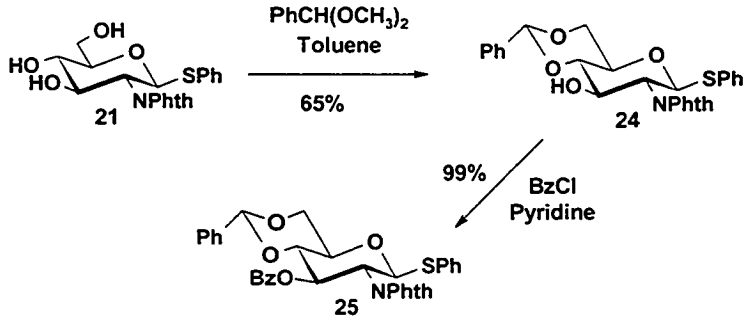
2.5.3.1 Glycosyl donor 25

Under kinetic conditions, benzylidene acetals are prepared using benzaldehyde dimethyl acetal while isopropylidene acetals[244] are made from 2-ethoxypropene,[245-247] 2-methoxypropene,[248-250] or 2,2-dimethoxypropane.[251] Acetalation reactions are acid catalysed, usually with *p*-toluenesulfonic acid[252] or camphorsulfonic acid[244] with heat, to remove the resulting methanol or ethanol pushing the reaction to completion. However, the acetal products are acid labile, more so for the six-membered ring isopropylidene acetal then six-membered ring benzylidene acetals. For these reasons, a benzylidene acetal was chosen to

simultaneously protect O-4 and O-6 of phenyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside.

2.5.3.2 Preparation of glycosyl donor 25

Phenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**25**) was prepared in two steps from compound **21** (Scheme 2.5.3.1). Acetalation of **21** with benzaldehyde dimethylacetal using a catalytic amount of *p*-toluenesulfonic acid in refluxing toluene afforded phenyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**24**) in 65% yield.[253] The reaction was driven to completion by removal of the byproduct methanol using a Dean-Stark apparatus. The presence of a sharp singlet in the ^{1}H NMR spectrum at 5.54 ppm (O_2CHPh) indicates the presence of the acetal hydrogen of the 4,6-benzylidene ring.[254] Further existence of acetal formation is shown in the ^{13}C NMR spectrum where an acetal carbon signal (O_2CHPh) appears at 102.1 ppm. Subsequent benzoylation of compound (**24**) using benzoyl chloride in pyridine at room temperature produced the target glycosyl donor, phenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**25**), in 99% yield. Proof of esterification of the remaining hydroxyl group (O-3) can be obtained from the ^{1}H NMR spectrum of compound **25**. The triplet representing H-3 has been shifted from 4.60 ppm for compound **24** to 6.21 ppm in the spectrum of the 3-*O*-benzoylated product. The ^{13}C NMR spectrum supports this conclusion as C-3 is much more deshielded in the product, 71.1 ppm. The appearance of a single signal in the carbonyl region, δ 165.8 (*C*OPh), of the spectrum confirmed a mono-*O*-benzoylated product.



Scheme 2.5.3.1 Synthesis of glycosyl donor 25

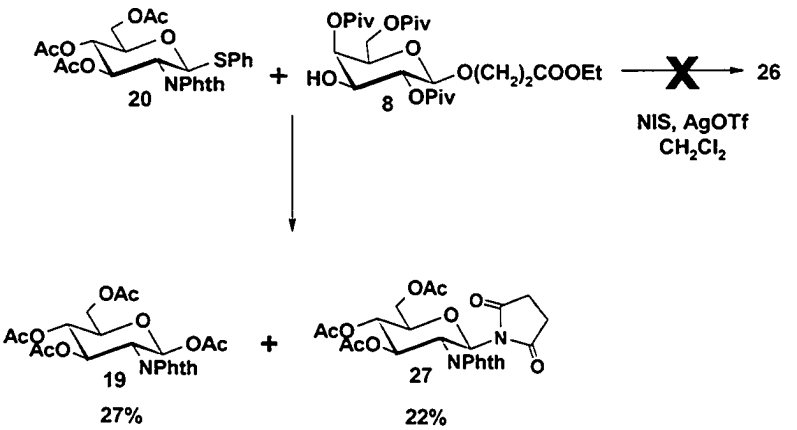
2.6 Synthesis of GlcNAcβ(1→3)Gal disaccharides

2.6.1 The sulfide glycosylation method

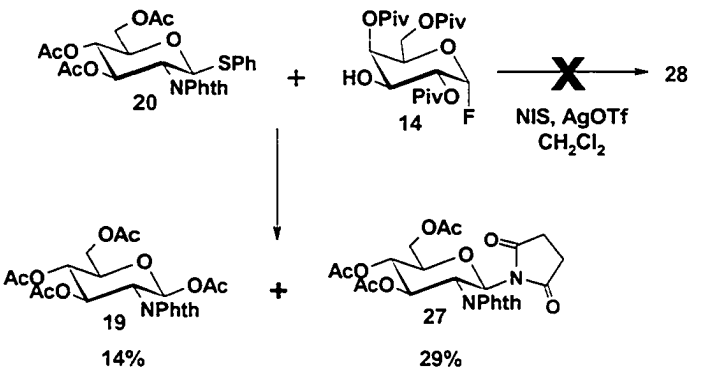
Glycosylation of the 2,4,6-tri-*O*-pivaloate glycosyl acceptor with glycosyl donor **20** did not give the desired disaccharide. The reaction of **8** with **20** using *N*-iodosuccinimide (NIS) as the electrophilic activator and silver triflate as the catalyst was carried out in dichloromethane at room temperature, Scheme 2.6.1.1. Three fractions were purified from the crude mixture by column chromatography. The first contained recovered glycosyl acceptor, compound **8**. The second product was identified as the *trans*-acetylated glycosyl donor, 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**19**) and was isolated in 27% yield. The third product from the reaction was determined to be *N*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)succinimide (**27**) and was isolated in 22% yield. In the ^{1}H NMR spectrum of **27**, the appearance a singlet at 1.82 ppm with a relative integral of four hydrogens shows the presence of the two methylene groups of the

five-membered ring succinamide. The signal for H-1 appears as a doublet centred at 5.56 ppm with the large coupling constant of 8.6 Hz, indicative of β-glycosides. The ^{13}C NMR spectrum of **27** further establishes the presence of the anomeric succinimide. The appearance of a signal at 14.5 ppm is representative of the methylene groups of the five-membered succinimide. Furthermore, the chemical shift of C-1 at 97.2 ppm is indicative of *N*-glycoside formation. The formation of **27** is a result of a reaction between the activated oxonium ion intermediate with the nucleophile succinimide. This result is an unexpected one as the lone pair on the nitrogen of succinimide is involved in resonance with the two adjacent carbonyl groups. Thus, this nitrogen would be expected to have low nucleophilicity. However, this problem was encountered by Wong and co-workers when a highly reactive glycosyl donor and an unreactive glycosyl acceptor was used.[157] This problem was also encountered by an honours student, Jonathan Watts, working on a similar project.[255]

Glycosylation of glycosyl acceptor **14** with glycosyl donor **20** using *N*-iodosuccinimide (NIS) as the electrophilic activator and silver triflate as the catalyst was carried out in dichloromethane at room temperature, Scheme 2.6.1.2. Three fractions were purified from the crude mixture by column chromatography. The first contained recovered glycosyl acceptor, compound **14**. The second contained the *trans* acetylated product **19** and was isolated in 14% yield The third isolated product was the glycosyl succinimide **27**, in 29% yield.



Scheme 2.6.1.1 **Attempt at glycosylation of glycosyl donor 20 with glycosyl acceptor 8**



Scheme 2.6.1.2 **Attempted glycosylation of glycosyl donor 20 with glycosyl acceptor 14.**

According to these results, this glycosylation method is not effective for the glycosylation of hindered nucleophiles. It was clearly established in section 2.4 that the pivaloate esters adjacent to position O-3 of galactose derivatives **8** and **14** cause a reduction

in reactivity at this site due to steric factors. Earlier, this reduced reactivity manifested itself during de-*O*-benzylation of compounds **7** and **9**. Here, it is shown that these factors also play a role in the formation of glycosidic linkages. In each case, it is clear that the glycosyl donor **20** has been activated by the promoter reagents. This is exemplified by the isolated products of reactions with other nucleophiles present in the reaction mixture (compounds **27** and **19**). These results demonstrate that a glycosylation reaction is not only dependent on the glycosyl donor used, but also on the availability of the nucleophile on the glycosyl acceptor.

2.6.2 The sulfoxide glycosylation method

Among the glycosyl donors recently developed, glycosyl sulfoxides have been shown to act successfully as glycosyl donors for the glycosylation of hindered nucleophiles. Sulfoxides were first introduced in 1989 by Kahne et al.[256] This group reported that even the most unreactive nucleophiles, such as phenols, can be coupled in high yield under mild conditions using β-glycopyranosyl sulfoxides as glycosyl donors. It was also noted that the reaction works well with both armed and disarmed glycosyl donors. Crich's group further expanded the usefulness of the sulfoxide glycosylation method by demonstrating that hindered tertiary alcohol acceptors can be glycosylated under these conditions.[257] More recently, Wipfs group showed that glycosyl sulfoxides are able to glycosylate tertiary butanol.[258] With these results in mind, it was hoped that the more reactive sulfoxide derivatives of compounds **20** and **25** would be able to react with a sterically hindered nucleophile.

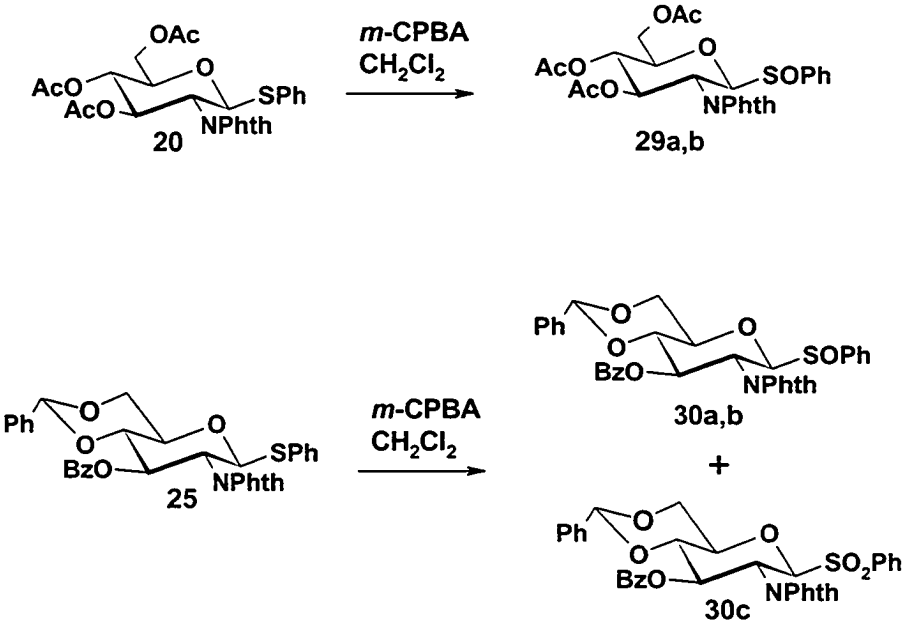
To date, a number of promoter systems for glycosyl sulfoxides have been described.

The most common reagents include; trifluoromethylsulfonyl (triflic) anhydride[256,259,260] (Tf_2O), trimethylsilyl triflate[261] (TMSTf), and benzenesulfenyl triflate[257] (PhSOTf) which are used as electrophiles in a stoichiometric amount. Other promotor reagents use a catalytic amount of triflic acid in the presence of large excess of methyl propiolate[262] or TEP (triethyl phosphite)[263] as a sulfenic acid (PhSOH) scavenger. Most recently, sulfoxides have been shown to be activated by Cp_2ZrCl_2-$AgClO_4$ to give good yields of glycosylated products.[264] Phenyl sulfoxides are usually made by the oxidation of the corresponding sulfides using hydrogen peroxide,[11] or *meta*-chloroperoxybenzoic acid,[12] or peroxyacetic acid at 25 °C.[191,192]

Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl sulfoxide was prepared as a mixture of diastereomers (**29a, 29b**) from compound **20** by oxidation with *m*-chloroperoxybenzoic acid (*m*CPBA),[190] Scheme 2.6.2.1. Careful control of stoichiometry at 1 eq. in dichloromethane at -78 °C to gave, in 96% yield, a mixture of the two sulfoxide diastereomers **29a** (*S* isomer) and **29b** (*R* isomer). The less polar diastereomer (**29a**) is present to a greater extent (R_f = 0.68, 0.54 in 5% acetone in dichloromethane). While the products of this reaction were not completely separated, ^1H and ^{13}C NMR spectra of purified fractions containing each diastereomer were obtained. These spectra were in good agreement with those reported earlier by Micheline Safatli.[169]

Phenyl 3-*O*- benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl sulfoxide was prepared as a mixture of diastereomers (**30a, 30b**) from compound **25** by oxidation with *m*-chloroperoxybenzoic acid,[190] in 89% yield, Scheme 2.6.2.1. A mixture of the two sulfoxide diastereomers **30a** and **30b** (R_f = 0.60, R_f = 0.55 respectively in 1:1 hexanes:ethyl acetate) were obtained in a ratio of 2:1 with a minor amount of a sulfone

byproduct, **30c**. The less polar diastereomer was tentatively assigned as the (S)-sulfoxide diastereomer, **30a**, with the more polar compound assigned as the (R)-sulfoxide isomer, **30b**. While the products of this reaction were not completely separated, ^{1}H and ^{13}C NMR spectra of purified fractions containing each diastereomer were obtained. The most obvious changes in the ^{1}H and ^{13}C NMR spectrum of **30a**, in comparison to the phenyl thioglycoside starting material (**25**), is shown by the chemical shifts associated with H-1 and C-1. They are 5.91 ppm (H-1) and 84.3 ppm (C-1) for compound **25** and 5.45 ppm (H-1) and 88.6 ppm (C-1) for compound **30a**. The chemical shifts for the remaining signals are relatively unchanged from starting material to product. The chemical shifts for H-1 (5.68 ppm) and C-1 (90.8) for compound **30b** show a similar pattern with little change in the chemical shifts of the remaining signals.



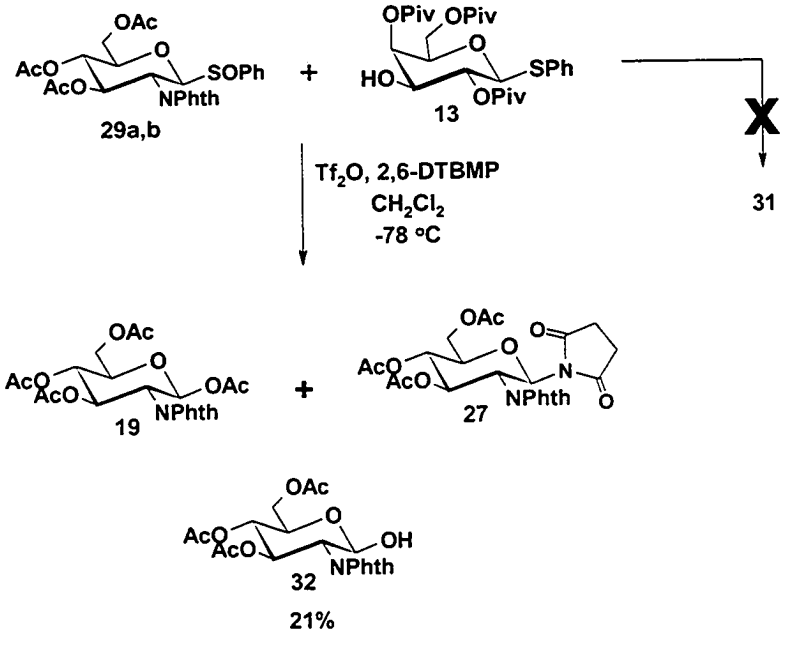
Scheme 2.6.2.1 Preparation of glycosyl sulfoxides 29a,b and 30a,b

Following a procedure reported by Voelter and coworkers,[265] the sulfoxide diastereomers **29a,b** were combined with the glycosyl acceptor **13** and a hindered acid scavenger, 2,6-di-*tert*butyl-4-methylpyridine (2,6-DTBMP), Scheme 2.6.2.2. Activation of the glycosyl sulfoxides is reported to occur rapidly at -78 °C to give a highly reactive glycosyl triflate, which then acts as the glycosyl donor.[190,257] The reaction was stirred and warmed up to -30 °C over a 45 minute period and the reaction quenched with saturated sodium hydrogen carbonate solution. The products were then separated by flash chromatography. Unfortunately the desired product, phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-pivaloyl-1-thio-β-D-galactopyranoside (**31**) was not isolated from the reaction mixture.

Four fractions, however, were purified from the crude mixture by column chromatography. The first contained recovered glycosyl acceptor, compound **13**. The second contained the *trans*-acetylated product, **19**, which was isolated in a low yield. The third isolated product was glycosyl succinimide **27**, also in low yield. The final product isolated was the hydrolysed glycosyl donor 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranose (**32**).

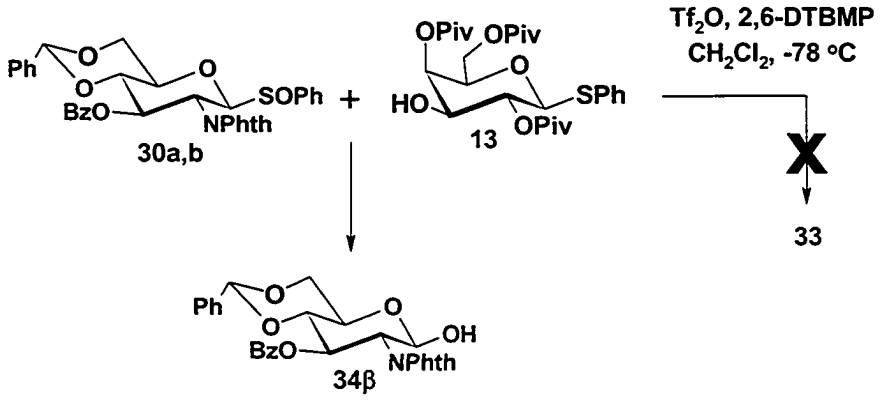
Following a procedure reported for the synthesis of compound **31**,[265] the sulfoxide diastereomers **30a,b** were combined with the glycosyl acceptor **13** and the acid scavenger 2,6-DTBMP. The reaction was treated with triflic anhydride and warmed up to -0 °C over a 45 minute period, Scheme 2.6.2.3. TLC analysis of the reaction mixture revealed the presence of five separate and distinct products. Unfortunately the desired product, phenyl (3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-

pivaloyl-1-thio-β-D-galactopyranoside (**33**) was not isolated from the reaction mixture



Scheme 2.6.2.2 Glycosylation of glycosyl donor 29a,b with glycosyl acceptor 13.

The first fraction contained recovered glycosyl acceptor, compound **13**. The second and third fractions contained an α/β mixture of hydrolysis products (**34α,β**), which was enriched in **34β**. The final two products were present in a small amount and were therefore not isolated or characterized.

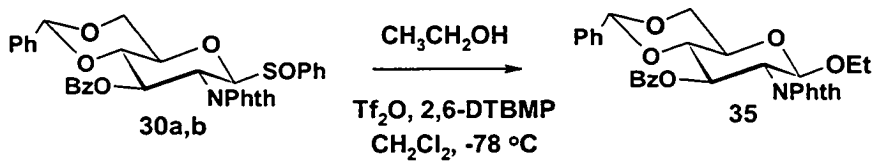


Scheme 2.6.2.3 Glycosylation of glycosyl donor 30a,b with glycosyl acceptor 13

In order to test whether the sulfoxide activation was proceeding correctly, the sulfoxide diastereomers **30a,b** and ethanol were combined with the acid scavenger 2,6-DTBMP, Scheme 2.6.2.4. The reaction was treated with triflic anhydride at -78 °C under nitrogen and warmed up to -0 °C. TLC analysis of the reaction mixture revealed the presence of one product on TLC which was determined to be the desired product, ethyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**35**), in 64% yield.

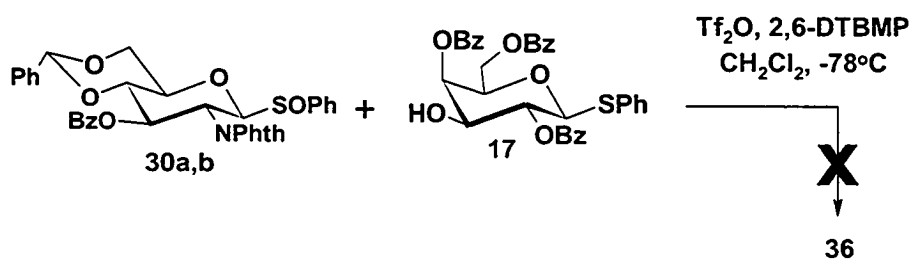
Proof of successful glycosylation can be found in the ^{1}H NMR spectrum of compound **35**. The appearance of a triplet at 1.07 ppm representing the methyl group of the ethyl glycoside. The presence of two diastereotopic proton signals, one at 3.60 ppm and the other as a part of a multiplet at 3.87 ppm, indicate the presence of the methylene portion of the ethyl glycoside. These protons were easily identified by a 2D-COSY experiment by coupling to the methyl signal at 1.07 ppm. The ^{13}C NMR spectrum supports the conclusion of successful glycoside formation. The signal for C-1 appears at 98.6 ppm, which is

characteristic of an acetal carbon. The appearance of signals at 65.8 ppm (OCH_2CH_3) and 15.2 (OCH_2CH_3) confirm the condensation of compound **30a,b** with ethanol.



Scheme 2.6.2.4 Glycosylation of glycosyl donor 30a,b with ethanol

Following a procedure reported for the synthesis of compound **31**, the sulfoxide diastereomers **30a,b** were combined with the glycosyl acceptor **17** and the acid scavenger 2,6-DTBMP, Scheme 2.6.2.5. The reaction was treated with triflic anhydride and warmed up to -0 °C over a 45 minute period. TLC analysis of the reaction mixture revealed the presence of seven separate and distinct products. Due to the number of products and the potential difficulties in purifying each fraction it was decided that the crude mixture would not be separated.



Scheme 2.6.2.5 Attempted glycosylation of glycosyl donor 30a,b with glycosyl acceptor 17

2.6.3 Conclusions

These experiments demonstrate that even very active glycosyl sulfoxides are not reactive enough to glycosylate the hindered pivaloate glycosyl acceptor. During the attempt at the formation of **31**, the major products observed resulted from reactions with other nucleophiles in the reaction mixture. The major product isolated results from a reaction with water. Both the *trans*-acetylated product (**19**) and the glycosyl succinimide (**27**) derivative were observed on TLC as minor products but were not quantified. It is feasible that these latter reactions do not occur at an appreciable rate at the low temperatures used in this experiment. It is also possible that the glycosyl triflate, determined to be the active species under these glycosylation conditions, is generated at -78 °C. This intermediate remains unreacted as the reaction mixture is warmed and quenched with a saturated sodium hydrogen carbonate solution. The introduction of water into the system at this point may account for the formation of the hydrolysed product, compound **32**. This hypothesis is supported by the attempt at synthesizing compound **33**. In this case, again, the major product formed was a result of condensation of the glycosyl donor with water giving compound **34β**.

As reported earlier the pivaloate esters adjacent to position O-3 of galactose derivative **13** cause a reduction in reactivity at this site due to steric factors. Here, further evidence indicates that these factors also play a role in the formation of glycosidic linkages. In each case, it is clear that the glycosyl sulfoxides **29a,b** and **30a,b** have been activated by the promoter reagents. A reaction with an unhindered nucleophile, ethanol, under these conditions with **30a,b** proceeds smoothly to give a good yield (64%) of the ethyl glycoside (**35**). This result further substantiates that a glycosylation reaction is not only dependent on

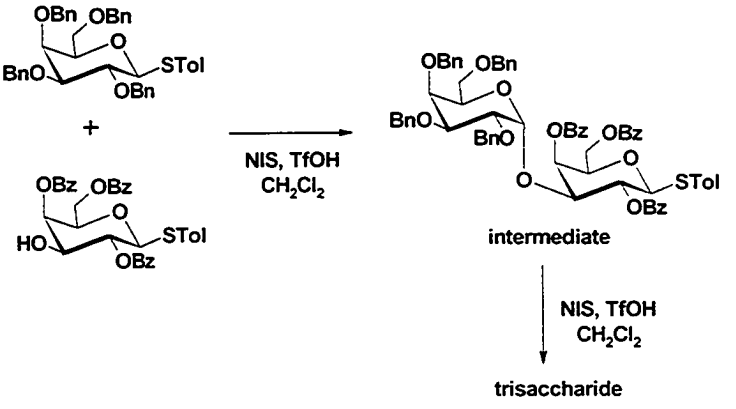
the glycosyl donor used, but that the availability of the nucleophile on the glycosyl acceptor is of major importance.

The final attempt at forming the β(1→3) linkage between GlcNAc and Gal using the sulfoxide glycosylation method failed to give the desired product, phenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (**35**). In this case a number of factors may play a role in preventing glycoside formation.

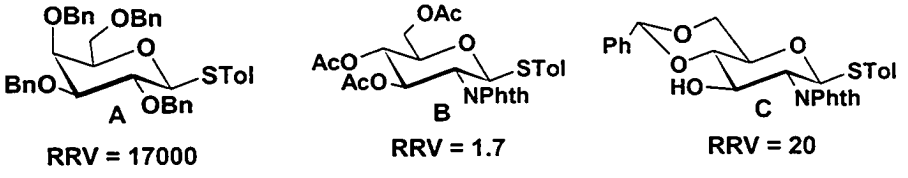
A reaction performed by Wong and co-workers[157] demonstrates that glycosylation of a *p*-methylphenyl 2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside can be achieved using a highly active glycosyl donor, *p*-methylphenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside, as a part of a longer synthetic pathway, Scheme 2.6.3.1. In this case, it is believed that the steric factors imparted by the neighboring benzoyl groups can be overcome by the use of a highly reactive glycosyl donor. As reported earlier, it is well established that the sufoxide method is capable of effecting glycosylation of hindered nucleophiles. At first glance, a conclusion may be drawn that glycosyl sulfoxides **29a** and **30a,b** should be more active than *p*-methylphenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside. Although a direct comparison cannot be made between these glycosyl donors, perspective can be gained by a comparison of the corresponding sulfides, Scheme 2.6.3.2.

According to Scheme 2.6.3.2, there is a significant difference in reactivity between the corresponding sulfide glycosyl donors. That used by Wong and co-workers (**A**) is clearly more active that those used in our synthetic methodology (**B** and **C**). It should also be noted

that **C** is unprotected at position O-3. Protection of this hydroxyl group by a benzoate ester should serve to deactivate the anomeric centre even further, thus making glycosyl sulfide **C** even less active as a glycosyl donor. With this taken into account, it is possible that



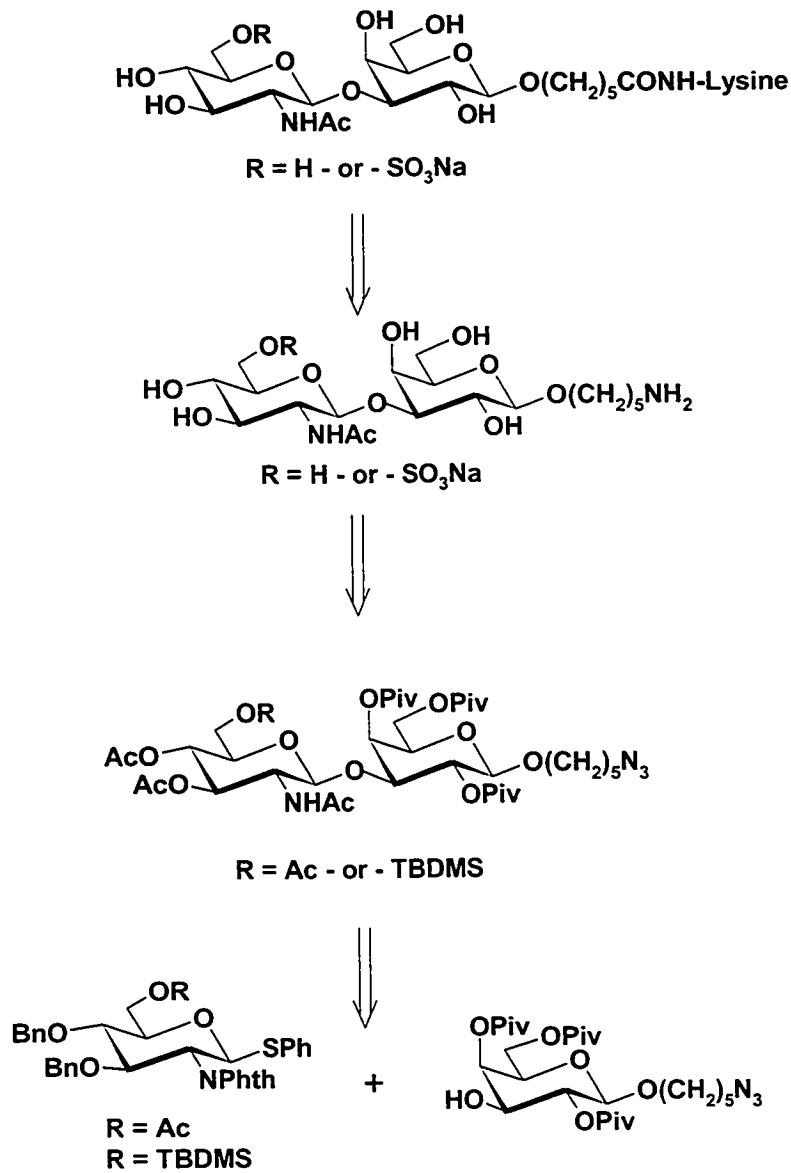
Scheme 2.6.3.1 **Glycosylation of *p*-methylphenyl 2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside using a highly reactive glycosyl donor**



Scheme 2.6.3.2 **Relative reactivity of glycosyl donors**[157]

conversion of unreactive glycosyl sulfides **20** and **25** into their sulfoxide cousins (**29a,b** and **30a,b**) does not impart the necessary reactivity required to react with a hindered glycosyl acceptor. Given that glycosyl sulfoxides are among the most active of glycosyl leaving groups it was decided that a less hindered glycosyl acceptor would be required to effect the

desired linkage.



Scheme 2.7 Retrosynthetic analysis for the second glycosylation approach in the synthesis of neutral and acidic disaccharides

2.7 Glycosyl acceptor 48β

2.7.1 General

Allyl ethers are commonly used protecting groups, and can be easily cleaved in the presence of other protecting groups.[194,266] They can be made under acidic or basic conditions: with allyl bromide and base or with allyltrichloroacetimidate and acid.[267] These types of ethers can be removed by isomerization with potassium *tert*-butoxide in dimethylsulfoxide (DMSO)[137,268] to prop-1-enyl ethers that are easily cleaved by mild acid or basic aqueous potassium permanganate.[268] However, allyl ethers can be cleaved in one step by isomerization and hydrolysis with palladium chloride and sodium acetate in acetic acid.[269] They can also be removed in the presence of a trace of *p*-toluenesulfonic acid and a catalytic amount of palladium on activated charcoal.[270] Furthermore, sulfinic acids or the corresponding salts are effective allyl scavengers in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium [$Pd(PPh_3)_4$] and are highly effective for facilitating the carbon-oxygen bond cleavage of allyl ethers.[271] With these observation in hand, it was decided that O-3 of thiogalactoside (**4**) would be selectively allylated using the chemistry of stannylene acetals and allyl bromide.

A suitable choice of protecting group is required for O-2, O-4, and O-6 that, upon removal of the allyl group at O-3, will allow glycosylation to take place without the complications of steric hindrance. A number of researchers have reported that formation of glycosidic linkages at position O-3 of galactose is easily accomplished when the neighboring protecting groups on O-2 and O-4 are benzyl ethers.[165,264,272-274] For these reasons, the benzyl

ether was chosen to protect the remaining hydroxyl groups of thiogalactoside **45**. This choice, however, introduces a problem of stereospecificity during the condensation of compound **47** with linker arms. The introduction summarized the evidence that β-galactose at the reducing end is a requirement for sperm binding. The presence of a C-2 non-participating benzyl group during glycosylation will result in the synthesis of predominantly α-galactose products, Scheme 1.7.1b. To solve this problem, the glycosylation of **47** with the linker arm will be performed in acetonitrile.[118,275] This procedure takes advantage of solvent participation in order to generate the desired β-linkage, Scheme 1.7.1c.

At this point alternative linker arm compounds were also investigated. Recent trends in the literature have taken advantage of the chemistry of diethyl squarate.[276-278] This molecule acts as a linking reagent by reacting with two stoichiometric equivalents of amine. Researchers have taken advantage of this property. In the first step, diethyl squarate is reacted with one equivalent of an oligosaccharide bearing an amino functionalized linker arm. In the second step, the oligosaccharide-squarate adduct is reacted with a second equivalent of amine, usually the γ-amino group of lysine on a protein. This two step procedure results in the coupling of the oligosaccharide to the carrier protein.

As discussed earlier a linker arm that can react with carbohydrate residues at one end and with diethyl squarate at the other will be required. 5-Azido-1-pentanol is such a bi-functional linker arm. This compound offers a primary hydroxyl group for glycosylation reactions at one end and an azido group at the other. In a later step, the azido functional group can be reduced to an amine to allow coupling with diethyl squarate. Furthermore, this compound maintains a five carbon spacer between each functional group allowing the

attached carbohydrates to be extended into the aqueous media surrounding the carrier protein. The following section describes several methods for the synthesis of 5-azido-1-pentanol followed by its participation as a glycosyl acceptor in a glycosylation reaction with compound **47**.

2.7.2 Synthesis of 5-azido-1-pentanol

The regioselective alkylation of diols and polyols using stannylene acetals was first encountered in this thesis in the selective benzylation of phenyl 1-thio-β-D-galactopyranoside (**4**) to form the 3-*O*-benzyl ether of compound **5**. This reaction has been widely used to selectively protect symmetrical diols. In particular, Mash and coworkers have prepared monobenzyl ethers of 1,n-alkane diols in good yield.[279] It was decided that the symmetrical diol, 1,5-pentanediol, would serve as a suitable substrate for selective alkylation in the synthesis of the target linker arm. Accordingly, stannylenation of 1,5-pentanediol (**37**) with dibutyltin oxide was performed in refluxing toluene overnight with the azeotropic removal of water using a Dean-Stark trap.[279] After draining the trap, the resulting dibutylstannylene acetal was directly treated with allyl bromide and tetrabutyl ammonium bromide as a catalyst. After refluxing overnight 5-allyloxy-1-pentanol (**38**) was isolated in 56% yield. In the ^{1}H NMR spectrum of compound **38**, the molecule has clearly lost its symmetry. The appearance of second order multiplets at 5.90 ppm (CH_2=C*H*CH_2O), 5.23 ppm (CH_2=CHCH_2O) and 3.95 ppm (CH_2=CHCH_2O) represent the signals for the allyl ether. The presence of two triplets at 3.59 ppm (CH_2OH) and 3.43 ppm ($CH_2OCH_2CH_2$) indicates that the molecule is unsymmetrical. This further demonstrates that the mono-*O*-allylated product has been

synthesized. These findings are confirmed by the ^{13}C NMR spectrum. Signals at 135.0 ppm and 117.0 ppm represent the sp^2 hybridized carbons of the allyl ether while the five signals representing the carbon chain each have their own distinct environment.

The free hydroxyl group of compound **38** was then converted into the toluenesulfonate ester to give compound **39** in a low yield of 46%. The appearance of two doublets (7.78 ppm and 7.33 ppm) in aromatic region of the ^1H NMR spectrum of **39** in conjunction with a singlet at 2.44 ppm (CH_3Ph) represent the signals of the toluenesulfonate group. Furthermore, the appearance of six signals in the aromatic region of the ^{13}C NMR spectrum as well as a singlet at 22.2 ppm (CH_3Ph) indicated success in the tosylation of **38**.

An S_N2 displacement of the toluenesulfonate group of compound **39** with sodium azide in *N,N*-dimethylformamide resulted in the formation of 5-allyloxy-1-azidopentane, compound **40**, in 88% yield. In the ^1H NMR spectrum of **40**, the disappearance of signals characteristic of the toluenesulfonate group (described for compound **39**) indicate success in its displacement by azide.

Removal of the allyl group to form the desired azido alcohol, compound **41**, proved to be more difficult than anticipated. Treatment of compound **40** with palladium(II) dichloride[280] and sodium acetate in acetic acid:water (20:1) produced a low yield (10%) of 5-azido-1-pentanol, **41**. Flash chromatography of the crude mixture (4:1 hexanes ethyl acetate) produced three fractions. The first fraction ($R_f = 0.90$) contained recovered starting material in 5% yield. The second fraction contained 1-acetoxy-5-azidopentane ($R_f = 0.80$, 7% yield), compound **42**. The final fraction contained the desired product compound **41**, 5-azido-1-pentanol ($R_f = 0.20$, 10% yield). In the ^1H NMR spectrum of **41**, the disappearance

of signals characteristic of the allyl group, described for compound **38**, as well as a change in the chemical shift for the signal representing the substituted methylene bearing the allyl ether (3.43 ppm for **38**, 3.66 ppm for **41**) confirmed its structure. This conclusion is supported by the ^{13}C NMR spectrum of **41**, allyl carbon signals disappeared and there was a distinctive change in the chemical shift of the *O*-methylene carbon signal, 72.0 ppm for compound **39** and 62.8 for azide derivative **41**.

An attempt at improving the yield for removal of the allyl group was made using the reagents described earlier for the removal of sterically hindered benzyl groups. Light catalysed α-bromination[215] of the allyl ether moiety of **40** using NBS and calcium carbonate in a two-phase system of carbon tetrachloride:water afforded the desired product, **41**, in a disappointing 22% yield.

Compound	37	38	39	40	41	42	43	44
R	OH	OH	OTs	N_3	N_3	OAc	I	Br
R1	OH	OAll	OAll	OAll	OH	N_3	OH	OH

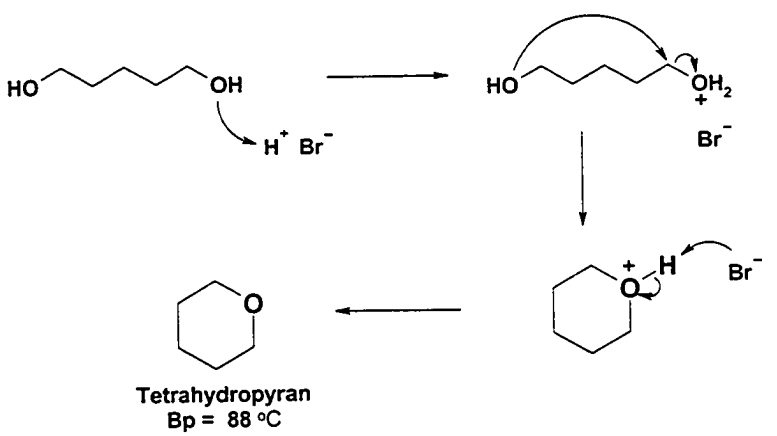
Figure 2.7.2 Derivatives of 1,5-pentanediol investigated during the synthesis of 5-azido-1-pentanol

Due to the difficulties in removing the allyl ether of **40** it was decided to attempt a selective substitution of one of the alcohols of 1,5-pentanediol. Marcantoni and co-workers[281] recently reported that 1,5-pentanediol was selectively iodinated using cerium trichloride heptahydrate and sodium iodide in refluxing acetonitrile. This group reported an excellent

yield (80%) of 5-iodo-1-pentanol (**43**) under these conditions. In our hands, however, these conditions provided the product in a very low yield of 8%.

A second attempt at monosubstitution was performed by treating compound **37** with 9 M hydrobromic acid in refluxing toluene for 24 h.[282] This reaction afforded the desired monosubstituted bromo alcohol, compound **44**, in 24% yield. A minor amount of 1,5-dibromopentane was also isolated but was not quantified. One possible explanation for the low yield of **44** and the lack of recovered starting material is proposed in Scheme 2.7.2. Acid catalysed cyclization of **37** could result in the formation of tetrahydropyran (bp = 88 °C). This compound would have been lost during work-up due to its low boiling point.

Compound **44** was then treated with sodium azide in *N*,*N*-dimethylformamide to afford the desired linker arm compound, 5-azido-1-pentanol (**41**) in 80% yield. The structure of **41** was confirmed by ^{1}H and ^{13}C NMR spectroscopy, which were in good agreement with data reported earlier. When combined, these final two experiments represent the easiest and most cost effective method for the preparation of the linker arm.



Scheme 2.7.2 Formation of tetrahydropyran during the bromination of compound 37

2.7.3 Preparation of glycosyl acceptor 48β

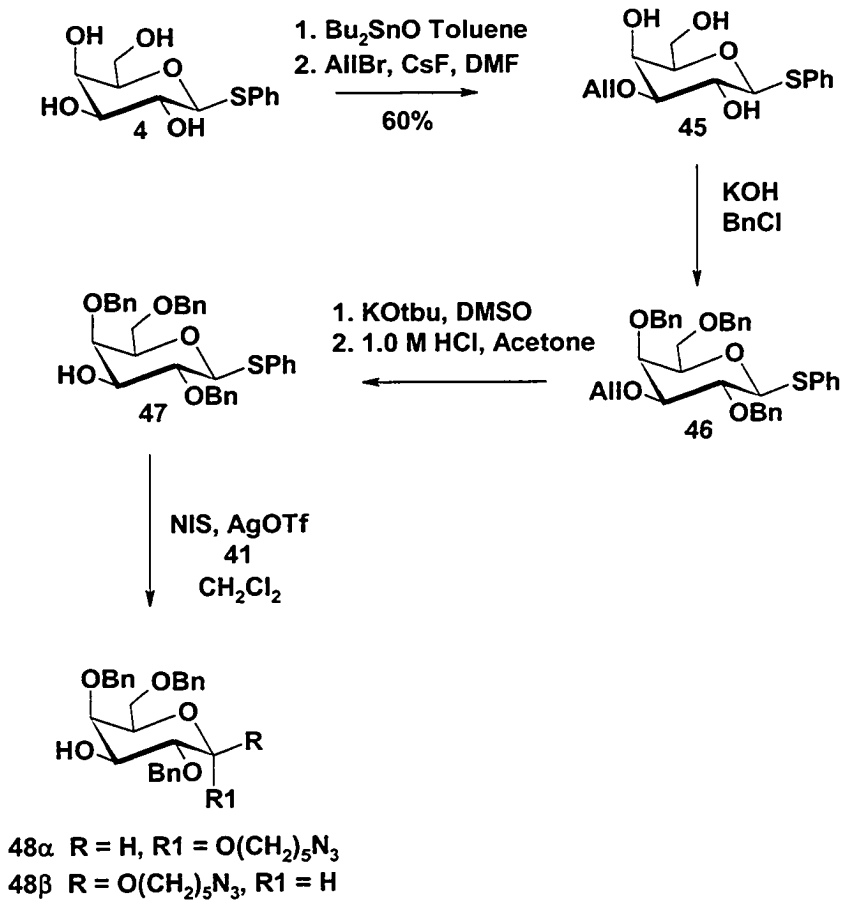
5-Azidopentyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**48β**), was prepared in four steps from fully deprotected thioglycoside **4** (see Scheme 2.7.3). Stannylenation of phenyl 1-thio-β-D-galactopyranoside (**4**) with dibutyltin oxide was performed in refluxing toluene overnight with the azeotropic removal of water using a Dean-Stark trap. After solvent removal, treatment of the resulting 3,4-*O*-dibutylstannylene acetal with allyl bromide and cesium fluoride in *N*,*N*-dimethylformamide at room temperature gave the 3-*O*-allylated galactose derivative (**45**) in 52% yield.[204] In the ^{1}H NMR spectrum of **45**, the three signals, one (8 lines) at 5.92 ppm and two overlapping (8 lines) at 5.27 ppm due to these protons CH_2=C*H*CH_2O and C*H*$_2$=$CHCH_2O$, respectively, reveal the presence of the allyl group. The deshielded signal of C-3 in the ^{13}C NMR spectrum at 81.1 ppm verifies that the allylation has taken place at O-3. The molecular formula of **45** was confirmed by chemical analysis.[205]

Benzylation of **45** using benzyl chloride and potassium hydroxide by stirring for 8 h at 100 °C afforded phenyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**46**) in 92% yield. The ^{1}H NMR spectrum shows the six methylene protons of three benzyl groups as AB quartets at 4.73 ppm, 4.41 ppm and 3.61 ppm. The signals of the three methylene carbons of the benzyl groups appear in the ^{13}C NMR spectrum at 75.5, 73.4 and 68.6 ppm. The observation of these signals prove that the tri-*O*-benzyl product was obtained. The molecular formula of **46** was confirmed by chemical analysis.

The removal of the allyl ether protecting group ($ROCH_2CH=CH_2$) at C-3 was performed by isomerization of compound **46** with potassium *tert*-butoxide in DMSO using the procedure of Gigg and Gigg[137] to form the *cis*-prop-1-enyl ether ($ROCH=CHCH_3$). The

prop-1-enyl ether was then refluxed with 0.1 M hydrochloric acid in acetone-water[137] for 45 minutes to afford phenyl 2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**47**) in an overall yield of 84%. The disappearance of the allyl protons and carbons from the ^{1}H and ^{13}C NMR spectra indicates that the removal of the allyl group was successfully achieved. In addition, the appearance of the signal due to the hydroxyl proton 3-OH in the ^{1}H NMR spectrum at 2.20 ppm supports this removal. Physical data of this compound were in full agreement with those published in the literature.[165]

5-Azidopentyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**48β**) was obtained in 73% yield by reacting thioglycoside (**47**), the glycosyl donor, with the linker compound 5-azido-1-pentanol (**41**) using NIS and AgOTf as promoter, conditions reported by Fraser-Reid and coworkers.[175] Since the benzyloxy group at C-2 does not act as a neighboring participating group, the reaction was performed in acetonitrile. This methodology was successful in the synthesis of the β product with only a small amount of the α-anomer (**48α**, 11%) present. In the ^{1}H NMR spectrum of compound **48β**, the coupling constant ($J_{1,2}$ = 7.3 Hz) of the doublet at 4.32 ppm is characteristic of β-glycosides. All of the protons of the linker arm moiety are present in the spectrum. The appearance of C-1 at 103.9 in the ^{13}C NMR spectrum is indicative of glycoside formation, while the appearance of four methylene carbons in the alkyl region, 24.6-34.2 ppm, as well as an additional methylene signal at 51.5 ppm (CH_2N_3) reaffirm the successful condensation of the glycosyl donor with 5-azido-1-pentanol. Optical rotations for of **48β** and **48α** were in good agreement with recently reported values.[264] The formulae of **48β** and **48α** were further confirmed by HRMS.



Scheme 2.7.3 Preparation of glycosyl acceptor 48β

2.7.4 Sulfide glycosylation method with glycosyl acceptor 48β

The glycosylation reaction between glycosyl acceptor **48β** and phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranoslide (**20**) (1.5 eq) was performed as previously described in the presence of NIS and a catalytic amount of TfOH.[157,175] The disaccharide derivative 5-azidopentyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1−3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**49**) was obtained in 1 h in 37% yield, Scheme 2.7.4. Evidence for the successful condensation of the reactants was

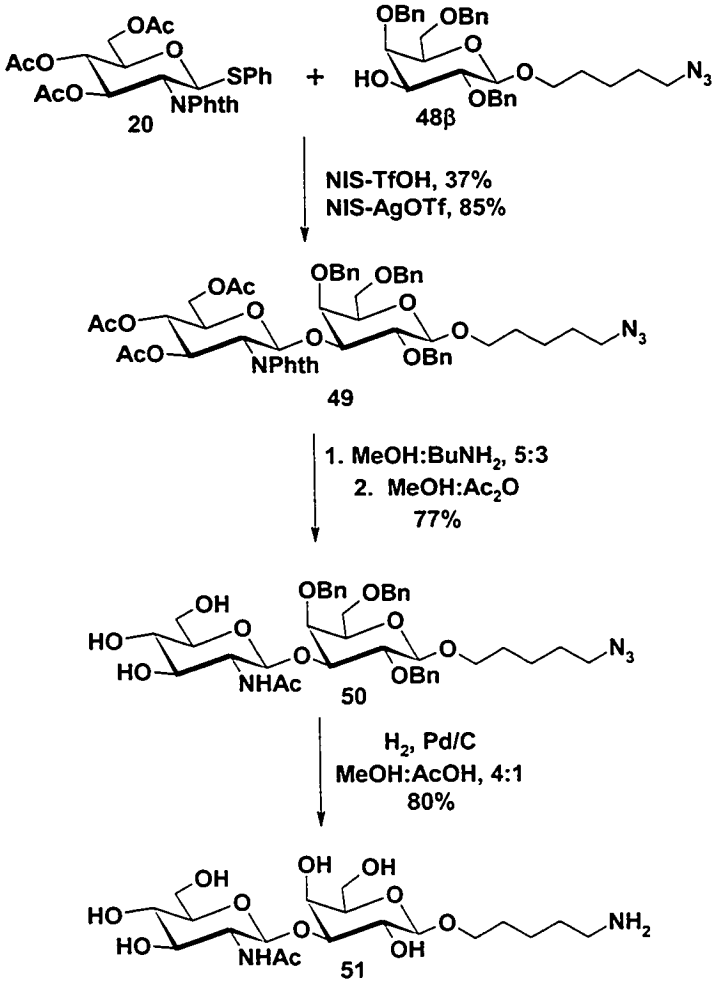
found in the ^{1}H NMR spectrum of **49**. The formation of the β-glycoside product was supported by the presence of the H-1' doublet at 5.71 ppm ($J_{1,2}$ = 8.4 Hz) while the signal for H-1 appears as another doublet at 4.24 ppm ($J_{1,2}$ = 7.7 Hz). The deshielded protons H-3' and H-4' are clearly shown at 5.85 ppm and 5.17 ppm respectively, while a triplet at 2.98 ppm (CH_2N_3) confirms the presence of the linker arm in the structure of **49**. The anomeric carbons C-1' and C-1 resonate at 99.4 ppm and 104.0 ppm in the ^{13}C NMR spectrum, respectively. The structure of **49** was further confirmed by HRMS. The low yield, however, prompted an investigation of other promoter systems to increase the yield of this glycosylation reaction.

The glycosylation reaction between glycosyl acceptor, **48β**, and 3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside, **23** (2.0 eq) was performed as previously described in the presence of NIS and AgOTf.[175] Using this promoter system, the disaccharide derivative 5-azidopentyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranosyide (**49**) was obtained in 1 h in 85% yield, Scheme 2.7.4.

Deprotection of the *N*-phthalimido group requires vigorous conditions which range from heating with methanolic or ethanolic hydrazine[145,283] to 4-aminobutane in refluxing methanol,[284-286] to sodium borohydride.[235,287] In order to cleave the phthalimido group and to saponify the ester groups simultaneously, compound **49** was treated with 4-aminobutane in methanol (3:5, v:v) at reflux for 48 h. After removal of the solvent under reduced pressure, the resulting aminoalcohol was stirred with acetic anhydride in methanol (1:10, v:v) for 24 h to selectively acetylate the amino group to give 5-azidopentyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**50**) in 77% yield, Scheme

2.7.4. In the ^{1}H NMR spectrum, the disappearance of the three singlets representing the methyl protons of the *O*-acetyl groups at 2.04, 2.01 and 1.85 ppm indicates the removal of the three acetates. The obvious change in chemical shift for the signals representing H-3' and H-4', 5.85 ppm and 5.17 ppm, respectively, for the starting material **49** and 3.60 ppm and 3.45 ppm, respectively, for the product **50**, indicates the removal of the electron withdrawing acetyl groups in the first step of the reaction. The appearance of an amide proton (AcN*H*) at 6.85 ppm shows that 2'-*N*-acetylation was successful during the second step of the reaction. In the ^{13}C NMR spectrum of **50**, the disappearance three signals in the carbonyl region of the spectrum at 170.7, 170.2, and 167.6 ppm are indicative of de-*O*-acylation. The appearance of a more deshielded signal at 172.0 ppm ($NCOCH_3$) is further evidence that 2'-*N*-acetylation was successful. The anomeric carbons C-1 and C-1' resonate at 105.2 and 104.4 ppm respectively, for compound **50**.

The benzyl ethers of compound **50** were removed by a catalytic hydrogenation using H_2(g) and 10% Pd/C in methanol:acetic acid (4:1), stirring for 21 h at atmospheric pressure and room temperature. The fully deprotected disaccharide derivative 5-aminopentyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (**51**) was obtained in 79% yield. The disappearance of the aromatic protons from the aromatic region, δ 7.34-7.23, is one of the indications that the benzyl groups were successfully removed. Reduction of the azido functional group is shown by the change in chemical shift of the adjacent methylene carbon in the ^{13}C NMR spectrum of **51**. For azide **50**, the signal appears at 52.6 ppm, while in the product amine, **51**, the signal has shifted to 40.0 ppm.



Scheme 2.7.4 **Preparation of neutral disaccharide 51.**

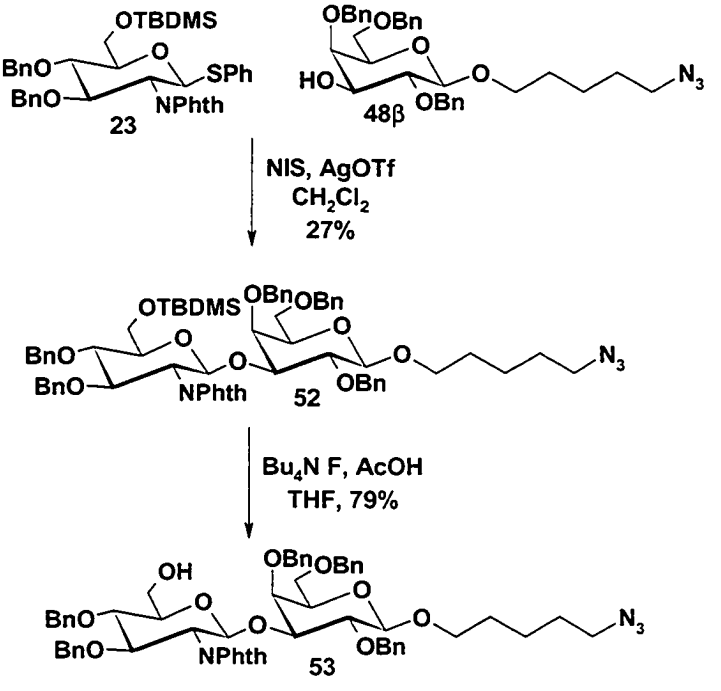
The glycosylation reaction between glycosyl acceptor **48β** and 3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside, **23**, (2.0 eq) was performed as previously described in the presence of NIS and AgOTf, Scheme 2.7.5. Three fractions were purified from the crude mixture by column chromatography using hexanes:ethyl acetate (4:1) as eluent. The first contained the desired product, 5-azidopentyl 3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-tert-butyldimethylsilyl-β-D-glucopyranosyl-

(1-3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**52**, R_f = 0.51), in 26% yield. The structure of **52** was confirmed by ^1H and ^{13}C NMR spectroscopy. In the ^1H NMR spectrum the doublet at 5.50 ppm ($J_{1,2}$ = 8.4 Hz) representing H-1' is indicative of β-glycoside formation. Singlet signals for the *tert*-butyldimethylsilyl ether appear at 0.92 ppm ($SiC(CH_3)_3$), and at 0.08 ppm and 0.06 ppm ($Si(CH_3)_2$) respectively. The presence of the linker arm was confirmed by a triplet at 3.00 ppm (CH_2N_3). Glycoside formation was further demonstrated by the presence of two acetal carbon signals in the ^{13}C NMR spectrum of **52**. The signal for C-1 appeared at 104.1 ppm and that for C-1' at 99.4 ppm. The five signals representing the α-carbons of each benzyl group were easily identified using the JMOD technique while all of the carbon signals representing the linker arm were present.

The second fraction (R_f = 0.35) recovered by flash chromatography contained the glycosyl acceptor (**48β**) in 53% yield. The final and most abundant product isolated (R_f = 0.30) contained a compound that could not be identified by ^1H and ^{13}C NMR spectroscopy. The presence of signals representing the *tert*-butyldimethylsilyl ether in both spectra indicate that this compound was related to the glycosyl donor. In the ^{13}C NMR spectrum, however, only three carbon signals in the aromatic region appeared. These signals were accompanied by a potential acetal carbon signal at 101.7 and a potential hemi-acetal carbon signal at 90.9 ppm. Taken together, the structure of the compound could not be unambiguously assigned.

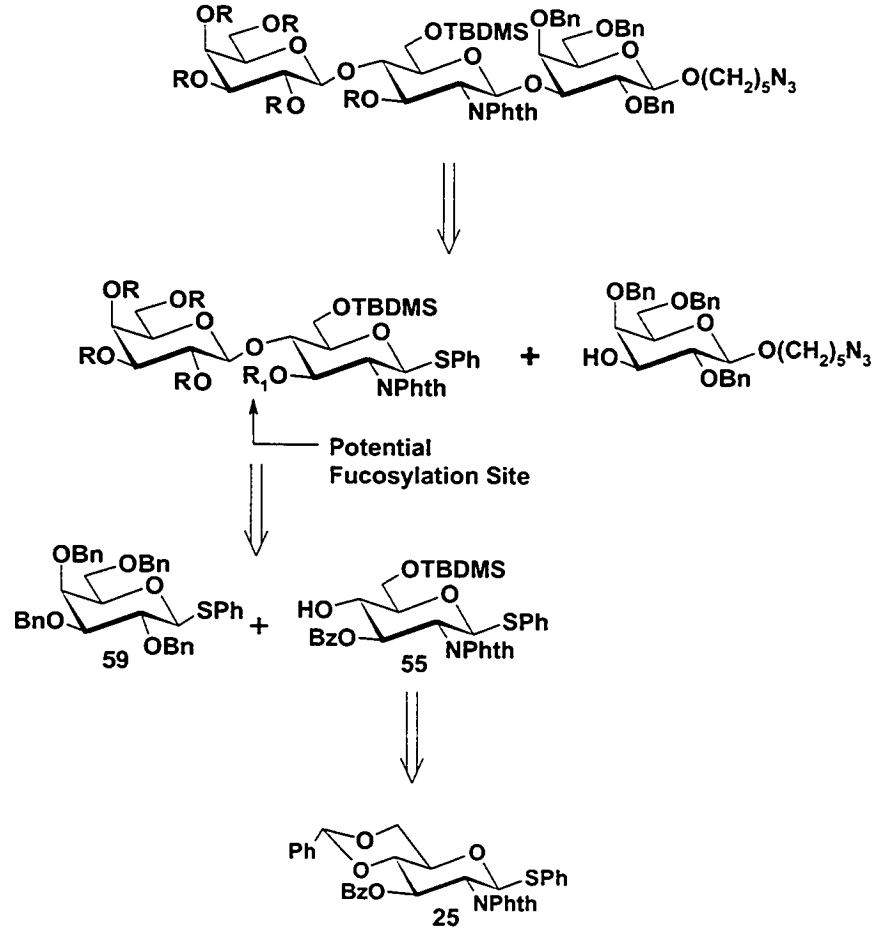
Removal of the *tert*-butyldimethylsilyl ether was accomplished by treating compound **52** with tetrabutylammonium fluoride in tetrahydrofuran[237] to give compound **53** in 79% yield. Removal of the silyl ether was confirmed in the ^1H NMR spectrum of **53**. The disappearance of signals at 0.92 ppm ($SiC(CH_3)_3$), 0.08 ppm and 0.06 ppm ($Si(CH_3)_2$) were

indicative of success in this transformation. The appearance of a broad triplet at 2.46 ppm represents the hydroxyl group which remains after de-*O*-silylation. These findings are confirmed in the ^{13}C NMR spectrum of **53**. The absence of signals at 29.3 ($SiC(CH_3)_3$), 26.0 ($SiC(CH_3)_3$), -4.9, and -5.2 (2 x $SiC(CH_3)_3$) show that the silyl ether has been successfully cleaved. The signal for C-1 appeared at 104.1 ppm and that for C-1' at 99.6 ppm.



Scheme 2.7.5 **Glycosylation of glycosyl donor 23 with glycosyl acceptor 48β and preparation of disaccharide 53.**

At this point in the synthesis further manipulations along this synthetic scheme were suspended in order to concentrate on the formation of the second glycosidic linkage. The reasons for this decision will be made more clear in the following sections.



Scheme 2.8 Retrosynthetic analysis for the synthesis of trisaccharides using an *N*-acetyllactosamine glycosyl donor

2.8 Synthesis of Galβ(1→4)GlcNAc disaccharides

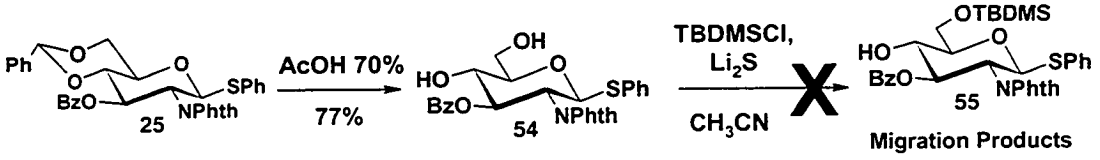
2.8.1 Synthesis of glycosyl acceptor 55

Deacetalation of phenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside, compound **25**, Scheme 2.8.1, was performed by refluxing in 70%

acetic acid in water[187] for 2 h to produce the desired product phenyl 3-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**54**) in 77% yield. The disappearance of the sharp singlet in the ^{1}H NMR spectrum at 5.54 ppm (O_2CHPh) indicates that the acetal hydrogen of the 4,6-benzylidene ring has been removed. Further evidence of deacetalation is shown in the ^{13}C NMR spectrum where the acetal carbon signal (O_2CHPh), which previously appeared at 102.1 ppm, is now absent.

An anticipated problem in the selective silylation of compound **54** arises in the potential for benzoate ester migration, which has been shown to occur under basic conditions.[157] At this point α-L-fucosylation of O-3 remained an option for the synthesis of higher oligosaccharides. The role of L-fucose in sperm-egg interactions had not been clearly defined so it was necessary to incorporate a protecting group strategy that would allow for the selective removal of the group at O-3 for fucosylation at a later stage in the synthesis. In order to prevent the anticipated migration of the benzoyl ester using TBDMSCl/pyridine, it was decided that an alternative method for silylation would be used. Consequently, compound **54** was treated with *tert*-butyldimethylsilyl chloride and lithium sulfide in acetonitrile, conditions reported by Olah et al. to be neutral.[288] The reaction was allowed to stir for 21 h where TLC, hexanes:ethyl acetate (1:1), of the reaction mixture revealed the presence of three compounds. The first compound ($R_f = 0.40$) was less polar than that of the starting material and was most likely a monosilylated product. The second and third compounds ($R_f = 0.20$, $R_f = 0.15$ respectively) were equal in intensity. The second compound corresponded to the starting material, 3-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**54**). Due to the similarities in polarity of the third compound with the

second compound it was postulated that this product was a result of benzoate migration. These compounds, however, were not isolated or characterized. Compound **55** was, therefore, not isolated. It was decided that the complications of benzoate migration would not be further investigated. Instead, the migrating benzoate ester was replaced by a benzyl ether and fucosylation would performed at an earlier stage in the synthesis if necessary.



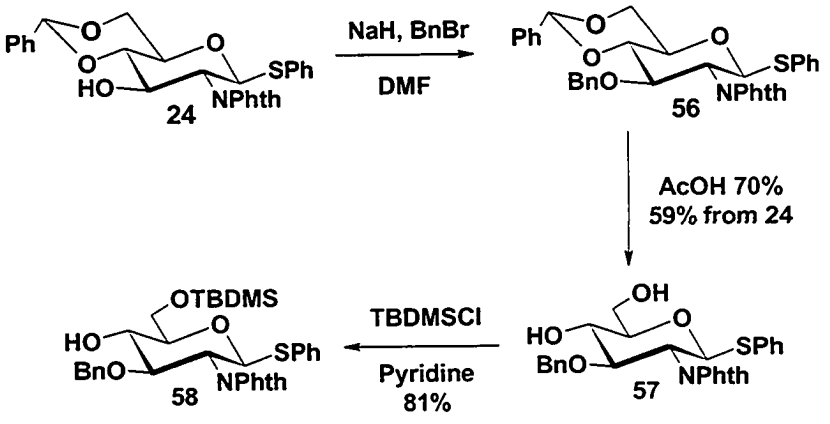
Scheme 2.8.1 Attempt at the synthesis of compound 55

2.8.2 Synthesis of glycosyl acceptor 58

Compound **58** was synthesized in three steps from phenyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**24**), Scheme 2.8.2. Benzylation of the remaining hydroxyl group, O-3, of **24** using sodium hydride and benzyl bromide in *N,N*-dimethylformamide afforded the desired product, namely phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**56**). A portion of the crude compound was purified by flash chromatography (R_f = 0.45) using hexanes ethyl acetate (2:1) as eluent. The structure of **56** was confirmed by comparing known ^1H and ^{13}C NMR data as well as the optical rotation, which were in good agreement with the reported values.[289]

The remaining portion of crude compound **56** was refluxed in 70% acetic acid for 2 h[187] to afford phenyl 3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**57**) in 59% yield from compound **24**. In the ^1H NMR spectrum of **57**, the disappearance of the sharp

singlet at 5.61 ppm (O_2CHPh) indicated that the acetal hydrogen of the 4,6-benzylidene ring has been removed. Further evidence of deacetalation is shown in the ^{13}C NMR spectrum



Scheme 2.8.2 Synthesis of glycosyl acceptor 58

where the acetal carbon signal (O_2CHPh), which previously appeared at 101.4 ppm, was now absent. The molecular formula of **57** was confirmed by HRMS.

The target glycosyl acceptor, phenyl 3-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**58**), was then prepared by treating compound **57** with TBDMSCl in pyridine[237] to give compound **58** in 81% yield. The structure of **58** was confirmed by ^1H NMR spectroscopy; two singlets at 0.13 ppm and 0.11 ppm represent the signals for the hydrogens belonging to the methyl groups of the TBDMS ether ($(CH_3)_2Si$). These signals were accompanied by a singlet at 0.93 ppm that represented the methyl groups of the *tert*-butyl portion of the TBDMS ether ($(CH_3)_3CSi$). Taken together, these results show that silylation has been achieved. The ^{13}C NMR spectrum of **58** also establishes the presence of the silyl group. The appearance of a highly shielded signal at -5.4 ppm is representative

of the two equivalent methyl groups of the 6-*O*-*tert*-butyldimethylsilyl ether ((CH_3)$_2$Si). The signal at 18.3 ppm identifies the quaternary carbon associated with the *tert*-butyl portion ((CH_3)$_3$*C*Si) of the silyl ether, while the signal at 26.0 ppm belongs to the three *tert*-butyl methyl groups ((CH_3)$_3$CSi). The molecular formula of compound **58** was confirmed by HRMS.

2.8.3 Synthesis of Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (59)

Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**59**) was prepared in 73% yield by treating compound **4** with an excess of sodium hydride and benzyl bromide in *N*,*N*-dimethylformamide. Recrystallization of **59** from ether:hexanes provided fine white needles. Physical data for this compound were in full agreement with those published in the literature.[290]

2.8.4 General

Before the first attempt at synthesising a disaccharide containing a Galβ(1→4)GlcNAc linkage is described several relevant concepts require review. At first glance it is obvious that both the glycosyl donor and glycosyl acceptor in this reaction bear the same glycosyl leaving group, a thiophenyl. Without further investigation, a reaction between these monomers would be expected to produce a mixture of disaccharides containing the desired dimer as well dimers resulting from the condensation of two glycosyl acceptors. In order to avoid this complication the concept of "armed" and "disarmed" glycosyl donors was employed.[150-153] This approach relies on the fact that C-2 ethers as in (**59**) activate and C-2 esters and amides

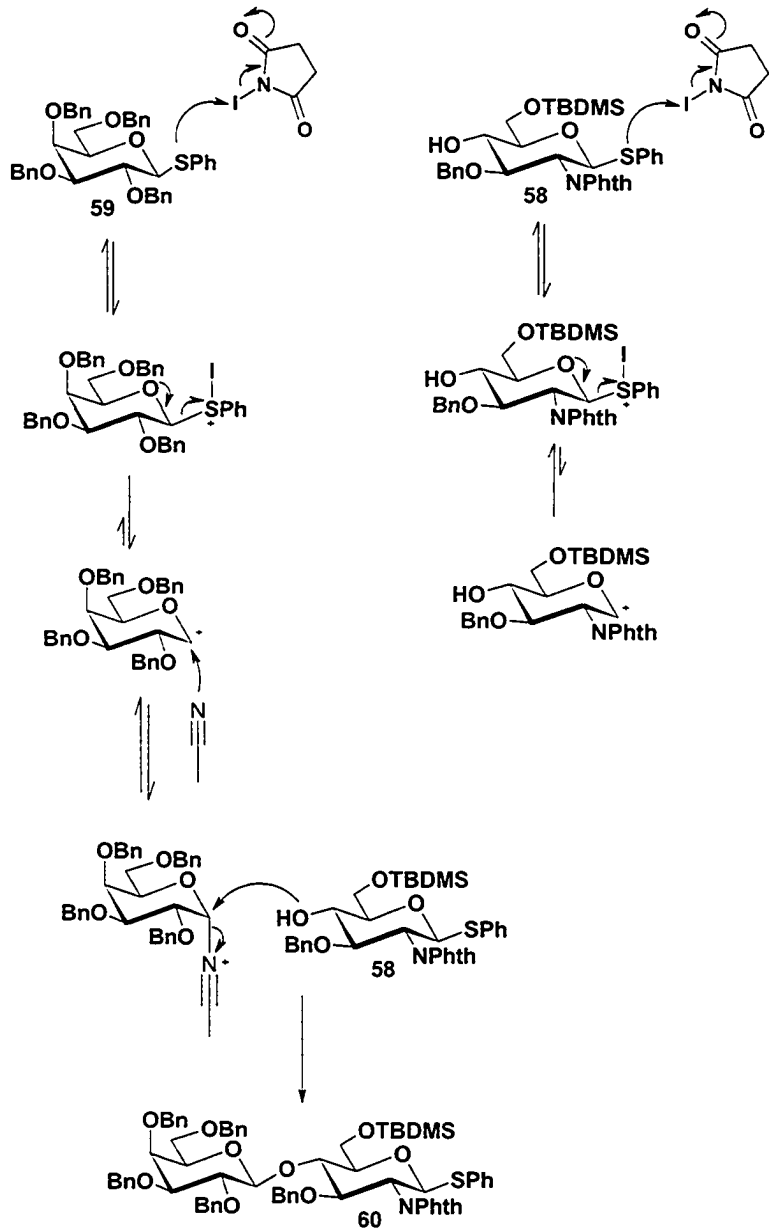
as in (**58**) deactivate the anomeric centre. Thus, coupling of the glycosyl donor **59**, which bears a C-2 benzyl ether protecting group, with the glycosyl acceptor **58**, which bears a C-2 phthalimide protecting group should proceed chemoselectively to give the desired cross-coupled product.

This is reinforced further by the concept of relative reactivity of glycosyl donors. Ley and co-workers[155,156] and then Wongs' group[157] have expanded the armed and disarmed glycosylation approach to include the entire protecting group complement of a given monomer, as well as the type of sugar core. According to the series of reactions outlined by Wong and coworkers,[157] the galactose core (**59**) is more reactive than the *N*-acetylglucosamine core (**58**). Glycosyl donor **59** has also been protected by four electron donating benzyl ethers. This choice of protecting group increases the relative reactivity of the galactose glycosyl donor. Conversely, glycosyl acceptor **58** is protected with a C-2 deactivating *N*-phthalimido group, an electron donating O-3 benzyl ether and a slightly electron donating O-6 silyl ether. Taken together, compound **59** is clearly more active as a glycosyl donor than compound **58**.

One drawback associated with the use of the more reactive phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**59**) arises from the control of stereospecificity during the glycosylation reaction. The O-2 protecting group is a non-neighboring-group participating benzyl ether. This situation was encountered earlier during the condensation of compound **47** with 5-azido-1-pentanol. To solve this problem the reaction between **59** and **58** was performed in acetonitrile at low temperature[118,291] in order to involve the participation of solvent to form the desired β-linked disaccharide.

A simplified mechanism for the formation of phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside using the concepts described above is depicted in Scheme 2.8.4.1. According to this mechanism, the lone pair of electrons on the sulfur atom of both compound **58** and **59** are iodinated by the promotor reagent NIS. Loss of the iodinated thiophenyl results in the formation of an oxocarbenium ion intermediate. Due to the lesser electron-withdrawing effect of the four benzyl groups protecting the hydroxyls of compound **59**, the oxocarbenium ion intermediate derived from this compound is more stable than that that formed from compound **58**. Consequently, the oxocarbenium ion intermediate of **59** reacts with the lone pair on the nitrogen of acetonitrile to form an α-nitrilium intermediate. In the final step, the β-face of the glycosyl nitrilium is attacked by the nucleophilic hydroxyl group on the glycosyl acceptor, **58**, to form the desired disaccharide, compound **60**.

Glycosylation of glycosyl acceptor **58** with glycosyl donor **59** using *N*-iodosuccinimide (NIS) as the electrophilic activator and silver triflate as the catalyst was carried out in acetonitrile at -35 °C for 12 h. Upon warming, TLC analysis of the reaction mixture revealed the presence of ten separate and distinct products. Due to the number of products and the potential difficulties in purifying each fraction it was decided that the crude mixture would not be resolved. Therefore, the desired product phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**60**) was not obtained. The reasons for the inability to form the target compound were not immediately apparent. Therefore, it was decided that a more simplified approach at forming the desired linkage β(1→4) would be investigated.



Scheme 2.8.4.1 Simplified mechanism for the formation of disaccharide 60

2.8.5 Synthesis of glycosyl donor 61

Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**61**) was prepared by treating β-D-galactopyranose pentaacetate (**2**) with the Lewis acid boron trifluoride diethyl

etherate and ethanethiol, as described by Ferrier *et al.*[185] The reaction afforded **61** in 95% yield. In the ^{1}H NMR spectrum of **61**, a multiplet at 2.73 ppm (SCH_2CH_3) and a triplet at 1.29 ppm (SCH_2CH_3) represent the glycosyl thioethyl group. In the ^{13}C NMR spectrum of **61** C-1 appears at 84.1 ppm, which is characteristic of thioglycosides. Signals at 24.4 ppm (SCH_2CH_3) and 14.9 (SCH_2CH_3) further confirm the condensation of **2** with ethanethiol. Physical data for this compound were in good agreement with those published in the literature.[292]

The second attempt at synthesising a disaccharide containing a Galβ(1−4)GlcNAc linkage also requires a review of relevant concepts. Firstly, in order to simplify the formation of the desired β-linked disaccharide, the non-participating benzyl ethers were replaced by neighboring-group participating acetate esters. This allows the reaction to be performed at a higher temperature in dichloromethane. Replacing the benzyl ethers with acetates introduces a problem in the chemoselective activation of the glycosyl donor, **61**, over the glycosyl acceptor, **58**. At first glance it is obvious that both the glycosyl donor and glycosyl acceptor in this reaction are thioglycosides. According to the "armed" and "disarmed" glycosylation strategy both compound **61**, which bears an O-2 deactivating ester and compound **58**, which bears a C-2 deactivating phthalimide, should be "disarmed" glycosyl donors. Furthermore according to the relative reactivity of glycosyl donors, glycosyl donor **61** has been protected by four electron withdrawing acetate esters. This choice of protecting group decreases the relative reactivity of **61** as a glycosyl donor. Conversely, glycosyl acceptor **58** is orthogonally protected with a C-2 deactivating *N*-phthalimido group, an electron donating O-3 benzyl ether and a slightly electron donating O-6 silyl ether. Taken

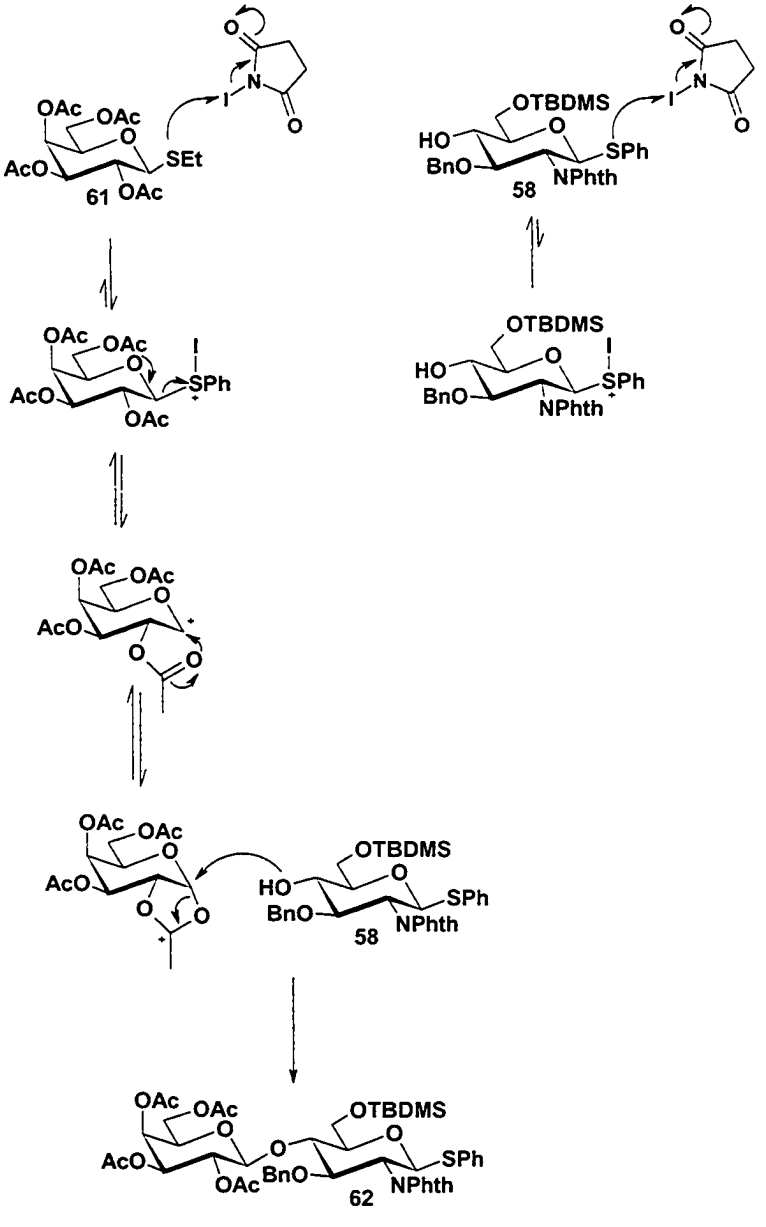
together, compound **61** is clearly less active as a glycosyl donor than compound **58**.

In order to solve this problem the differences in the sizes and nucleophilicities of the glycosyl leaving groups of the reactants was exploited. This methodology has been successfully employed in chemoselective glycosylations based on the differences in reactivities between ethyl and *p*-tolyl thioglycosides[159] as well as ethyl and dicyclohexylmethyl thioglycosides.[154] In both cases, the less hindered and more nucleophilic ethyl thioglycosides are selectively activated in the presence of the more hindered *p*-tolyl and dicyclohexylmethyl groups. One drawback is that this method has been shown to be incapable of activating a disarmed ethyl thioglycoside in the presence of an armed dicyclohexylmethyl thioglycoside.[154] This problem has been addressed in that both of the reactants, **61** and **58**, are disarmed.[150]

A simplified mechanism for the chemoselective activation of glycosyl donor **61** in the presence of compound **58** is depicted in Scheme 2.8.5.1. In the first step, the sulfur atom of ethyl thioglycoside **61** is iodinated by the promoter reagent NIS. This step is chemoselective as the iodination of the lone pair on the sulfur of phenyl thioglycoside **58** is slowed due to resonance delocalization with the aromatic ring. Thus, in theory glycosyl donor **61** is selectively activated and continues along the mechanistic pathway to give the cross-coupled product phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**62**).

Glycosylation of glycosyl acceptor **58** with glycosyl donor **61** using *N*-iodosuccinimide (NIS) as the electrophilic activator and silver triflate (in acetonitrile) as the catalyst was carried out in dichloromethane at 0 °C for 20 min, Scheme 2.8.5.2. Upon

warming, TLC analysis, toluene:ethyl actetate (2:1) of the reaction mixture revealed the presence of four products.



Scheme 2.8.5.1 Simplified mechanism for the formation of disaccharide 62

These fractions were purified from the crude mixture by column chromatography. The first contained recovered glycosyl acceptor, compound **58**. The second fraction contained a disaccharide derivative that resulted from the condensation of the glycosyl acceptor with one or more molecules of the glycosyl donor. This compound (**63**) was classified as a byproduct by the absence of signals characteristic of the *tert*-butyldimethylsilyl ether in the ^{1}H NMR spectrum. The presence of signals in the aromatic region indicate that the C-2 phthalimido of **58** remains, while an AB quartet representing the methylene hydrogens of the O-3 benzyl ether are also observed. The signal for H-1' appears at 4.70 ppm indicating the success in forming a β-glycoside. The spectrum is complicated by the fact that H-1 appears as a singlet at 5.51 ppm. In the ^{13}C NMR spectrum of **63**, two acetal carbons appear at 102.3 ppm and 99.5 ppm. These signals are accompanied by C-1 of the phenyl thioglycoside at 84.4 ppm. Taken together, this indicates that **63** may be a trisaccharide derivative or a mixture of products. This is contradicted by the number of carbohydrate carbon signals in the region between 74.0 and 56.0 ppm, of which there are nine. Since the chemical shift of the signal representing C-6 has not shifted significantly (64.7 ppm in the starting material, **58** to 61.4 ppm in the product), the desired glycosidic linkage may have been formed, followed by de-*O*-silylation. Due to the conflicting nature of the spectrum, the structure of **63** could not be unambiguously assigned. The evidence obtained, however, indicates that this compound may be phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**63**).

The third fraction contained a disaccharide product that resulted from the condensation of two molecules of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside

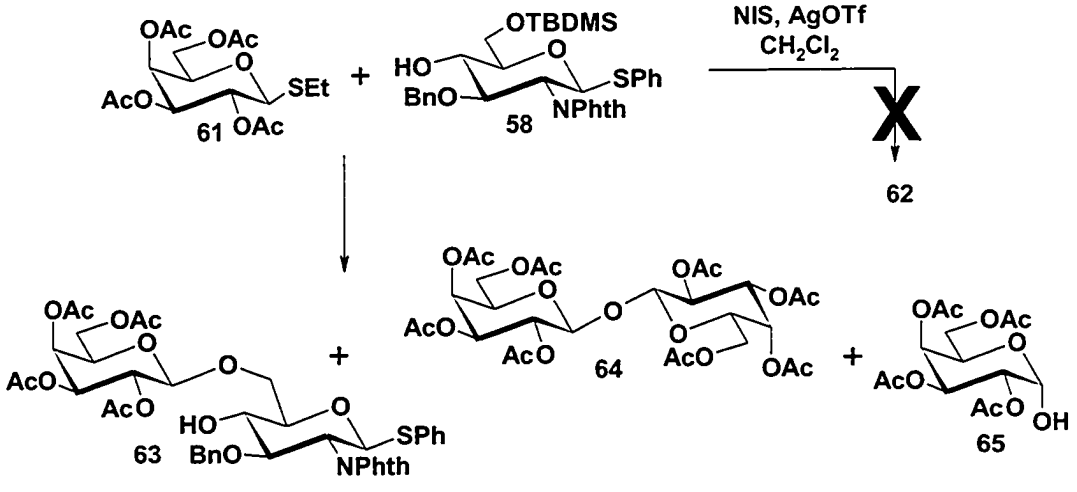
(**61**) followed by hydrolysis, compound **64**. In the ^{1}H NMR spectrum of this byproduct, the absence of signals in the aromatic region indicates that this byproduct does not contain the 3-O-benzyl or the C-2 phthalimido groups associated with the glycosyl acceptor. The anomeric proton signal for H-1 appears at 5.43 ppm and has a small coupling constant, which is indicative of an α-linkage. The anomeric proton signal for H-1' appears at 4.58 ppm and has a large coupling constant, which is indicative of a β-linkage. In the ^{13}C NMR spectrum of compound **64**, the presence of an acetal carbon signal at 101.5 ppm indicated the formation of a glycosidic linkage, while a signal at 90.7 ppm is representative of a hemi-acetal carbon resulting from hydrolysis. Due to the complexity of the spectra, the structure of **64** could not be unambiguously assigned. Given the observed data this compound was tentatively assigned as the non-reducing disaccharide, compound **64**.

The fourth product isolated was determined to be 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranose (**65**). In the ^{1}H NMR of **65**, H-1 appears as a doublet at 6.30 ppm ($J_{1,2}$ = 3.7 Hz) which is characteristic of an α-linkage. In the ^{13}C NMR of **65**, C-1 appears at 92.1 ppm, which is characteristic of a hemi-acetal carbon signal.

Unfortunately the desired disaccharide product phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-6-O-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**62**) was not obtained. The byproduct resulting from cross coupling maintains the thiophenyl moiety of the glycosyl acceptor indicating that the ethyl thioglycoside was clearly activated in the presence of the phenyl thioglycoside. The loss of the *tert*-butyldimethylsilyl ether indicates that this group may not be stable to these vigorous conditions. The lack of coupling to the correct hydroxyl group (O-4 of compound **58**) may

also indicate that this site is hindered by the neighboring C-2 phthalimide, O-3 benzyl ether and the O-6 silyl ether. As a consequence removal of the silyl ether on O-6 of the glycosyl acceptor is observed along with removal of the O-6 acetate of the glycosyl donor. This latter reaction may account for the apparent self condensation of two molecules of glycosyl donor. Hydrolysis of the glycosyl donor also seems to be a viable alternative in this reaction.

In order to circumvent the complications observed in the chemoselective activation of one glycosyl leaving group over another, the system was further simplified. Thus, in order to achieve successful glycosylation of the glycosyl acceptor **58** an alternative glycosyl donor was investigated.



Scheme 2.8.5.2 Glycosylation of glycosyl donor 61 with glycosyl acceptor 58

2.8.6 The bromide glycosylation method

Since 1901, the Koenigs-Knorr method[183] has been the most popular method for forming glycosidic bonds and modifications of it are still being developed. A glycosyl halide was originally used as the glycosyl donor and silver salts were used as the activating reagents. Since then, many other types of activators have been described in the literature. These include silver salts (e.g. AgOTf, Ag_2O, Ag_2CO_3 or $AgNO_3$), mercury salts (e.g. $Hg(CN)_2$, $Hg(Br_2)$, $Hg(I_2)$) and Lewis acids (e.g. $SnCl_4$, $BF_3.OEt_2$).[119] The stability and reactivity of glycosyl halides depends on the halide, the anomeric configuration and on the protecting groups. Compared with glycosyl bromides or chlorides, glycosyl fluorides are more stable and thus easier to handle. Glycosyl fluorides were reviewed in section 2.4.2. Glycosyl iodides have also been recently been found to act as excellent glycosyl leaving groups in the synthesis of various oligosaccharides.[291,293-297]

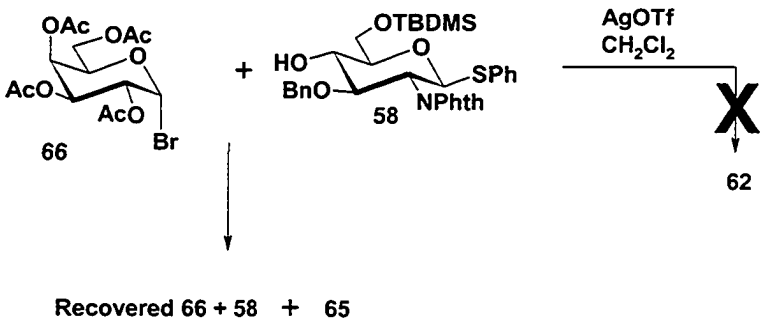
2.8.7 Synthesis of glycosyl donor 66

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**66**) was prepared by treating ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**61**) with bromine in dichloromethane, as described by Jung et al.[298] The reaction afforded **66** in 26% yield with hydrolysis resulting in the major byproduct, compound **65**. In the ^1H NMR spectrum of **66**, H-1 appears as a doublet at 6.71 ppm. The small coupling constant ($J_{1,2}$ = 4.3 Hz) is indicative of α-bromoglycosides. In the ^{13}C NMR of **66**, C-1 appears at 88.2 ppm. Other NMR and physical data for this compound were in good agreement with those published in the literature.[299]

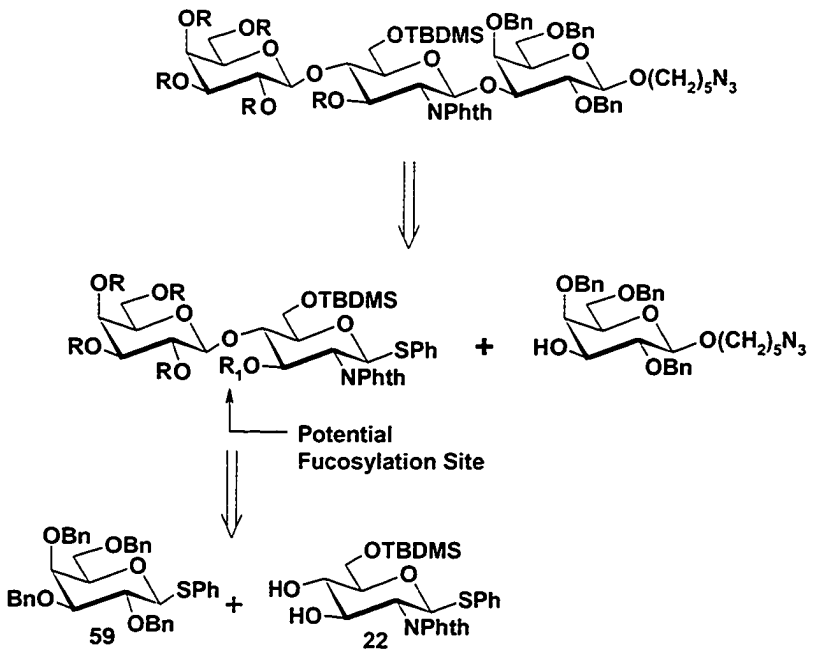
Glycosylation of glycosyl acceptor **58** with glycosyl donor **66** using AgOTf (in acetonitrile) as the electrophilic activator and the acid scavenger 2,6-DTBMP was carried out in dichloromethane at 0 °C, Scheme 2.8.7.1. After no visible reaction had occured at 0 °C for 2 h, the reaction was warmed to room temperature. Stirring at this temperature for 1 h showed no visible change in the reaction mixture on TLC, hexanes ethyl acetate (3:1). The reaction was then quenched with solid tetraethylammonium chloride and purified by flash chromatography. Three fractions were isolated from the crude mixture. The first fraction contained recovered glycosyl acceptor (R_f = 0.50, 99%). The second fraction contained recovered glycosyl donor (R_f = 0.30, 32%). The third fraction contained a minor amount of hydrolysed glycosyl donor (**65**, R_f = 0.10). Unfortunately, the desired product phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-6-O-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**62**) was not obtained.

The extended reaction time and excess of glycosyl donor were incapable of glycosylating the glycosyl acceptor. Recovery of the glycosyl acceptor indicates that the *tert*-butyldimethylsilyl ether is stable to these glycosylation conditions. A small amount of hydrolysis (**65**) shows that the glycosyl donor has been activated. Recovery of a portion of the glycosyl donor indicates that in the absence of other available nucleophiles the oxocarbenium ion intermediate reacts with bromide to regenerate the glycosyl donor. This observation implies that the hydroxyl group of the glycosyl acceptor is unavailable for glycosylation as seen in the glycosylation of pivaloated galactose derivatives. This conclusion is supported by recent findings reported by Crich.[300] This group has demonstrated that the 4-hydroxy group of *N*-acetylglucosamine derivatives is a very poor nucleophile in

glycosylation reactions To solve this problem it was decided that the steric complement of the protecting groups surrounding O-4 had to be reduced in order to achieve glycosylation of GlcNAc.



Scheme 2.8.7.1 **Glycosylation of glycosyl donor 66 with glycosyl acceptor 58**



Scheme 2.9 **Retrosynthetic analysis for the synthesis of trisaccharides using regioselective glycosylation of compound 22 to form an *N*-acetyllactosamine glycosyl donor**

2.9 Regioselective glycosylation of *N*-acetylglucosamine derivatives

2.9.1 General

A number of researchers have demonstrated the usefulness of reactions in which O-4 of *N*-acetylglucosamine is preferentially glycosylated over O-3 when both hydroxyl groups are free. It has been postulated that this regioselectivity is based on steric hindrance where the protecting groups on C-2 and O-6 affect the availability of O-3 for glycosylation. In general, the protecting group of choice for C-2 is the bulky *N*-phthalimido group. The groups protecting O-6 have been variable and give variable yields of O-4 glycosylated products. These groups include benzyl ethers,[301-304] 2-(naphthylmethyl) ethers[302] and TBDPS ethers.[305]

To employ this strategy, it was decided that an intermediate in the synthesis of glycosyl donor **23**, namely phenyl 2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**22**), would act as a suitable glycosyl acceptor. Compound **22** bears a C-2 *N*-phthalimido group and an O-6 TBDMS ether which comprise the requisite functionality for regioselective glycosylation of O-4 over O-3.

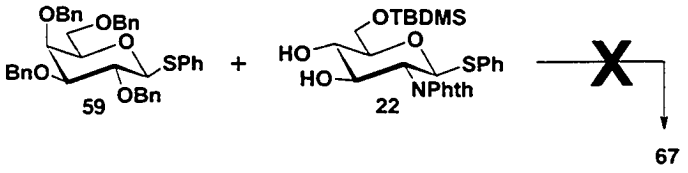
2.9.2 Regioselective glycosylation reactions

The first attempt at the regioselective synthesis of a disaccharide containing a Galβ(1→4)GlcNAc linkage bears similarities to the glycosylation reaction described in section 2.8.4. This reaction took advantage of the chemoselective activation of phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**59**) over a less reactive glycosyl acceptor, compound **58**. In addition, this reaction was performed in acetonitrile to enhance the stereoselectivity

of the reaction to favor the formation of the β-linked disaccharide. Glycosylation of phenyl 2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**22**) with phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**59**) combines these concepts and adds regioselectivity which will enhance the formation of the desired product.

Another addition to the procedure for this reaction involves the use of low temperatures to reduce the formation of byproducts and enhance the formation of phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**67**). Acetonitrile was used in several previous experiments to induce the formation of β-linkages when non-participating benzyl ethers reside on the glycosyl donor. The freezing point of acetonitrile is -45 °C. Hence the low temperature limit for these reaction is the freezing point of the solvent. To increase this limit it was decided that the reaction would be performed in butyronitrile which freezes at -112 °C.

Regioselective glycosylation of glycosyl acceptor **22** with glycosyl donor **59** using *N*-iodosuccinimide (NIS) as the electrophilic activator and silver triflate (in acetonitrile) as the catalyst was carried out in butyronitrile at -63 °C, Scheme 2.9.2.1. Stirring at this temperature for 90 min produced no visible change on TLC. The reaction was then warmed to -30 °C and stirred for an additional 5 h where TLC analysis revealed the presence of seven separate and distinct products. Due to the number of products and the potential difficulties in purifying each fraction it was decided that the crude mixture would not be resolved. Therefore the desired product compound **67** was not isolated. The reasons for the inability to form the target compound were, again, not immediately apparent.

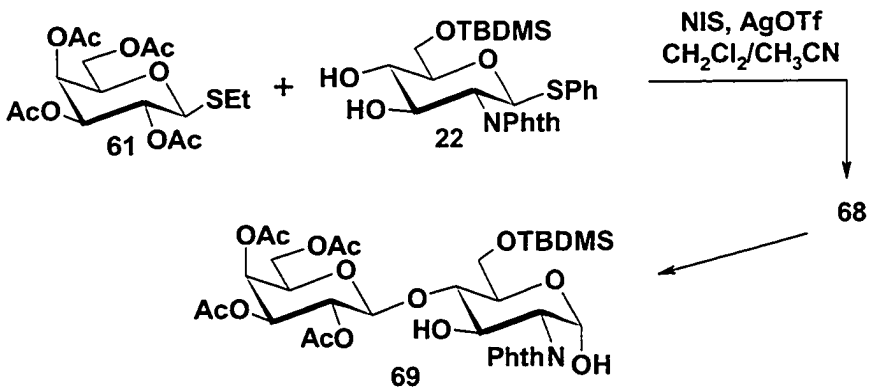


Scheme 2.9.2.1 **Attempted regioselective glycosylation of glycosyl acceptor 22 with glycosyl donor 59**

The second attempt at the regioselective synthesis of a disaccharide containing a Galβ(1−4)GlcNAc linkage follows the changes reported in section 2.8.5. Compound **61** was chosen as the glycosyl donor since it is protected by neighboring group participating acetate esters and can be chemoselectively activated in the presence of compound **22**. This is due to the differences in the nucleophilicities of the sulfur atoms in the glycosyl leaving groups.

Glycosylation of glycosyl acceptor **22** with glycosyl donor **61** using *N*-iodosuccinimide (NIS) as the electrophilic activator and silver triflate (in acetonitrile) as the catalyst was carried out in dichloromethane at 0 °C for 2 h, Scheme 2.9.2.2. Upon warming, TLC analysis [hexanes:ethyl actetate (1:2)] of the reaction mixture revealed the disappearance of both reactants and the appearance of seven products. The major product (R_f = 0.55) of these seven was purified from the crude mixture by column chromatography. This compound (**69**) could not have been the desired product because of the location of the signal for H-1 (6.54 ppm) in its ^{1}H NMR spectrum. The presence of signals in the aromatic region indicate that the C-2 phthalimido of **58** remains. The signal for H-1' appears at 4.79 ppm indicating the success in forming β-glycoside. The presence of the *tert*-butyldimethylsilyl ether is shown by signals at 0.91 ppm ($SiC(CH_3)_3$), 0.13 ppm, and 0.08 ppm ($Si(CH_3)_2$). Furthermore,

four singlets between 2.25-2.17 ppm represent the signals for the methyl groups of the four acetate esters (4 x CH_3CO). In the ^{13}C NMR spectrum of **69**, one acetal carbon appears at 101.7 ppm while a hemi-acetal carbon appears at 90.9 ppm. These signals indicate that this compound is dimeric and may be hydrolysed at the reducing end. The six carbonyl signals between 170.2-167.8 ppm indicate the presence of the four acetate esters and the C-2 phthalimide. These signals are accompanied by the signals representing the *tert*-butyldimethylsilyl ether which appear at 25.9 ppm ($SiC(CH_3)_3$), 18.3 ppm ($SiC(CH_3)_3$), and -5.2 (2 x $SiC(CH_3)_2$). The chemical shift of the signal representing C-2 has shifted from 54.4 ppm, which may be a result of hydrolysis at C-1. This evidence indicates that this compound is (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-deoxy-2-phthalimido-6-*O*-tert-butyldimethylsilyl-α-D-glucopyranose (**69**).



Scheme 2.9.2.2 **Attempted regioselective glycosylation of glycosyl acceptor 22 with glycosyl donor 61**

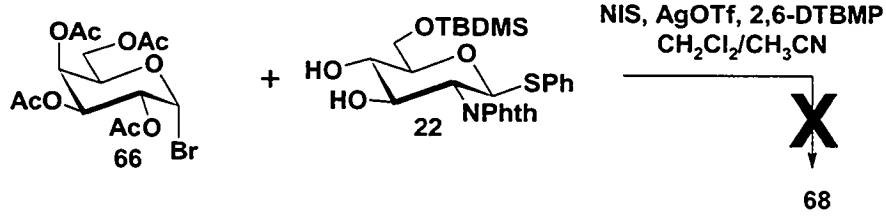
In order to circumvent the complications that were again observed in the chemoselective activation of the two thioglycosides, glycosylation using 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**66**) as the glycosyl donor was investigated.

Glycosylation of glycosyl acceptor **22** with glycosyl donor **66** using AgOTf (in acetonitrile) as the electrophilic activator and the acid scavenger 2,6-DTBMP was carried out in dichloromethane at -45 °C, Scheme 2.9.2.3. After no visible reaction had occurred at -45 °C for 30 min, the reaction was warmed to 0 °C. Stirring at this temperature for 3 h showed the consumption of the glycosyl donor and the appearance of two products. The reaction was then quenched with solid tetraethylammonium chloride and purified by flash chromatography. The major component was isolated and determined to be recovered glycosyl acceptor (R_f = 0.50, 29%). The second component could not be separated or identified but was similar in R_f on TLC to compound **65**, 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose. Therefore, the desired product phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-deoxy-2-phthalimido-6-O-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**68**) was not obtained.

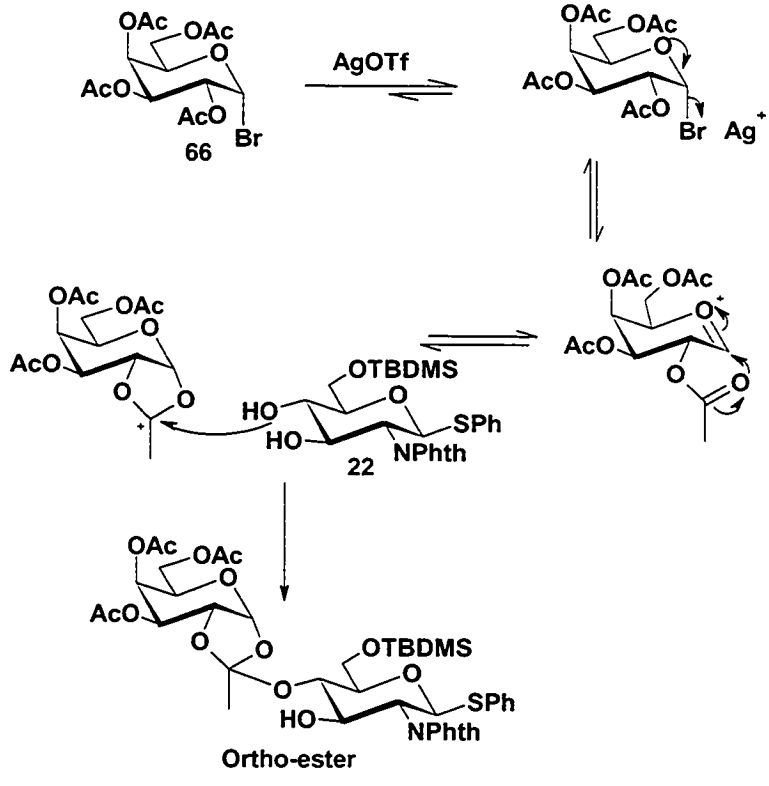
The extended reaction time and excess of glycosyl donor were incapable of glycosylating the glycosyl acceptor. Recovery of the glycosyl acceptor further confirms that the *tert*-butyldimethylsilyl ether is stable to these glycosylation conditions. A small amount of hydrolysis product (**65**) shows that the glycosyl donor has been activated. Recovery of a portion of the glycosyl donor indicates that in the absence of other available nucleophiles the oxocarbenium ion intermediate reacts with bromide to regenerate the glycosyl donor.

A number of researchers have demonstrated the formation of orthoesters when glycosyl donors bearing C-2 acetates are used in glycosylation reactions, Scheme 2.9.2.4. This problem has been solved by the use of C-2 benzoate protecting groups instead of acetates.[306] It was decided that in order to eliminate this possibility a fourth glycosyl donor, 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (**71**) would be used to glycosylate

compound **22**. This glycosyl donor is a glycosyl bromide, which requires activation conditions that have been shown to be compatible with the TBDMS ether.



Scheme 2.9.2.3 **Attempted regioselective glycosylation of glycosyl acceptor 22 with glycosyl donor 66**



Scheme 2.9.2.4 **Mechanism for the formation of ortho-esters during glycosylation reactions**

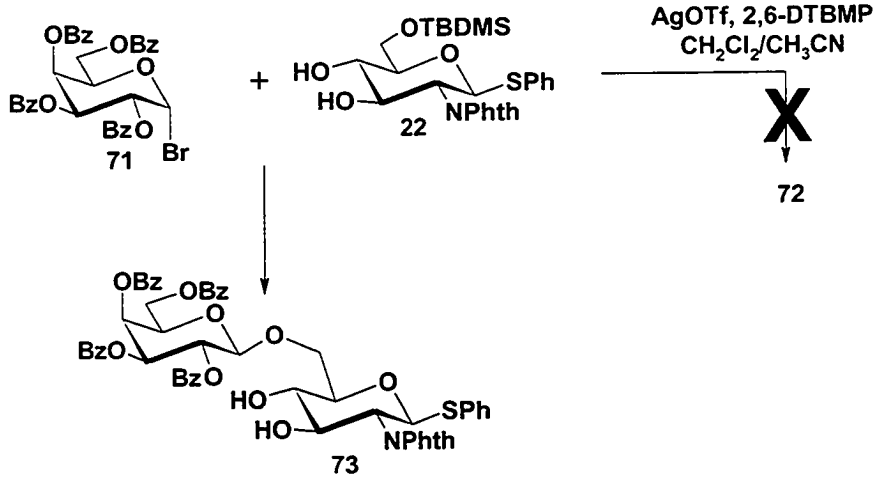
2.9.3 Synthesis of 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (71)

2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (**71**) was prepared in two steps from D-galactose (**1**) by a procedure reported by Fletcher.[307] In the first step compound **1** was treated with benzoyl chloride in pyridine at 0 °C to give D-galactopyranose pentabenzoate (**70**), which was used without further purification. Crude **70** was then treated with aqueous hydrobromic acid in dichloromethane to give 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (**71**) in 74% yield from D-galactose. NMR spectral data for this compound were in good agreement with those published in the literature.[308]

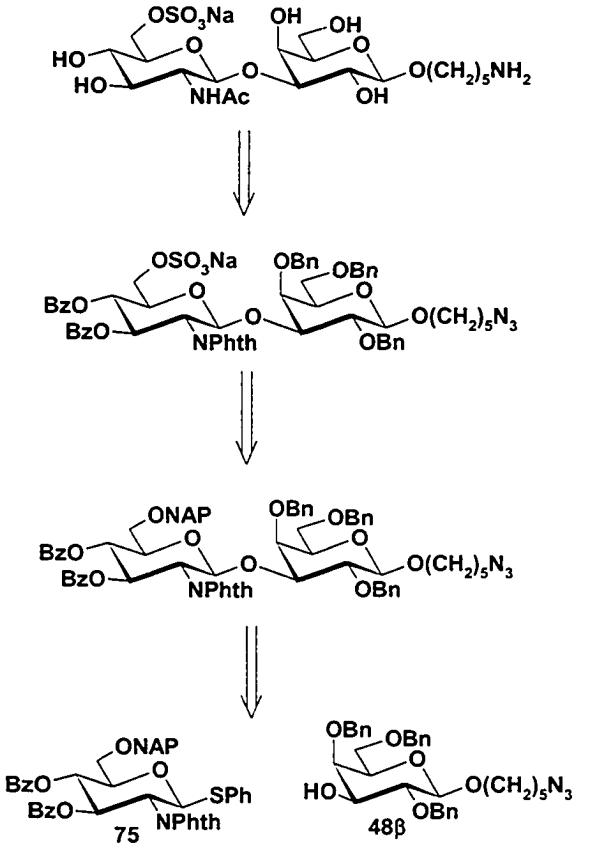
Glycosylation of glycosyl acceptor **22** with glycosyl donor **71** using AgOTf (in acetonitrile) as the electrophilic activator and the acid scavenger 2,6-DTBMP was carried out in dichloromethane at -0 °C, Scheme 2.9.3.1. After no visible reaction had occurred at 0 °C for 30 min, the reaction was warmed to room temperature. Stirring at this temperature for 12 h showed the consumption of the glycosyl donor and the appearance of four products. The reaction was then quenched with triethylamine (2 mL) and purified by flash chromatography using hexanes:ethyl acetate (1:2). The major disaccharide component isolated was found in fraction four (R_f = 0.20) and was determined to be phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**73**). In the ^{1}H NMR spectrum of **73** H-1' appears as a doublet at 5.59 ppm ($J_{1,2}$ = 10.8 Hz), which is indicative of β-glycoside formation. The disappearance of signals that represent the TBDMS ether at 0.92 ppm ($SiC(CH_3)_3$), 0.13, and 0.11 ppm (2 x $SiC(CH_3)_2$) indicate that the silyl group has been removed. In the ^{13}C NMR spectrum of **73** C-1' appears at 101.7, which is characteristic of glycoside formation. The signal for C-1 appears at 83.8 ppm and is

indicative of thioglycosides. The characteristic carbon signals of the TBDMS ether are absent, confirming the loss of this protecting group. Furthermore, the chemical shift for C-6 has been changed from 62.2 ppm (compound **21**) to 69.4 ppm in the product, **73**. This change indicates that glycosylation has taken place at O-6.

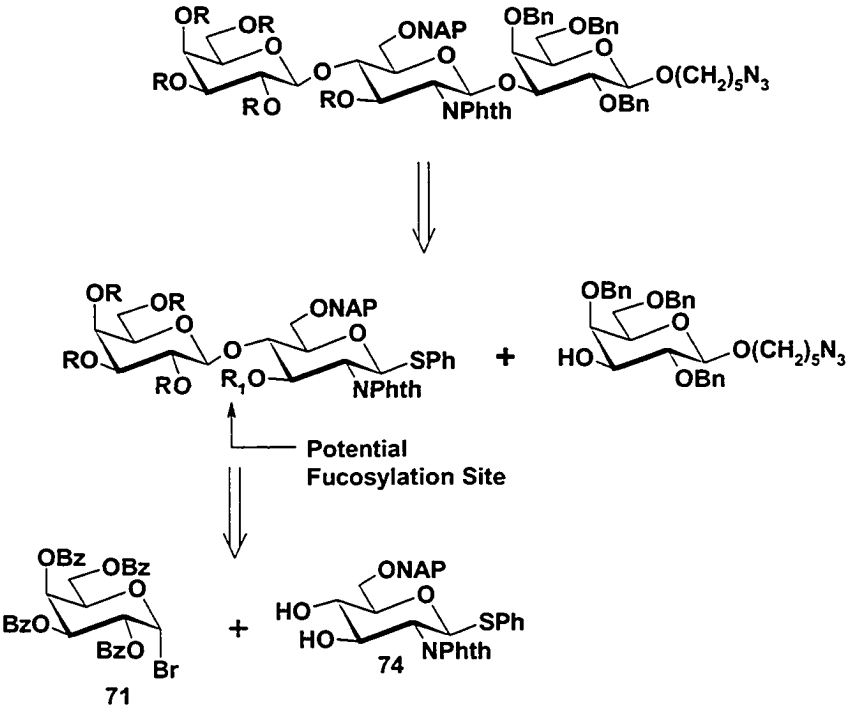
With these results in hand, it was clear that the TBDMS ether chosen to protect O-6 of *N*-acetylglycosamine derivatives was unstable to these glycosylation conditions. The evidence for this is the inability to isolate the desired disaccharide phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-deoxy-2-phthalimido-6-O-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (**72**), Scheme 2.9.3.1. In order to solve this problem the TBDMS ether was replaced by a novel and potentially more stable protecting group, the 2-naphthylmethyl ether.



Scheme 2.9.3.1 **Attempted regioselective glycosylation of glycosyl acceptor 22 with glycosyl donor 71**



Scheme 2.9.3.2 **Retrosynthetic analysis for the synthesis of an acidic disaccharide**



Scheme 2.9.3.3 **Retrosynthetic analysis for the synthesis of trisaccharides using regioselective glycosylation to form an *N*-acetyllactosamine glycosyl donor**

2.10 Glycosyl acceptor 74 and glycosyl donor 75

2.10.1 The 2-naphthylmethyl ether

The protection of alcohols as 2-naphthylmethyl ethers (NAP) was first reported by Spencer and co-workers[214] in 1998. This protecting group has been further used by Xia et al.[302,309] as an alternative benzyl type protecting group for the protection of carbohydrate

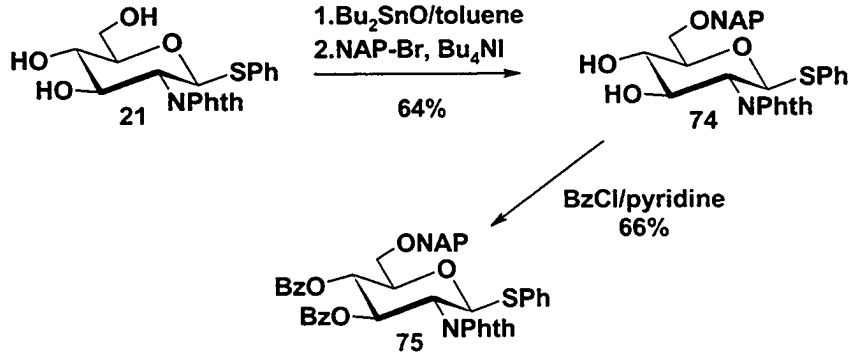
hydroxyl grouops. The normal method for the introduction of ethers uses sodium hydride and the corresponding alkyl bromide in dimethyl formamide. This method has been successful in the introduction of naphthylmethyl ethers using 2-bromomethylnaphthalene (NAP-Br).[310] Other bases have also been uses for naphthylation such as KOH in THF.[311] Other methods include the formation of (2-naphthyl)methylene acetals using 2-naphthaldehyde or 2-(dimethoxymethyl)naphthalene with a catalytic amount of p-toluenesulfonic acid and concomitant removal of water or methanol.[312] Formation of the acetal between O-4 and O-6 of galactose derivatives can be followed by reductive ring opening to form 6-O-naphthylated products or 4-O-naphthylated products.[310] Still others have employed the use of stannylene ethers to selectively introduce the 2-naphthylmethyl ether on primary alcohols over secondary ones.[302]

Several conditions currently exist for the selective removal of the NAP group. Catalytic hydrogenation has been shown to remove the NAP group selectively in the presence of benzyl ethers.[214] Oxidative methods have also been explored which use ammonium cerium(IV) nitrate (CAN)[310] or, more commonly, 1,2-dichloro-4,5-dicyanobenzoquinone (DDQ).[309-311] The NAP ether has also been shown to be selectively cleaved in the presence of a variety of other protecting groups including, acetyl and pivaloyl esters, *N*-phthalimido amides and benzyl ethers.[312] These findings show that the NAP group can be easily introduced at the primary position and removed using conditions that do not affect other protecting groups used in the current synthetic strategy. Therefore, it was decided that this group would be explored as an alternative to the *tert*-butyldimethylsilyl ether.

2.10.2 Synthesis of glycosyl acceptor 74 and glycosyl donor 75

Compound **74** was prepared from phenyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**21**) in 64% yield using a two-step method, Scheme 2.10.2.1. Stannylenation of **21** with dibutyltin oxide was performed in refluxing toluene overnight with the azeotropic removal of water using a Dean-Stark trap. Addition of 2-(bromomethyl)naphthalene and tetrabutylammonium iodide to the refluxing solution containing the 4,6-*O*-dibutylstannylene acetal resulted in the formation of phenyl 2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**74**) in 64% yield. In the ^{1}H NMR spectrum of compound **74**, the appearance of an AB quartet at 4.68 ppm indicates the presence of the methylene (OCH_2NAP) hydrogens of the 2-naphthylmethyl group. In the ^{13}C NMR spectrum of **74**, the appearance of a second methylene signal at 73.8 ppm (OCH_2NAP) showed the presence of the 2-naphthylmethyl group. All other chemical shifts in the ^{1}H and ^{13}C NMR spectra of **74** were in good agreement with reported data.[302]

Glycosyl donor **75** was prepared by treating compound **74** with benzoyl chloride in pyridine at 0 °C to afford phenyl 3,4-di-O-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**75**) in 66% yield. In the ^{1}H NMR spectrum of compound **75**, H-3 (6.27 ppm) and H-4 (5.61 ppm) are highly deshielded when their chemical shifts are compared to compound **21**. This demonstrates that benzoylation has taken place at these sites. In the ^{13}C NMR spectrum of **75**, two additional carbonyl signals at 165.9 ppm and 165.4 ppm show the presence of the two benzoyl esters. The molecular formula of **75** was confirmed by HRMS.

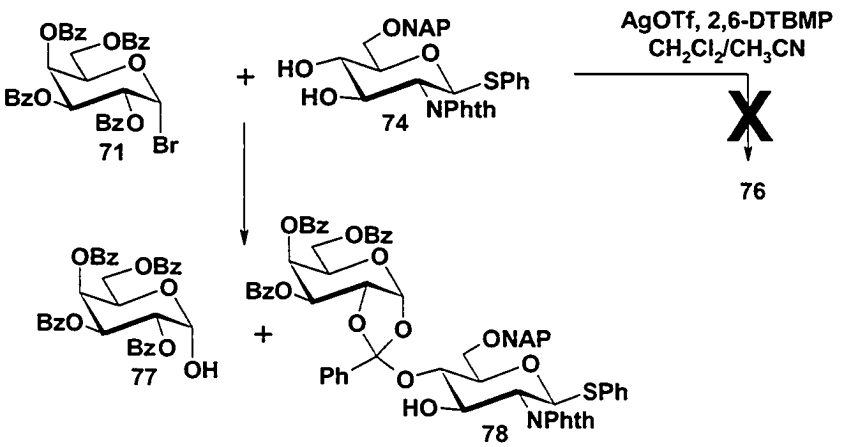


Scheme 2.10.2.1 Synthesis of glycosyl acceptor 74 and glycosyl donor 75

Glycosylation of glycosyl acceptor **74** with glycosyl donor **71** using AgOTf (in acetonitrile) as the electrophilic activator and the acid scavenger 2,6-DTBMP was carried out in dichloromethane at 0 °C, Scheme 2.10.2.2. After no visible reaction had occurred at 0 °C for 20 min, the reaction mixture was warmed to room temperature and stirred at this temperature for 12 h. TLC showed that the glycosyl donor had been consumed and three products appeared. The reaction was then quenched with triethylamine (2 mL) and purified by flash chromatography using hexanes:ethyl acetate (1:2). The major disaccharide component isolated was fraction two (R_f = 0.30) which was tentatively assigned as the orthoester **78**. In the ^{1}H NMR spectrum of **78**, H-1' appears as a doublet at 6.10 ppm ($J_{1,2}$ = 4.9 Hz), which is indicative of α-glycoside formation. The signal for H-1 resonates at 5.67 ppm ($J_{1,2}$ = 10.4 Hz). The appearance of a singlet at 4.78 ppm that represents the methylene of the 2-naphthylmethyl (OC*H*$_2$NAP) ether indicates the presence of this functional group. In the ^{13}C NMR spectrum of **78**, C-1' appears at 99.0 ppm, which is characteristic of glycoside formation. The signal for the ortho-ester carbon atom resonates at 121.1 ppm[306] while the

signal for C-1 appeared at 83.4 and was indicative of thioglycosides. Furthermore, the signal representing the methylene of the 2-naphthylmethyl ether is evident at 73.8 ppm.

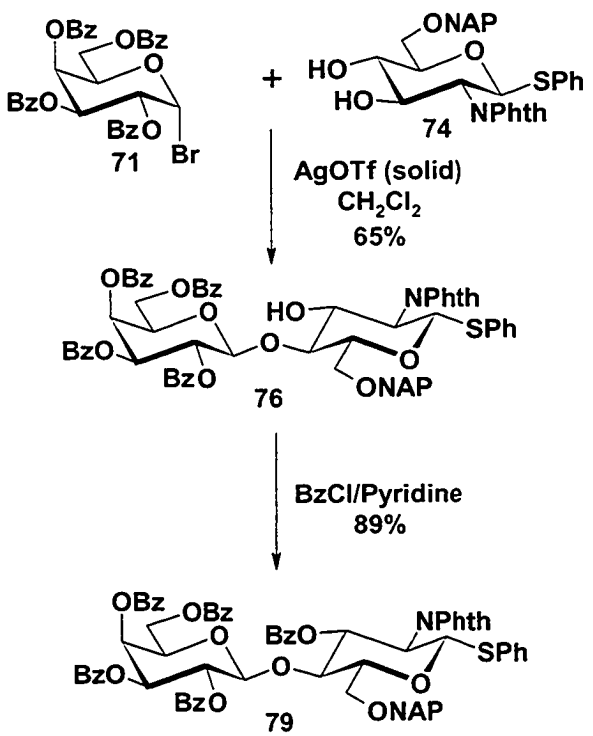
Fraction one contained hydrolysed glycosyl donor 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranose (**77**) in 59% yield. The third fraction contained recovered glycosyl acceptor, **74**, in 48% yield.



Scheme 2.10.2.2 Attempted regioselective glycosylation of glycosyl acceptor 74 with glycosyl donor 71

Glycosylation of glycosyl acceptor **74** with glycosyl donor **71** using AgOTf (solid) as the electrophilic activator was carried out in dichloromethane at room temperature for 30 min, Scheme 2.10.2.3. It appeared from TLC that the glycosyl donor had been consumed and two products formed with some glycosyl acceptor remaining. The reaction was then quenched with triethylamine (0.5 mL) and purified by flash chromatography using hexanes:ethyl acetate (2:1). The major disaccharide component isolated (R_f = 0.33), was phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1-4)-2-deoxy-6-*O*-(2-

naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**76**) in 65% yield. In the ^{1}H NMR spectrum of **76**, H-1' appeared as a doublet at 4.90 ppm ($J_{1,2}$ = 8.0 Hz), which is indicative of β-glycoside formation. The signal for H-1 resonated at 5.62 ppm ($J_{1,2}$ = 10.5 Hz). The methylene of the 2-naphthylmethyl (OC*H*$_2$NAP) ether appeared as an AB quartet with one signal at 4.40 ppm and the other at 4.30 ppm. All of the signals for H-2', H-3', and H-4' were relatively unchanged in comparison to those of the glycosyl donor (**71**). In the ^{13}C NMR spectrum of **76**, C-1' appeared at 102.1 ppm while the signal for C-1 appears at 83.6 ppm and is characteristic of thioglycosides. Furthermore, the signal representing the methylene of the 2-naphthylmethyl ether is evident at 73.2 ppm.



Scheme 2.10.2.3 **Regioselective glycosylation of glycosyl donor 71 with glycosyl acceptor 74 and synthesis of glycosyl donor 79**

2.10.3 Synthesis of *N*-phthalimidolactosamine glycosyl donor 79

Compound **79** was prepared from phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**76**), using benzoyl chloride in pyridine to give *N*-phthalimidolactosamine glycosyl donor, 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**79**) in 89% yield, Scheme 2.10.2.3. The ^{1}H NMR spectrum of **79** confirms that the glycosidic linkage formed in section 2.10.2 is at position 4 of *N*-acetylglucosamine. The signal for H-3 appears as a triplet at 6.14 ppm in the product (**79**), which is more deshielded than that shown for the starting material (**76**), 4.64 ppm. This deshielding effect demonstrates that benzoylation has taken place at O-3. In the ^{13}C NMR spectrum of **79** C-1' appears at 100.7 ppm while the signal for C-1 appears at 83.6 ppm. The molecular formula of **79** was confirmed by high resolution ESMS.

2.10.4 Synthesis of acidic disaccharide 83

The glycosylation reaction between glycosyl acceptor, **48β**, and phenyl 3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-6-*O*-(2-naphthylmethyl)-1-thio-β-D-glucopyranoside (**75**) (1.6 eq) was performed as previously described in the presence of NIS and AgOTf. Using this promoter system, the disaccharide derivative 5-azidopentyl 3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-6-*O*-(2-naphthylmethyl)-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**80**) was obtained in 2 h in 74% yield, Scheme 2.10.4.1. Evidence for the successful condensation of the reactants was found in the ^{1}H NMR spectrum of **80**. The formation of the β-glycoside product was supported by the presence of the H-1' doublet at

5.88 ppm ($J_{1,2}$ = 8.4 Hz) while the signal for H-1 appears as a doublet at 4.23 ppm ($J_{1,2}$ = 7.7 Hz). The deshielded protons H-3' and H-4' were evident at 6.30 and 5.62 ppm respectively, while a triplet at 3.00 ppm (CH_2N_3) confirmed the presence of the linker arm in the structure of **80**. The glycosidic carbons C-1' and C-1 resonate at 104.1 ppm and 99.5 ppm in the ^{13}C NMR spectrum, respectively. All of the signals for the methylene carbons of the linker arm, the three benzyl groups, C-6, C-6' and the 2-naphthylmethyl group were clearly visible by the JMOD technique. The structure of **80** was further confirmed by ESMS.

Acidic disaccharide **84** was prepared in four steps from compound **80** using literature procedures, Scheme 2.10.4.1. Removal of the 2-naphthylmethyl group protecting O-6' was performed using DDQ in dichloromethane:methanol (4:1) to give the product 5-azidopentyl 3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**81**) in 59% yield. The ^1H NMR spectrum of **81** clearly showed the disappearance of the signal at 4.63 ppm which formerly represented the methylene hydrogens associated with the 2-naphthylmethyl ether. The ^{13}C NMR spectrum supported this conclusion. The disappearance of one of the methylene carbon signals at 74.0 ppm in the JMOD of **81** shows that removal of the NAP group has been achieved.

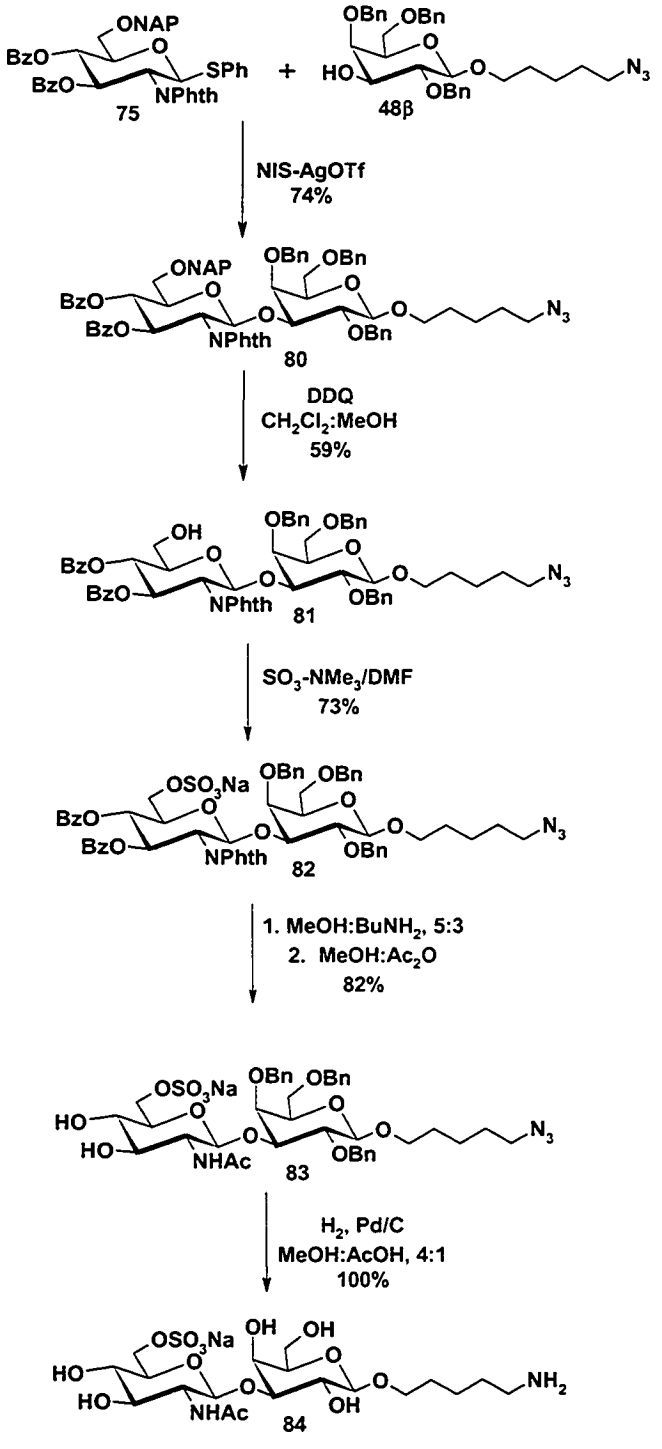
Subsequent sulfation was performed by treating compound **81** with sulfur trioxide-trimethylamine complex in DMF. This transformation afforded sodium 5-azidopentyl 3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-6-*O*-sulfonate-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**82**) in 73% yield. The most obvious indication that the transformation was successful comes from the change in polarity on TLC [(R_f = 0.50, dichloromethane:methanol (9:1)] of **82** in comparison to the non-sulfated starting material,

81 [(R_f = 0.45, toluene:ethyl acetate (8:1)]. Further comparisons between the ^{1}H NMR and ^{13}C NMR spectra of **82** and that for compound **81** show that sulfation has occurred at O-6'. The signals for H-6' appear at 3.80 (H-6a'), 3.71 (H-6b') for compound **81**. Sulfation results in the deshielding of these protons ultimately causing their signal to resonate at 4.32-4.20 (H-6a',b') for compound **82**. This deshielding effect is also shown in the ^{13}C NMR spectrum of **82**, in which the signal for C-6' resonates at a 67.8 ppm in comparison to compound **81** where C-6' appears at 61.3 ppm.

In order to cleave the *N*-phthalimido group and to saponify the ester groups simultaneously, compound **82** was treated with 4-aminobutane in methanol (3:5, v:v) at reflux for 48 h. After removal of the solvent under reduced pressure, the resulting amino compound was stirred with acetic anhydride in methanol (1:10, v:v) for 24 h to selectively acetylate the amino group to give 5-azidopentyl 2-acetamido-2-deoxy-6-*O*-sulfate-β-D-glucopyranosyl-(1−3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**83**) in 82% yield. In the ^{1}H NMR spectrum of **83**, the disappearance of signals formerly representing H-3' and H-4' at 6.24 ppm and 5.49 ppm respectively for the starting material (**82**) indicated the removal of the electron withdrawing benzoate esters groups in the first step of the reaction. The presence of a sharp singlet at 1.71 ppm demonstrated the presence of the hydrogens of the acetyl group (CH_3CO), confirming that re-*N*-acylation has been successful in the second step. In the ^{13}C NMR spectrum, the disappearance three signals in the carbonyl region of the spectrum at 165.9 ppm and 165.4 ppm are indicative of de-*O*-benzoylation. The appearance of a signal at 23.2 (CH_3CONH) also confirms that re-*N*-acetylation has been achieved.

To remove the benzyl ethers and reduce the azido group, compound **83** was subjected

to catalytic hydrogenation using H_2(g) and 10% Pd/C in methanol:acetic acid (4:1). Stirring for 21 h at atmospheric pressure and room temperature under these conditions gave the fully deprotected acidic disaccharide derivative, sodium 5-aminopentyl 2-acetamido-2-deoxy-6-*O*-sulfonate-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (**84**) in quantitative yield (100%). In the ^{1}H NMR spectrum of **84**, the disappearance of the aromatic protons from the aromatic region between 7.34-7.23 ppm is one of the indications that the benzyl groups were successfully removed. This was confirmed by the ^{13}C NMR spectrum in which no aromatic carbon signals exist. Successful reduction of the azido functionality was shown by the change in the chemical shift of the substituted methylene signal. For azide derivative **83**, the signal for the methylene attached to the nitrogen atom appeared at 52.2 ppm, while in the product amine **84**, the corresponding signal resonates at 40.0 ppm (CH_2NH_2). The molecular formula of **84** was confirmed by ESMS.



Scheme 2.10.4.1 Synthesis of acidic disaccharide 84

2.10.5 Synthesis of trisaccharide 85

The glycosylation reaction between glycosyl acceptor, **48β**, and phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**79**) (1.5 eq) was performed as previously described in the presence of an excess amount of NIS (4.5 eq.) and AgOTf (4.5 eq.) for an extended reaction time of 3 days, Scheme 2.10.5.1. Four fractions were purified from the crude mixture by column chromatography using toluene:ethyl acetate (10:1) as eluent. The first two fractions contained an α/β mixture of glycosyl donor hydrolysis products, **86α** and **86β** (R_f = 0.50, 0.40).

The third fraction contained the desired product 5-azidopentyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-(3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**85**) (R_f = 0.33) in 21% yield. Since the *N*-phthalimido protecting group at C-2 is a neighboring group participating group, the reaction was stereoselective, favoring the formation of the desired β product. In the ^{1}H NMR spectrum of compound **85**, H-1' appeared at 4.81 ppm. The most deshielded proton signals in the spectrum belonged to H-3' (6.14 ppm), H-1'' (5.74 ppm), and H-4'' (5.60 ppm). All of the protons of the linker arm moiety are visible in the spectrum with the triplet at 2.99 ppm representing the azido substituted methylene signal. In the ^{13}C NMR spectrum, the appearance of three acetal carbon signals 104.2 (C-1), 100.8 (C-1''), and 99.3 ppm (C-1') at 101.3 indicated success in the synthesis of the trisaccharide. The appearance of three methylene carbons in the alkyl region, 24.6-34.2 ppm show the presence of the linker arm moiety in the structure. Seven signals between 178.5-165.0 confirm the presence of the

five carbonyl carbons of the benzoate esters and two *N*-phthalimido carbonyl groups, confirming the presence of these groups in the product. The molecular formula of **85** was confirmed by high resolution ESMS.

The fourth fraction (R_f = 0.25) recovered by flash chromatography contained the glycosyl acceptor **48β** in 32% yield.

Initial attempts at the synthesis of trisaccharide **85** failed hence an excess of promoter reagents and an excessive amount of time (described above) was required to obtain the desired product, albeit in low yield. The observed change in the color of the reaction mixture was an indication that the promoter reagents had indeed activated the glycosyl leaving group. This is evidenced by the appearance of **86α** and **86β** on TLC, which result from a reaction between the oxonium ion intermediate and atmospheric water or water on the TLC plate. After the lengthy reaction time, the solution became colorless, indicating the completion of the reaction. The eventual intrusion of water over this period of time resulted in **86α** and **86β** being the major products, with a low yield of compound **85** obtained.

To solve this problem, it was decided that the reaction rate must be increased in order to form more of the desired product, **85**, and prevent the eventual reaction of the glycosyl donor with water, which results from the excessive reaction time. Since the reaction takes place in a low boiling solvent, dichloromethane, heating the solution was not considered as an option. Due to the cost of the electrophilic activator, AgOTf, increasing the concentration of this reagent was also not a viable alternative. In order to effectively increase the reaction rate to an appreciable rate it was decided that the electrophilic activator triflic acid would be employed. This reagent is traditionally used in a catalytic amount, so it was hoped that an

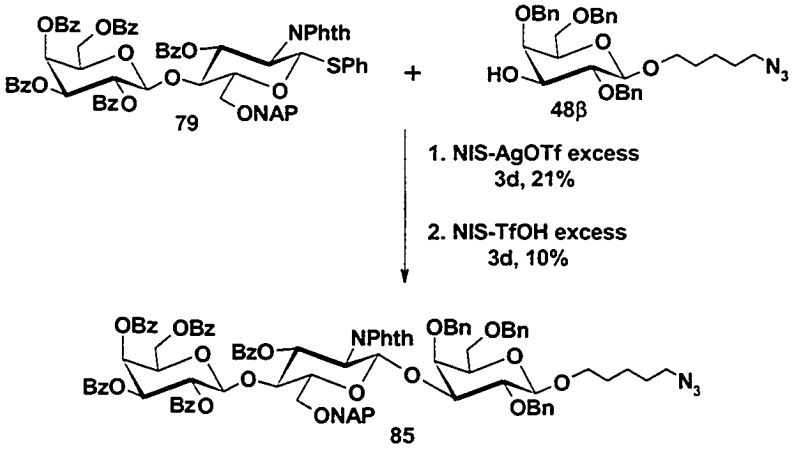
excess of this promoter might solve the problem.

The second glycosylation reaction between glycosyl acceptor, **48β**, and phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**79**), (1.5 eq) was performed, as previously described, in the presence of an excess amount of NIS (3.0 eq.) and TfOH (0.2 eq.) for an extended reaction time of 3 days, Scheme 2.10.5.1. Three fractions were purified from the crude mixture by column chromatography using toluene:ethyl acetate (10:1) as eluent. The first two fractions contained an α/β mixture of glycosyl donor hydrolysis products, **86α** and **86β** (R_f = 0.50, 0.40). The third fraction contained the desired product 5-azidopentyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**85**) (R_f = 0.33) in only 10% yield.

Clearly altering the promoter system failed to give a better yield of trisaccharide **85**. As in the first attempt, the observed change in the color of the reaction mixture was an indication that the promoter reagents had indeed activated the glycosyl leaving group. This was again evidenced by the appearance of **86α** and **86β** resulting in a low yield of compound **85**.

A recent report by Wong and co-workers suggested that glycosyl donor reactivity can be related to the chemical shift of the anomeric proton.[157] These researchers demonstrated this phenomenon using *p*-methylphenylthio (STol) glycosides where relative glycosyl donor reactivity was found to decrease as the chemical shift of H-1 increased. According to this observation, a comparison of the chemical shifts of LacNAc derivative **79** (δ H-1 = 5.81 ppm)

and GlcNAc glycosyl donor **75** (δ H-1 = 5.93 ppm) shows a slight difference in reactivity in favor of donor **79**. Since the condensation of **75** with **48β** proceeded smoothly to give **80** in good yield and the most distinctive difference between **75** and **79** is the β(1→4) linked 2,3,4,6-tetra-*O*-benzoyl-galactosyl moiety, we felt that steric effects were playing a role in preventing alcohol acceptor **48β** from coupling with the oxonium ion intermediate resulting from the activation of compound **79**. Furthermore, upon the addition of promoter reagents during attempts at forming trisaccharide **85** the reaction mixture turned light pink to dark purple. This color persisted throughout each three-day reaction. Monitoring the reaction by TLC over the three day period showed the formation of mainly glycosyl donor hydrolysis products. The former observation indicates that donor **79** is being activated by the promoter reagents, which is in agreement with donor reactivity and the ^{1}H-NMR chemical shift of the anomeric proton. The latter indicates that it is possible for **79** to react with smaller nucleophiles such as water. With these arguments in hand, it was necessary to reduce the steric bulk of **79** to solve the problem. This was accomplished by the conversion of the benzoate ester groups to smaller acetate esters.



Scheme 2.10.5.1 Synthesis of trisaccharide 85

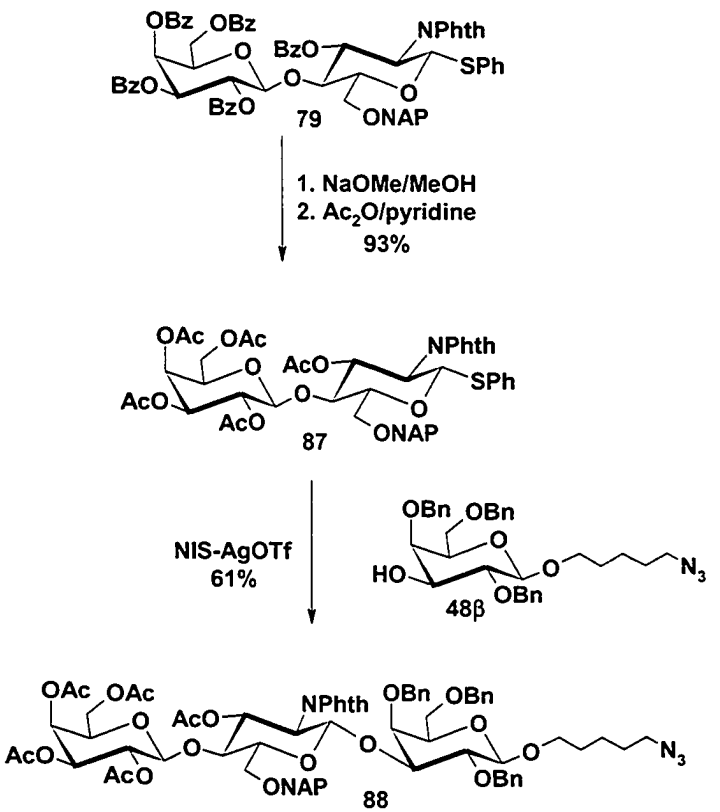
2.11 Synthesis of neutral and acidic trisaccharides

2.11.1 Synthesis of *N*-phthalimidolactosamine glycosyl donor 87

Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside was prepared in a two-step procedure. Compound **79** (Scheme 2.11.1.1) was stirred in dry methanol and treated with a solution of sodium methoxide in methanol. De-*O*-benzoylation was easily monitored by the dissolution of the starting material. Upon completion of the reaction the solution was neutralized and the solvent evaporated. The crude mixture was then treated with acetic anhydride in pyridine to afford compound **87** in 93% yield. The structure of **87** was confirmed by comparing the ^{1}H and ^{13}C NMR spectral data with that for the known compound.[302]

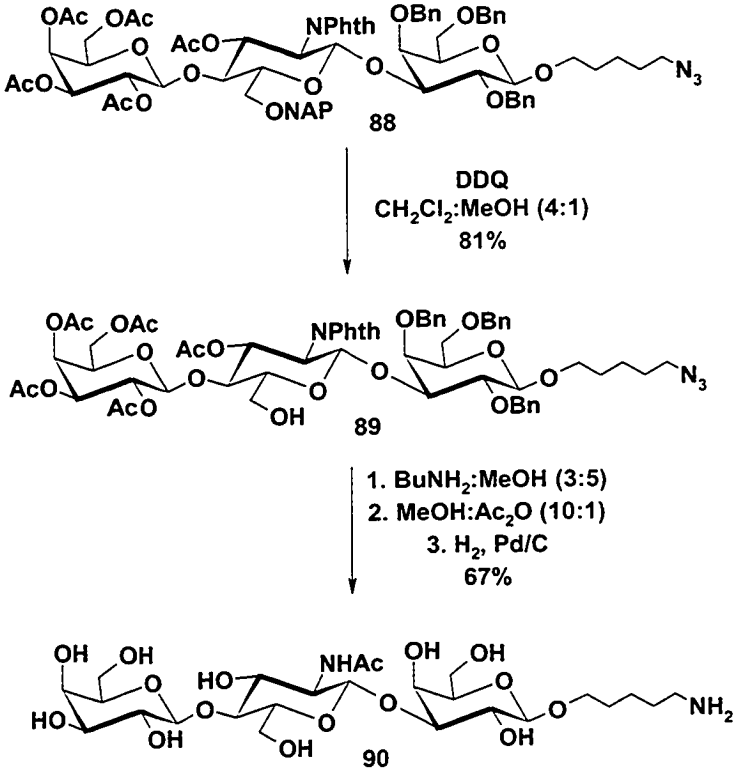
The glycosylation reaction between glycosyl acceptor, **48β**, and phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**87**) (1.5 eq), was performed as previously described in the presence of NIS (4.5 eq.) and AgOTf for 15 min, Scheme 2.11.1.1. The resulting crude mixture was purified by flash chromatography using hexanes:ethyl acetate as eluent to give the desired trisaccharide 5-azidopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**88**) (R_f = 0.50) in 74% yield. Since the *N*-phthalimido protecting group at C-2 is a neighboring participating group, the reaction was stereoselective, favoring the formation of the desired β product. Evidence for the successful condensation

of the reactants was found in the ^{1}H NMR spectrum of **88**. The formation of the β-glycoside product was supported by the presence of the H-1' doublet at 5.79 ppm ($J_{1,2}$ = 8.4), while a triplet at 3.01 ppm (CH_2N_3) confirmed the presence of the linker arm in the structure of **88**. The glycosidic carbons C-1, C-1', C-1'' resonated at 104.2, 99.3, and 100.4 ppm respectively, in the ^{13}C NMR spectrum. All of the signals for the methylene carbons of the linker arm, the three benzyl groups, C-6, C-6', C-6'' and the 2-naphthylmethyl group are clearly visible in the ^{13}C NMR spectrum recorded by the JMOD technique. The molecular formula of **88** was further confirmed by high resolution FABMS.



Scheme 2.11.1.1 **Synthesis of *N*-phthalimidolactosamine glycosyl donor and condensation with glycosyl acceptor 48β to form trisaccharide 88**

Removal of the 2-naphthylmethyl group protecting O-6' was performed using DDQ in dichloromethane:methanol (4:1) to give the product 5-azidopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**89**) in 81% yield, Scheme 2.11.1.2. The ^{1}H NMR spectrum of **89** clearly shows the disappearance of an AB quartet centered at 4.40 ppm, which formerly represented the methylene hydrogens associated with the 2-naphthylmethyl ether. The ^{13}C NMR spectrum supports this conclusion. The disappearance of one of the methylene carbon signals at 74.0 ppm in the JMOD of **89** shows that removal of the NAP group has been achieved.



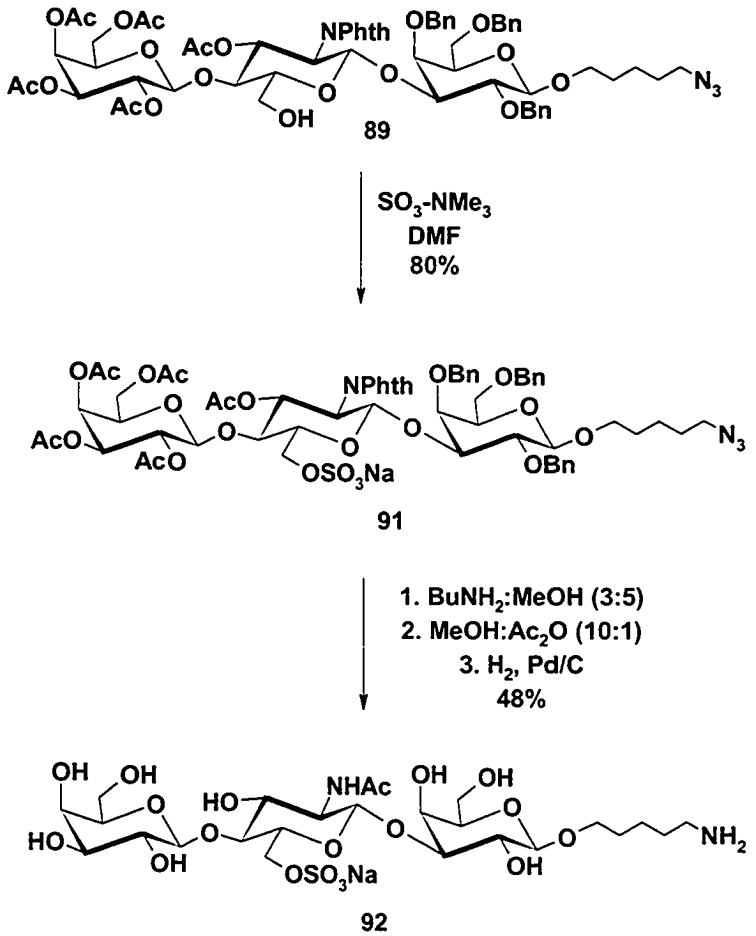
Scheme 2.11.1.2 Synthesis of neutral trisaccharide 90

Once the neutral disaccharide **90** was synthesized from compound **89** in a two-step procedure according to Scheme 2.11.1.2. Cleavage of the *N*-phthalimido group and saponification of the ester groups of **89** was performed in refluxing 1-aminobutane in methanol (3:5, v:v). After removal of the solvent under reduced pressure the resulting amino compound was stirred with acetic anhydride:methanol (1:10, v:v) for 24 h to selectively acetylate the amino group. Subsequent removal of the solvent was followed by reduction of the benzyl ethers and the azido group by catalytic hydrogenation using H_2(g) and 10% Pd/C in methanol:acetic acid (4:1). Stirring for 21 h at atmospheric pressure and room temperature under these conditions gave the fully deprotected acidic disaccharide derivative, 5-aminopentyl β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (**90**) in 67% yield. The molecular formula of **90** was confirmed by high resolution ESMS.

Acidic disaccharide **92** was synthesized from compound **89** in a three-step procedure according to Scheme 2.11.1.3. Regioselective sulfation was performed by treating compound **89** with sulfur trioxide-trimethylamine complex in *N*,*N*-dimethylformamide. This transformation afforded sodium 5-azidopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-2-phthalimido-6-*O*-sulfonate-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**91**) in 80% yield. The most obvious indication that the transformation was successful comes from the change in polarity on TLC (R_f = 0.30, dichloromethane:methanol, 9:1) of **91** in comparison to the non-sulfated starting material, **89** (R_f = 0.30, hexanes:ethyl acetate, 1:1). This deshielding effect of sulfation is also shown in the ^{13}C NMR spectrum of **91**, in which the signal for C-6' resonates at a 66.1 ppm in

comparison to compound **89** where C-6' appears at 61.1 ppm.

Deprotection of compound **91** was performed in the manner reported for the synthesis of compound **90**. De-*O*-acetylation followed by re-*N*-acetylation and reduction produced the target acidic trisaccharide, sodium 5-aminopentyl β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-6-*O*-sulfonate-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (**92**), in an overall yield of 48%. In the ^{1}H NMR spectrum of **92**, the presence of a sharp singlet at 1.98 ppm demonstrates the presence of the hydrogens of the *N*-acetyl group (CH_3CO), confirming that re-*N*-acylation has been successful in the second step. The disappearance of the aromatic protons from the aromatic region between 7.34-7.23 ppm indicates that the benzyl groups were successfully removed. In the ^{13}C NMR spectrum, the appearance of a signal at 23.2 (CH_3CONH) confirms that re-*N*-acylation has been achieved. Removal of the benzyl groups is confirmed as no aromatic carbon signals exist. Successful reduction of the azido functionality is shown by the change in the chemical shift of the substituted methylene signal. For azide derivative **89** the signal appears at 52.4 (CH_2N_3), while in the product compound **92** the signal resonates at 39.3 ppm (CH_2NH_2). The molecular formula of **92** was confirmed by HRMS.



Scheme 2.11.1.3 **Sulfation and deprotection of trisaccharide 89: Synthesis of acidic trisaccharide 92**

3. Conclusions

The work described in this thesis has demonstrated and solved the problems encountered during formation of the linkages required for the synthesis *Zona Pellucida* oligosaccharides and has culminated in the synthesis of neutral and acidic disaccharides and trisaccharides. The target molecules containing terminal neutral and acidic (6-O-sulfonate) 2-deoxy-2-acetamido-β-D-glycopyranosyl unit linked to O-3 of a β-galactopyranosyl derivative were prepared. Early attempts at glycosylating galactose derivatives to form the desired linkage unearthed a problem associated with steric hindrance. This problem was first encountered during the removal of the benzyl ether protecting group on O-3 of ethyl 6-(2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyloxy)hexanoate (**8**). A seldom used method for de-*O*-benzylation, *N*-bromosuccinimide and light in a two-phase system with aqueous calcium carbonate was investigated and proved to be exceptional in effecting the desired transformation. This success was expanded to encompass a number of substrates demonstrating that this method was compatible with many groups not previously studied: thiophenyl glycosides, glycosyl fluorides, silyl ethers, *N*-phthalimides, acetyl, benzoyl, and pivaloyl esters. However, it proved impossible to form glycosidic bonds to 2,4,6-tri-O-pivaloate protected galactopyranosyl derivatives, even using very active glycosyl donors previously shown to react with hindered glycosyl acceptors.

To solve the problem of steric hindrance, glycosyl acceptor **48β**, 5-azidopentyl-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside, was synthesized in seven steps. The key reaction in the formation of this molecule was the stereoselective glycosylation of phenyl 2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**47**) at low temperature in acetonitrile with 5-azido-1-pentanol

(**41**). This reaction formed the β-glycoside in good yield (73%) with a minor amount of the α-glycoside **48α** (11%).

The synthesis of the neutral disaccharide derivative **49**, 5-azidopentyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside, was completed in 85% yield by condensing phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**20**) with acceptor **48β** using the NIS-AgOTf promoter system. Subsequent deprotection of **49** afforded 5-aminopentyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (**51**).

For the convergent synthesis of trisaccharides, protected βGal(1→4)βGlcNAc (LacNAc) disaccharides were chosen as the targets for eventual condensation with glycosyl acceptor **48β**. A number of LacNAc derivatives were investigated. It was found that the *tert*-butyldimethylsilyl ether protecting group was not stable to various conditions used for the formation of glycosidic bonds, particularly *N*-iodosuccinimide and silver triflate or triflic acid. This problem was solved by selecting the 2-naphthylmethyl ether as the protecting group on O-6 of GlcNAc derivatives.

The 2-naphthylmethyl group was introduced regioselectively on O-6 of phenyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**21**) to form phenyl 2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**74**) in 66% yield using the chemistry of stannylene acetals, conditions that had not been previously reported. Compound **74** proved to be a useful intermediate in the synthesis of both disaccharides and trisaccharides. Benzoylation of **74** gave phenyl 3,4-di-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**75**), which was condensed with

glycosyl acceptor **48β** in the synthesis of acidic disaccharide derivative 5-azidopentyl 3,4-di-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**80**) in 74% yield. De-*O*-2-naphthylmethylation of **80** using DDQ proceeded smoothly and in good yield to give 5-azidopentyl 3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**81**) which was sulfated and deprotected to yield the acidic disaccharide target, sodium 5-aminopentyl 2-acetamido-6-*O*-sulfonate-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (**84**).

Compound **74** also proved to be a useful substrate for regioselective galactosylation with 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (**71**) using solid AgOTf in dichloromethane. This reaction afforded phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**76**) in 65% yield. Benzoylation of the remaining hydroxyl group (O-3) afforded the desired LacNAc disaccharide **79**, phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside.

The condensation of **79** with glycosyl acceptor **48β** uncovered a another problem associated with steric hindrance. It was found that **79** reacted with the glycosyl acceptor in poor yield (only 22%) to give 5-azidopentyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside trisaccharide (**85**). This problem was solved

by changing the protecting groups on the galactopyranosyl ring of **79**. De-*O*-benzoylation followed by re-*O*-acetylation gave phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (**87**). Condensation of **87** with glycosyl acceptor **48β** afforded trisaccharide 5-azidopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**88**) in good yield (61%).

It is interesting to note that such a distant change had a dramatic impact on the success of the glycosylation reaction. Presumably, the large benzoyl groups influence the conformations of the groups on the thiophenyl galactosyl ring slowing the glycosidation reaction. This observation emphasizes how closely balanced success and failure are when the substrates are fully protected with large protecting groups. It suggests that protecting groups should be chosen for minimal size and minimal conformational mobility if other considerations are not more important.

De-*O*-2-naphthylmethylation of **88** afforded 5-azidopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (**89**), which was used in the synthesis of both the neutral and acidic trisaccharides. Compound **89** was deprotected to give the target neutral trisaccharide, 5-aminopentyl β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (**90**). Compound **89** was also regioselectivly sulfated and deprotected to afford the acidic trisaccharide target, sodium 5-aminopentyl-β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-6-*O*-sulfate-β-D-glucopyranosyl-(1→3)-β-D-

galactopyranoside (**92**).

4. Future Work

A. The development of a successful synthetic methodology for the synthesis of neutral and acidic disaccharides and trisaccharides can lead to further efforts in synthesizing larger and potentially more immunogenic oligosaccharide molecules.

B. Saturation transfer difference NMR (STD-NMR) techniques[313-318] can be performed to determine the binding capacity of the synthetic materials described in this thesis. Through a collaboration with members of the biology department at Dalhousie University, the sera of grey seals immunized with pZP can be obtained and used in this assay. These experiments may determine if anti-carbohydrate antibodies exist in the sera of these animals.

C. Using STD-NMR techniques the binding properties of the various sperm proteins discussed in the introduction (Sp56, β-(1→4)-galactosyltransferase, acrosin, aryl sulfatase) can be obtained and tested against synthetic materials. These experiments may elucidate the structures responsible for the binding of these proteins to the ZP.

D. The linker arm present at the reducing end of each oligosaccharide synthesized can be used for conjugation to a protein carrier. Some candidates for conjugation include Bovine Serum Albumin or pZP3, which has been cloned by collaborators in the Biology department at Dalhousie University. Such a conjugate vaccine may induce the production of anti-carbohydrate antibodies capable of binding to the oligosaccharide sequences responsible for sperm binding.

E. The linker arm moiety also offers the potential for conjugation of synthetic materials to a series of short peptide sequences. A number of researchers[52,107-110] have used synthetic peptides to circumvent the problems associated with ovarian failure when whole

protein vaccines are used as immunogens. A combination of synthetic oligosaccharides conjugated to various synthetic peptides may provide an even better alternative to these procedures.

5. Experimental Section

5.1 General methods.

^{1}H and ^{13}C NMR spectra were run at 300 K on Bruker AC-250 or AMX-400 NMR instruments operating at 250.13 and 62.9 MHz, and 400.16 and 100 MHz, respectively. The samples were made up in concentrations between 10 and 15 mM in chloroform-*d* unless otherwise specified. Chemical shifts are given in parts per million (ppm +/-0.01) relative to TMS (tetramethylsilane) in the case of ^{1}H NMR spectra, and to the central line of $CDCl_3$ (δ 77.23) for the ^{13}C NMR spectra. When MeOH or D_2O was used acetone was used as an external chemical shift reference for ^{13}C NMR (δ) spectra; in the case of ^{1}H NMR spectra, water (δ 4.80) was used as an internal chemical shift reference. Spectral assignments were made by first-order analysis and COSY and HETCOR experiments. Compounds whose mass spectra were obtained using electron ionization (70.0 eV) were measured on a CEC 21-110B mass spectrometer. Compounds whose mass spectra were obtained by positive ion mode electrospray ionization were measured on a Micromass ZabSpec Hybrid Sector-TOF mass spectrometer. Compounds whose mass spectra were obtained by Fab ionization were measured with a JEOL JMS–AAX505H mass spectrometer. Microanalyses were performed by the Canadian Microanalytical Service Ltd, Vancouver, B.C.

5.2 Materials

Dichloromethane was first dried with calcium chloride, then refluxed over calcium hydride for one hour, fractionally distilled and stored over molecular sieves (4 Å). *N,N*-dimethylformamide was stored over activated molecular sieves (4 Å) for 72 h before

distillation under reduced pressure from freshly activated molecular sieves (4 Å). Toluene was stored over calcium hydride for 3 h then distilled onto activated molecular sieves (4 Å). Benzyl chloride was dried with $MgSO_4$, refluxed over calcium hydride and then fractionally distilled under reduced pressure, collecting the middle fraction and then storing over calcium hydride. Pyridine was refluxed over calcium hydride for 2 h and then distilled over freshly activated 3 Å molecular sieves. Dimethyl sulfoxide, dried with activated 4 Å molecular sieves, was distilled under reduced pressure. Thin layer chromatography was performed on 0.25 mm thick Whatman G/UV silica gel aluminum plates. Components were visualized by spraying with a 2% ceric sulfate solution in 1 M H_2SO_4 followed by heating on a hot plate until discolouration occurred. Flash chromatography was performed on silica gel TLC standard grade (230-400 mesh).

5.3 Experimental

5.3.1 β-D-Galactopyranose pentaacetate (2)

Compound **2** was prepared by the method reported by Wolfrom et al.,[202] mp 140-142 °C, lit.[202] mp 142 °C.

5.3.2 Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (3)

Compound **3**, prepared as a colourless syrup by the procedure described by Ferrier *et al.*,[185] was used without purification: ^{1}H NMR (400 MHz) δ 7.52-7.30 (2m, 5H, Ar-H), 5.41 (br d, 1H, H-4), 5.23 (t, 1H, H-2), 5.04 (dd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 3.4 Hz, H-3), 4.71 (d, 1H,

$J_{1,2}$ = 10.0 Hz, H-1), 4.14 (AB of ABX system, ddd, 2H, $J_{6a,6b}$ = -11.9 Hz, $J_{5,6a} = J_{5,6b}$= 6.3 Hz, H-6a,b), 3.93 (distorted t, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 2.11, 2.09, 2.04, 1.97 (4s, 12H, 3 x CH_3CO); ^{13}C NMR (400 MHz): δ 170.3, 170.1, 170.0, 169.4 (4 x CH_3CO), 132.6-128.1 (Ar-C), 86.6 (C-1), 76.7 (C-5), 74.4 (C-2), 72.0 (C-3), 67.3 (C-4), 61.6 (C-6), 20.8, 20.6, 20.4, 20.1 (4 x CH_3CO).

5.3.3 Phenyl 1-thio-β-D-galactopyranoside (4)

Compound **3** was deacetylated by the method described by Janaki *et al.*[203] to give the title compound: mp 96-98 °C, lit.[203] mp 99-100 °C; ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.56-7.18 (3m, 5H, Ar-H), 4.58 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 3.91 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 2.4 Hz, H-4), 3.73 (AB of ABX system, m, 2H, $J_{6a,6b}$ = -11.5 Hz, $J_{5,6}$ = 6.1 Hz, H-6a,b), 3.60 (t, 1H, H-2), 3.54 (m, 1H, H-5), 3.49 (dd, 1H, $J_{2,3}$ = 9.3 Hz, $J_{3,4}$ = 3.4 Hz, H-3); ^{13}C NMR (400 MHz) δ 132.2-128.0 (Ar-C), 90.3 (C-1), 80.6 (C-5), 76.4 (C-2), 71.0 (C-4), 70.4 (C-3), 62.6 (C-6).

5.3.4 Phenyl 3-*O*-benzyl-1-thio-β-D-galactopyranoside (5)

Phenyl 1-thio-β-D-galactopyranoside[205] (20.13 g, 0.07401 mol) and dibutyltin oxide (19.57 g, 0.0786 mol, 1 mol eq) were refluxed overnight in toluene (500 mL) with azeotropic removal of water. The solvent was removed and the residue was dried at 0.1 torr for 1 h. Cesium fluoride (16.60 g, 0.1093 mol, 1.45 eq) was added followed by a solution of distilled benzyl bromide (15.82 g, 0.09248 mol, 1.0 eq) in *N*,*N*-dimethylformamide (300 mL) and the reaction mixture was stirred until TLC, ethyl acetate:acetone (4:1), indicated that the starting material had been completely consumed (3.5 h). Distilled water (2 mL) was added and the

solvents evaporated. The residue was taken up in ethanol and the mixture filtered through celite. The filtrate was concentrated to a dark brown syrup that was purified by flash chromatography using 1:1 hexanes:ethyl acetate as eluent. Crystallization (ethyl acetate) produced fine colourless needles (15.9 g, 60 %): mp 156-160 °C, lit[205] 161-163 °C; $[\alpha]_D$ -8.4°(*c* 2.0, CH_3OH), lit[205] $[\alpha]_D$ -8.5°; ^{1}H NMR (acetone-d_6) δ 7.22-7.60 (m, 10H, CH_2*Ph*,S*Ph*), 4.69, 4.79 (AB 2d, 2H, J_{AB} = -12.2 Hz, OCH_2Ph), 4.67 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 4.22 (td, 1H, $J_{4,5}$ = 1.0 Hz, $J_{3,4}$ = 3.4 Hz, H-4), 3.84 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.5 Hz, H-2), 3.77 (d, 2H, $J_{5,6}$ = 5.8 Hz, H-6a,b), 3.64 (td, 1H, $J_{4,5}$ = 0.92 Hz, $J_{5,6} = J_{5,6'}$ = 6.0 Hz, H -5), 3.36 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{2,3}$ = 9.2 Hz, H-3); ^{13}C NMR (acetone-d_6) δ 127.2 - 131.6 (CH_2*Ph*, S*Ph*), 88.9 (C-1), 83.4 (C-3), 79.6 (C-5), 71.6 (CH_2Ph), 69.3 (C-2), 66.4 (C-5), 61.8 (C-6).

5.3.5 Phenyl 3-*O*-benzyl-2,4,6-tri-*O*-pivaloyl-1-thio-β-D-galactopyranoside (6)

Compound **5** (7.19 g, 0.01986 mol) was dissolved in dry pyridine (50 mL) and pivaloyl chloride (8.81 g, 9.0 mL, 0.07307 mol, 3.7 eq.) was added dropwise. The reaction was stirred at a bath temperature of 75 °C for 12 h. The reaction mixture was poured into ice water (150 mL) that was then extracted with dichloromethane (150 mL). The organic layer was washed with hydrochloric acid (1.0 M, 3 x 150 mL), saturated sodium hydrogen carbonate (3 x 150 mL), and water (2 x 150 mL), then dried and concentrated. The residue was applied to a flash chromatography column and eluted with 20 % ethyl acetate in hexanes. The title compound was obtained as pure colourless crystals (10.36 g, 0.0168 mol, 85 %), recrystallized from methanol-water: mp 101-2 °C; $[\alpha]_D$ +21.3° (*c* 2.0, CH_2Cl_2); ^{1}H NMR δ 1.14, 1.21, 1.22 (3s, 3 x 9H, 3 x CO(CH_3)$_3$), 3.64 (dd, 1H, $J_{2,3}$ = 9.3 Hz, $J_{3,4}$ = 3.3 Hz, H-3),

3.93 (broad td, 1H, $J_{5,6} = J_{5,6'} = 6.7$ Hz, H-5), 4.16 (m, 2H, H-6a,b), 4.36, 4.67 (AB 2d, 2H, $J_{AB} = -11.3$ Hz, OCH_2Ph), 4.68 (d, 1H, $J_{1,2} = 10.1$ Hz, H-1), 5.16 (t, 1H, $J_{1,2} = J_{2,3} = 9.8$ Hz, H-2), 5.54 (broad dd, 1H, $J_{3,4} = 3.2$ Hz, H-4), 7.2-7.6 (m, 10H, CH_2Ph, SPh); ^{13}C NMR δ 26.8, 26.9, 27.0 (3 x $CO(CH_3)_3$), 38.3, 38.5, 38.8 (3 x $OC(CH_3)_3$), 62.0 (C-6), 65.5 (C-4), 67.9 (C-2), 71.2 (CH_2Ph), 74.6 (C-5), 78.2 (C-3), 86.1 (C-1), 127.4-137.0, (CH_2Ph, SPh) 176.4, 177.2, 177.8 (3 x CO); Anal. Calcd for $C_{34}H_{46}O_8S$: C, 66.42; H, 7.54; S, 5.22. Found: C, 66.57; H, 7.52; S, 5.68.

5.3.6 Ethyl 6-(3-*O*-benzyl-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyloxy)hexanoate (7).

Compound **6** (0.81 g, 1.317 mmol) was dissolved in dry dichloromethane (40 mL) under nitrogen in a glove bag and ethyl 6-hydroxyhexanoate (0.428 mL, 2.635 mmol) was added followed by *N*-iodosuccinimide (0.341 g, 1.515 mmol), silver triflate (0.677 g, 2.635 mmol), and powdered 4Å molecular sieves. The reaction vessel was then stoppered with a rubber septum and covered with aluminum foil. After 3h, the mixture was filtered over celite and the filtrate was washed with saturated sodium hydrogen carbonate (3 x 30 mL) and water (3 x 30 mL). The organic layer was dried and concentrated to a residue that was applied to a flash chromatography column. Elution with 17% ethyl acetate in hexanes gave the title compound ($R_f = 0.43$) as a colorless syrup (0.739 g, 84%): $[\alpha]_D$ +18.0° (*c* 3.7, $CHCl_3$); ^1H NMR δ 1.17, 1.20, 1.21 (3s, 3 x 9H, 3 x $COC(CH_3)_3$), 1.24 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 1.29-1.41 (complex m, 2H, $OCH_2CH_2CH_2$), 1.5-1.7 (complex m, 4H, $OCH_2CH_2CH_2CH_2$), 2.28 (t, 2H, J = 7.3 Hz, $OCOCH_2$), 3.42 (complex m, 1H, one OCH_2CH_2), 3.58 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{2,3}$ = 9.9 Hz, H-3), 3.78-3.89 (complex m, 2H, one OCH_2CH_2, H-5), 4.05-4.22

(complex m, 4H, OCH_2CH_3, H-6a,b), 4.40 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 4.37, 4.68 (AB 2d, 2H, J_{AB} = -11.3 Hz, OCH_2Ph), 5.13 (dd, 1H, $J_{1,2}$ = 8.2 Hz, $J_{2,3}$ = 10.1 Hz, H-2), 5.50 (br d, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 7.2-7.4 (m, 5H, Ph); ^{13}C NMR δ 14.4 (OCH_2CH_3), 24.8 (CH_2CH_2COOEt), 25.7 ($OCH_2CH_2CH_2$), 27.1-27.7 (9 x $COC(CH_3)_3$), 29.4 (OCH_2CH_2), 34.3 (CH_2COOEt), 38.9, 38.9, 39.2 (3 x $COC(CH_3)_3$), 60.4, (OCH_2CH_3), 61.9 (C-6), 65.6 (C-4), 69.6 (OCH_2CH_2), 70.3 (C-2), 71.1 (C-5), 71.6 (CH_2Ph), 77.5 (C-3), 101.6 (C-1), 127.7, 127.8, 128.3, 128.3, 137.5 (CH_2Ph), 173.8 ($COCH_2$), 176.9, 177.7, 178.1 (*3 x* $COC(CH_3)_3$),; EIMS m/z: 664 (not observed, M^+), 505 (2.4%, M^+ - $O(CH_2)_5COOEt$); HRMS for $C_{28}H_{41}O_8$: calcd 505.2801. Found, 505.2806.

5.3.7 Ethyl 6-(2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyloxy)hexanoate (8)

Compound 7 (0.068 g, 0.102 mmol) was dissolved in carbon tetrachloride (10 mL) and water (5 mL). NBS (2.5 equiv, 0.047 g, 0.250 mmol) was then added, followed by calcium carbonate (2.4 equiv, 0.024 g, 0.246 mmol). The solution was then purged with nitrogen for one hour. Using a 350 W incandescent light, the reaction mixture was irradiated for 15 min while monitoring by TLC. The mixture was then poured into water (20 mL) and extracted with dichloromethane (3 x 20 mL). The combined extracts were dried ($MgSO_4$), concentrated, and the residue purified by flash chromatography on silica gel using as eluent, hexanes:ethyl acetate (R_f 0.50), to give the title compound (0.057 g, 95%) as a colorless syrup: $[\alpha]_D$ +3.2° (*c* 6.1, CH_2Cl_2); ^1H NMR δ 1.25 (t, 3H, J = 6.8 Hz, OCH_2CH_3), 1.29-1.41 (complex m, 2H, $OCH_2CH_2CH_2$), 1.5-1.8 (complex m, 4H,$OCH_2CH_2CH_2CH_2$), 2.02, 2.04, 2.13 (3s, 3 x 3H, 3 x $COCH_3$), 2.30 (t, 2H, J = 7.3 Hz, $OCOCH_2$), 3.41-3.50 (complex m, 1H,

one OCH_2CH_2), 3.80-3.92 (m, 3H, one OCH_2CH_2, H-3, H-5), 4.10 (q, 2H, J = 7.0 Hz, OCH_2CH_3), 4.12 (AB part of ABX, 2H, H-6a,b), 4.46 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 4.90 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 10.1 Hz, H-2),, 5.30 (br d, 1H, $J_{3,4}$ = 3.5 Hz, H-4); ^{13}C NMR: δ 14.3 (OCH_2CH_3), 21.1 (CH_2CH_2COOEt), 24.7 ($OCH_2CH_2CH_2$), 25.6 (OCH_2CH_2), 27.2-27.3 (9 x $C(CH_3)_3$) 29.4 (CH_2COOEt), 39.8, 39.0, 39.4 (3 x $C(CH_3)_3$) 60.3 (OCH_2CH_3), 61.9 (C-6), 69.4, 69.7 (C-4, OCH_2CH_2), 71.1, 72.1 (C-3, C-5), 72.9 (C-2), 101.1 (C-1), 173.7 ($COCH_2$), 178.0, 178.2, 179.2 (3 x $COC(CH_3)_3$); EIMS m/z: 574 (not observed, M^+), 415 (5.3%, M^+ - $O(CH_2)_5CO_2Et$); HRMS for $C_{21}H_{35}O_8$: calcd 415.2332. Found, 415.2333.

5.3.8 3-*O*-Benzyl-2,4,6-tri-*O*-pivaloyl-α-D-galactopyranosyl fluoride (9)

Compound **6** (0.5215 g, 0.848 mmol) was dissolved in dry dichloromethane (20 mL) and cooled with a $CaCl_2$ - ice bath that maintained the temperature at 0 °C. Diethylaminosulfur trifluoride (0.155 mL, 1.27 mmol) was added dropwise to the stirred solution followed by *N*-bromosuccinimide (NBS) (0.196 g, 1.10 mmol). After 40 min, the reaction mixture was poured into a saturated solution of sodium hydrogen carbonate (50 mL). The aqueous mixture was extracted with ether (4 x 25 mL) and the combined extracts were dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography on silica gel using hexanes:ethyl acetate (5.5:1) to give the title compound (R_f = 0.94) as pure colourless crystals (0.3675 g, 88%) that were recrystallized from ether-hexanes: mp 103-104 °C; $[\alpha]_D$ +73.0° (*c* 0.9, CH_2Cl_2); ^{1}H NMR δ 1.21 (s, 3 x 9H, 3 x $CO(CH_3)_3$), 3.99 (dd, 1H, $J_{3,4}$ = 3.20 Hz, $J_{2,3}$ = 10.2 Hz, H-3), 4.13 (d, 2H, H-6a,b), 4.34 (broad t, 1H, $J_{5,6a} = J_{5,6b}$ = 6.7 Hz, H-5), 4.46, 4.71 (AB 2d, 2H, J_{AB} = -11.1 Hz, OCH_2Ph), 5.16 (ddd, 1H, $J_{1,2}$ = 2.6 Hz, $J_{2,3}$

=10.2 Hz, $J_{2,F}$ = 24.7 Hz, H-2), 5.64 (broad d, 1 H, $J_{3,4}$ = 2.5 Hz, H-4), 5.64, 5.86 (2d, 1H, $J_{1,2}$ = 2.6, $J_{1,F}$ = 53.9 Hz, H-1), 7.2-7.6 (m, 5H, CH_2Ph); ^{13}C NMR: δ 27.2, 27.2, 27.3 (3 x $CO(CH_3)_3$), 39.0, 39.3 (3 x $COC(CH_3)_3$), 61.6 (C-6), 66.0 (C-4), 69.4 (d, $^2J_{C,F}$ = 24.0 Hz, C-2), 69.4 (C-5), 71.9 (CH_2Ph), 73.0 (C-3), 104.7 (d, $^1J_{C,F}$ = 226.7 Hz, C-1), 127.2, 127.5, 127.8, 128.3, 129.1, 137.3 (CH_2Ph),177.3, 177.9, 177.9 (3 x OCO); ^{19}F NMR: δ -150.52 (dd, $J_{H1,F}$ = 53.4 Hz, $J_{H2,F}$ = 25.1 Hz,F); EIMS m/z: 524 (1.0%, M^+), 489 (0.5%, M^+ - HF -CH_3), 439 (1%7, M^+ - *t*-BuCO); Anal. Calcd for $C_{28}H_{41}O_8F$: C, 64.10; H, 7.88. Found: C, 64.22; H, 7.71.

5.3.9 Phenyl 3-*O*-benzyl-2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (10)

Acetic anhydride (470 mL) was added slowly to a stirred solution of compound **5** (15.67 g, 0.04353 mol) in pyridine (470 mL) at 0°C and the solution was left for 12 h, then poured into an ice-cold saturated solution of sodium hydrogen carbonate (900 mL). The resulting mixture was extracted with dichloromethane (3 x 500 mL) and the combined extracts were washed with hydrochloric acid (0.8 M, 3 x 250 mL), dried and concentrated to a dark yellow solid (25.69 g). Recrystallization from hexanes:ethyl acetate (3:1) yielded the title compound as colourless needles (19.05 g, 90.2%): $[\alpha]_D$ +63.4° (*c* 3.1, $CHCl_3$); mp 100-101°C; ^1H NMR δ 2.06, 2.08, 2.16 (3s, 3 x 3H, 3 x $COCH_3$), 3.57 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{2,3}$ = 9.5 Hz, H-3), 3.83 (td, 1H, $J_{4,5}$ = 1.7 Hz, $J_{5,6a}$ = $J_{5,6b}$ = 6.3 Hz, H -5), 4.17 (d, 2H, $J_{5,6}$ = 6.4 Hz, H-6a,b), 4.62 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 4.40, 4.68 (AB 2d, 2H, J_{AB} = -12.3 Hz, OCH_2Ph), 5.16 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.8 Hz, H-2), 5.54 (dd, 1H, $J_{4,5}$ = 1.1 Hz, $J_{3,4}$ = 3.4 Hz, H-4), 7.23-7.55 (m, 10H, CH_2Ph,S*Ph*); ^{13}C NMR δ: 20.8, 20.8, 21.0 (3 x $COCH_3$), 62.3 (C-6), 66.0

(C-4), 68.8 (C-2), 71.3 (*C*H_2Ph), 74.7 (C-5), 77.6 (C-3), 86.6 (C-1), 127.9-137.3 (CH_2*Ph*, S*Ph*), 169.4, 170.4, 170.5 (CO); EIMS m/z: 488 (not observed, M^+), 379 (60.6%, M^+ - SPh); HRMS for $C_{19}H_{23}O_8$: calcd 379.1393. Found, 379.1377. Anal. Calcd for $C_{25}H_{28}O_8S$: C, 61.46; H, 5.78. Found: C, 61.50; H, 5.62.

5.3.10 Ethyl 6-(2,4,6-tri-O-acetyl-3-O-benzyl-β-D-galactopyranosyloxy)hexanoate (11)

Compound **10** (21.28 g, 0.0441 mol) was dissolved in dry dichloromethane (200 mL) containing activated 3Å powdered molecular sieves in a 2 necked round bottomed flask wrapped in aluminum foil. Ethyl 6-hydroxyhexanoate (14.78 g, 15.0 mL, 0.09222 mol, 2.0 eq) was added, followed by *N*-iodosuccinimide (11.46 g, 0.0511 mol, 1.15 eq), followed by a solution of silver triflate (12.76 g, 0.0497 mol, 1.10 eq) in dry toluene (100 mL). The reaction mixture was stirred at room temperature under a stream of N_2 (g) until starting material was consumed (1.5 h). The reaction mixture was filtered through celite, and the filtrate was washed with saturated sodium hydrogen carbonate solutions (2 x 250 mL), followed by water (2 x 250 mL). The water layers were back extracted with CH_2Cl_2 (2 x 250 mL), combined, dried, and concentrated to yield a dark yellow residue. Flash chromatography on silica gel using hexanes:ethyl acetate (2:1) gave the title compound as a syrup (4.58 g, 19.5%): $[\alpha]_D$ +36.6° (*c* 4.2, $CHCl_3$); ^{1}H NMR 1.24 (t, 3H, J = 7.0 Hz, OCH_2CH_3), 1.29-1.41 (complex m, 2H, $OCH_2CH_2CH_2$), 1.5-1.7 (complex m, 4H, $OCH_2CH_2CH_2CH_2$), 2.02, 2.06, 2.13 (3s, 3 x 3H, 3 x $COCH_3$), 2.27 (t, 2H, J = 7.3 Hz, $OCOCH_2$), 3.45 (complex m, 1H, one OCH_2CH_2), 3.54 (dd, 1H, $J_{3,4}$ = 3.5 Hz, $J_{2,3}$ = 9.9 Hz, H-3), 3.79 (t, 1H, $J_{5,6}$ = $J_{5,6'}$ = 6.8 Hz, H-5), 3.82-3.94 (m, 1H, one OCH_2CH_2), 4.11 (q, 2H,

J = 7.0 Hz, OCH_2CH_3), 4.17 (AB part of ABX, 2H, H-6a,b), 4.35 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), 4.40, 4.69 (AB 2d, 2H, J_{AB} = -12.2 Hz, OCH_2Ph), 5.11 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 5.51 (br d, 1H, $J_{3,4}$ = 3.4 Hz, H-4), 7.2-7.4 (m, 5H, CH_2Ph); ^{13}C NMR δ: 14.3 (OCH_2CH_3), 20.7, 20.8, 20.8 (3 x $COCH_3$), 24.6 (CH_2CH_2COOEt), 25.4 ($OCH_2CH_2CH_2$), 29.1 (OCH_2CH_2), 34.2 (CH_2COOEt), 60.3 (OCH_2CH_3), 62.0 (C-6), 65.9 (C-4), 69.6 (OCH_2CH_2), 70.5 (C-2), 70.9 (C-5), 71.3 (OCH_2Ph), 76.6 (C-3), 101.4 (C-1), 127.8-137.6 (CH_2Ph), 169.4, 170.5, 170.6 (3 x $COCH_3$), 173.6 ($COCH_2$); EIMS m/z: 538 (not observed, M^+), 379 (11.6%, M^+ - $O(CH_2)_5CO_2Et$); HRMS for $C_{19}H_{23}O_8$: calcd 379.1393. Found, 379.1369.

5.3.11 Phenyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-1-thio-β-D-galactopyranoside (12)

Compound **5** (1.95 g, 5.38 mmol) was dissolved in pyridine (150 mL) and benzoyl chloride (2.81 mL) was added. The reaction was stirred for 12 h with the exclusion of moisture. When TLC analysis, hexanes:ethyl acetate (3:1), indicated that the starting material had been totally consumed. The solution was poured into ice-cold water (150 mL). The mixture was extracted with CH_2Cl_2 (3 x 25 mL) and the combined extracts were washed with hydrochloric acid (1.0 M, 3 x 150 mL) and water (150 mL). The solution was dried and concentrated to a dark yellow syrup which was purified by flash chromatography using hexanes:ethyl actate(3:1) to yield the title compound (R_f = 0.45) as colorless syrup (3.05 g, 84%); ^1H NMR δ 3.84 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{2,3}$ = 9.5 Hz, H-3), 4.16 (td, 1H, $J_{4,5}$ = 1.1 Hz, $J_{5,6}$ = $J_{5,6b}$ = 5.3 Hz, H-5), 4.44-4.61 (m, 2H, $J_{5,6a}$ = 6.4 Hz, H-6a,b), 4.48, 4.68 (AB 2d, 2H, $J_{A,B}$ = -12.6 Hz, OCH_2Ph) 4.85 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 5.54 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 9.8 Hz, H-2), 5.93 (br d, 1H, $J_{3,4}$ = 3.2 Hz, H-4), 7.06-8.10 (m, 25H, 3x $COPh$, CH_2Ph, SPh); ^{13}C NMR

δ 20.8, 20.8, 21.0 (3 x $COCH_3$), 63.3 (C-6), 67.0 (C-4), 69.6 (C-2), 71.1 (OCH_2Ph), 75.3 (C-5), 77.5 (C-3), 86.2 (C-1), 127.9-137.2 (Ar-C), 165.3, 165.9, 166.3 (3 x *C*OPh).; EIMS m/z: 674 (not observed, M^+), 566 (7.7%, M^+ - SPh); HRMS for $C_{34}H_{29}O_8$: calcd 565.1862. Found, 565.1883.

5.3.12 Phenyl 2,4,6-tri-*O*-pivaloyl-1-thio-β-D-galactopyranoside (13)

Compound **6** (1.00 g, 0.16 mmol) was dissolved in carbon tetrachloride (10 mL) and water (5 mL). NBS (2.5 equiv, 0.049 g, 0.276 mmol) was then added, followed by calcium carbonate (4.4 equiv, 0.072 g, 0.716 mmol). The solution was then purged with nitrogen for one hour. Using a 350 W incandescent light, the reaction mixture was irradiated for 15 min while monitoring by TLC. The mixture was then poured into water (25 mL) and extracted with dichloromethane (3 x 30 mL). The combined extracts were dried ($MgSO_4$), concentrated, and the residue purified by flash chromatography on silica gel using hexanes:ethyl acetate (5.5:1) as eluent (R_f = 0.23), to give the title compound (0.081 g, 95%) as a colorless syrup, which crystallized from hexane to give fine colorless needles: mp 105-106 °C; $[\alpha]_D$ -5.0° (*c* 1.2, $CHCl_3$); ^1H NMR δ 1.27, 1.20 (2s, 3 x 9H, 3 x $CO(CH_3)_3$), 3.91 (m, 2H, H-3, H-5), 4.12 (m, 2H, H-6a,b), 4.70 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 4.92 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.8 Hz, H-2), 5.32 (broad dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 0.9, H-4), 7.2-7.6 (m, 5H, S*Ph*); ^{13}C NMR δ 27.1 (3 x $CO(CH_3)_3$), 38.7, 38.9, 39.2 (3 x $COC(CH_3)_3$), 62.1 (C-6), 69.5 (C-4), 70.2 (C-2), 73.0, 74.9 (C-3, C-5),86.6 (C-1), 128.3-133.2, (S*Ph*), 178.0, 178.1, 178.6 (3 x OCO); HRMS for $C_{27}H_{40}O_8S$: calcd 524.2444. Found, 524.2437.

5.3.13 2,4,6-Tri-*O*-pivaloyl-β-D-galactopyranosyl fluoride (14)

Compound **9** (0.229 g, 0.56 mmol) was reacted as outlined for compound **13** to give the title compound (0.187 g, 87%) as a colorless syrup: R_f = 0.50 (16% ethyl acetate in hexanes); $[\alpha]_D$ +52.5° (*c* 3.9, CH_2Cl_2); ^{1}H NMR δ 1.20, 1.28, 1.29 (3s, 3 x 9H, 3 x CO(CH_3)$_3$), 4.11 (d, 2H, H-6a,b), 4.28 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{2,3}$ = 10.4 Hz, H-3), 4.37 (broad t, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 6.7 Hz, H-5), 4.98 (ddd, 1H, $J_{1,2}$ = 2.6 Hz, $J_{2,3}$ =10.4 Hz, $J_{2,F}$ = 24.4 Hz, H-2), 5.44 (broad d, 1 H, $J_{3,4}$ = 3.4 Hz, H-4), 5.63, 5.84 (2d, 1H, $J_{1,2}$ = 2.9, $J_{1,F}$ = 53.9 Hz, H-1); ^{13}C NMR δ 27.1, 27.2, 27.4 (3 x CO(*C*H$_3$)$_3$, 38.9, 39.2, 39.5 (3 x CO*C*(CH$_3$)$_3$), 61.4 (C-6), 67.0 (C-3), 69.5 (2C, C-4, C-5), 70.5 (d, $^2J_{C,F}$ = 23.8 Hz, C-2), 104.8 (d, $^1J_{C,F}$ = 227.7 Hz, C-1),178.1, 178.4, 178.9 (3 x O*C*O); EIMS m/z: 434 (not observed, M^+), 414 (7.6%, M^+ - HF), 399 (1.5%, M^+ - HF - CH_3), 333 (2.8 %. M^+ - OCO*t*Bu); HRMS for $C_{21}H_{34}O_8$: calcd 414.2253. Found, 414.2245.

5.3.14 Ethyl 6-(2,4,6-tri-*O*-acetyl-β-D-galactopyranosyloxy)hexanoate (15)

Compound **11** (2.00 g, 3.43 mmol) was reacted as outlined for compound **13** to give the title compound (1.26g, 83%) as a colorless syrup: $[\alpha]_D$ +7.7° (*c* 1.3, CH_2Cl_2); ^{1}H NMR δ 1.25 (t, 3H, J = 6.8 Hz, OCH_2CH_3), 1.29-1.41 (complex m, 2H, $OCH_2CH_2CH_2$), 1.5-1.8 (complex m, 4H, $OCH_2CH_2CH_2CH_2$), 2.02, 2.04, 2.13 (3s, 3 x 3H, 3 x $COCH_3$), 2.29 (t, 2H, J = 7.3 Hz, $OCOCH_2$), 3.48 (X of ABXY, 1H, OCH_2CH_2), 3.79-3.93 (complex m, 3H, OCH_2CH_2, H-3, H-5), 4.12 (q, 2H, J = 7.2 Hz, OCH_2CH_3),4.15 (AB part of ABX, 2H, H-6a,b), 4.41 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.97 (dd, 1H, $J_{1,2}$ = 8.4 Hz, $J_{2,3}$ = 9.8 Hz, H-2), 5.33 (br d, 1H, $J_{3,4}$ = $J_{4,5}$ = 3.2, H-4); ^{13}C NMR δ 14.4 (OCH_2CH_3), 20.8, 20.9, 21.1 (3 x $COCH_3$), 24.7

(CH_2CH_2COOEt), 25.5 ($OCH_2CH_2CH_2$), 29.2 (OCH_2CH_2), 34.23 (CH_2COOEt), 60.4 (OCH_2CH_3), 62.1 (C-6), 69.9 (C-4, OCH_2CH_2), 71.0, 71.4 (C-3, C-5), 72.8 (C-2), 101.1 (C-1), 170.7, 171.1, 171.1 (3 x $COCH_3$), 173.6 ($COCH_2$); EIMS m/z: 448 (not observed, M^+), 403 (0.9%, M^+ - OEt), 289 (14.8%, M^+ - $O(CH_2)_5CO_2Et$); HRMS for $C_{18}H_{27}O_{10}$ and $C_{12}H_{17}O_8$: calcd 403.1604 and 289.0923. Found, 403.1633 and 289.0987, respectively.

5.3.15 Phenyl 2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (16)

Compound **10** (0.197 g, 0.404 mmol) was reacted as outlined for compound **13** to give the title compound (0.122 g, 76%) as a colorless syrup, crystallized from hexanes:ethyl acetate to give fine colorless needles: mp 120-121 °C R_f = 0.21 (hexanes:ethyl acetate, 3:2); $[\alpha]_D$ +14.6° (*c* 1.0, chloroform); ^1H NMR δ 2.05, 2.15, 2.73 (3s, 3 x 3H, 3 x $CO(CH_3)$), 3.89 (m, 2H, H-3, H-5), 4.13, 4.16 (AB part of ABX pattern, 2H, $J_{5,6}$ = 7.4 Hz, $J_{5,6'}$ = 5.5 Hz, $J_{6a,6b}$ = -11.5 Hz, H-6a,b), 4.68 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 5.04 (t, 1H, $J_{1,2} = J_{2,3}$ = 9. Hz, H-2), 5.36 (dd, 1 H, $J_{3,4}$ = 3.5 Hz, $J_{4,5}$ = 0.94 Hz, H-4), 7.20-8.10 (3m, 6H, S*Ph*); ^{13}C NMR δ 20.8, 21.2, 29.7 (3 x $CO(CH_3)$), 62.4 (C-6), 70.1 (C-4), 70.8 (C-2), 72.1, 74.9 (C-5, C-3), 86.3 (C-1), 128.1-132.4 (S*Ph*),171.2, 171.2, 178.5 (3 x OCO); EIMS m/z: 398 (0.5%, M^+), 289 (100%, M^+ - SPh); HRMS for $C_{18}H_{22}O_8S$: calcd 398.1035. Found, 398.1054.

5.3.16 Phenyl 2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (17)

Compound **12** (0.414 g, 0.614 mmol) was dissolved in carbon tetrachloride (100 mL) and water (15 mL). NBS (4.1 eq., 0.45 g, 2.53 mmol) was then added, followed by calcium carbonate (5.1 eq., 0.32 g, 3.15 mmol). The solution was then purged with nitrogen for one

hour. Using a 350W incandescent light, the reaction mixture was irradiated for 20 min while monitoring by TLC. The mixture was then poured into water (25 mL) and extracted with dichloromethane (3 x 30 mL). The combined extracts were washed with hydrochloric acid (1.0 M, 2 x 100 mL) and water (2 x 100 mL), dried ($MgSO_4$), concentrated, and the residue purified by flash chromatography using hexanes:ethyl acetate (3:2) to give the title compound (0.227 g, 55%) as a colorless syrup.; ^{1}H NMR δ 4.15 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{2,3}$ = 9.5 Hz, H-3), 4.21 (br t, 1H, $J_{5,6a} = J_{5,6b}$ = 6.4 Hz, H -5), 4.42-4.60 (m, 2H, H-6a,b), 4.93 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1), 5.30 (t, 1H, $J_{1,2} = J_{2,3}$ = 9.6 Hz, H-2), 5.78 (broad d, 1H, $J_{3,4}$ = 2.7), 7.2-7.6 (m, 20H,2 x CO*Ph*, S*Ph*); ^{13}C NMR δ 63.1 (C-6), 70.9 (C-4), 71.8 (C-2), 73.2 (C-3), 75.5 (C-5), 85.6 (C-1), 127.3-134.0 (Ar-C), 166.3, 166.3, 167.0(3 x *C*OPh); EIMS m/z: 584 (not observed, M^+), 475 (26.5%, M - SPh), 105 (100 %, COPh). HRMS for $C_{27}H_{23}O_8$: calcd 475.1393. Found, 475.1407.

5.3.17 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (19)

D-Glucosamine hydrochloride (**8**, 100 g, 0.46 mol) was dissolved in methanol (200 mL) and a sodium hydroxide solution (18.6 g, 0.46 mol in 100 mL H_2O) was added. The reaction mixture was cooled to 10 °C and a solution of phthalic anhydride (80 g, 0.54 mol) in acetone (600 mL) was added to the reaction mixture. The temperature was kept between 10 and 20 °C while the reaction was stirred for 30 min. Then $NaHCO_3$ (80 g, 0.95 mol) and more phthalic anhydride (30 g, 0.20 mol) were added to the stirred solution. The reaction was allowed to warm up to room temperature and its progress was monitored by TLC. After 4 h, the reaction was complete and it was quenched by adding concentrated hydrochloric acid (60

mL) until an approximate pH of 1 was reached. The solution was evaporated to about half volume and was left in the freezer overnight. A white precipitate was obtained which was filtered using a sintered glass funnel and dried in *vacuo* overnight (108.66 g, 76%).

This white solid, 2-(2'-carboxybenzamido)-2-deoxy-D-glucopyranose, as reported by Lemieux,[145] was used to prepare the title compound by the procedure described by Wolfrom *et al.*[202] except that no precipitate was obtained on pouring the solution onto cracked ice (2 L). An extraction was then performed using dichloromethane (2 x 1 L). The organic layer was dried and evaporated to yield an orange syrup which contained the β-anomer (major) and some α-anomer (minor). The crude product was purified by MPLC using hexanes:ethyl acetate (1:1) as eluent to give the β-product as bright yellow solid which was crystallized, using hexanes:ethyl acetate (1:1) mixture, as long and thick colorless needles (160.79 g, 73%): mp 90-92 °C; lit. mp 90-94 °C;[145] lit. mp 74-75 °C (from ethanol);[235] $[\alpha]^{25}_{D}$ +64.4° (*c* 0.52, $CHCl_3$); lit. $[\alpha]^{22}_{D}$ +65.5° (*c* 1.0, chloroform);[145] lit. $[\alpha]^{25}_{D}$[235] +70.5° (*c* 1.3, $CHCl_3$); ^{1}H NMR δ 7.86-7.74 (AA'BB' 2m, 4H, N*Phth*-H), 6.52 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1), 5.89 (dd, 1H, $J_{3,4}$ = 9.2 Hz, $J_{2,3}$ = 10.4 Hz, H-3), 5.22 (t, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 4.48 (dd, 1H, $J_{1,2}$ = 8.8 Hz, $J_{2,3}$ = 10.7 Hz, H-2), 4.38 (A of ABX pattern, dd, 1H, $J_{6a,6b}$ = -12.5 Hz, $J_{5,6a}$ = 4.3 Hz, H-6a), 4.13 (B of ABX pattern, dd, 1H, H-6b), 4.35 (X of ABX system, ddd, 1H, H-5), 2.12, 2.05, 2.01, 1.87 (4s, 12H, 4 x CH_3CO); ^{13}C NMR δ 170.7, 170.1, 169.5, 168.6 (4 x CH_3CO), 167.1 (2 x NCO), 134.5, 130.8, 123.8 (N*Phth*-C), 89.7 (C-1), 72.6 (C-5), 70.5 (C-3), 68.2 (C-4), 61.4 (C-6), 53.5 (C-2), 20.7, 20.6, 20.4, 20.1 (4 x CH_3CO).

5.3.18 Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (20)

Compound **20** was prepared from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**9**, 63.76 g, 0.134 mol) in 69% yield (48.29 g) by the method reported by Ferrier:[185] mp 140-141 °C (from 15% ethyl acetate in hexanes); lit. mp 145-146 °C (from MeOH);[236] $[\alpha]^{25}_{D}$ +49.4° (*c* 0.88, $CHCl_3$); lit.[236] $[\alpha]_D$ +53.0° (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.86-7.74 (AA'BB', 4H, N*Phth*-H), 7.45, 7.26 (2m, 5H, Ar-H), 5.80 (t, 1H, $J_{2,3} = J_{3,4}$ = 10.4 Hz, H-3), 5.72 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 5.15 (t, 1H, $J_{4,5}$ = 10.1 Hz, H-4), 4.36 (t, 1H, $J_{1,2}$ = 10.4 Hz, H-2), 4.26 (AB of ABX pattern, m, 2H, $J_{5,6}$ 4.9 Hz, $J_{6a,6b}$ = -12.5 Hz, H-6), 3.91 (X of ABX system, ddd, 1H, H-5), 2.11, 2.03, 1.84 (3s, 9H, 3 x CH_3CO); ^{13}C NMR δ 170.6, 170.1, 169.5 (3 x CH_3CO), 167.0 (2 x N*C*O), 134.4, 128.9, 128.4, 123.1 (Ar-C), 133.3, 130.9, 123.7 (N*Phth*, S*Ph*), 83.0 (C-1), 75.9 (C-5), 71.6 (C-3), 68.7 (C-4), 62.2 (C-6), 53.5 (C-2), 20.8, 20.6, 20.4 (3 x CH_3CO).

5.3.19 Phenyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (21)

Compound **20** (15.95 g, 0.030 mol) was dissolved in dry methanol. A 3.4 mL aliquot of a solution of NaOMe/MeOH (3.0 M, 0.24 g Na in 3.5ml MeOH) was then added and the reaction allowed to proceed overnight. TLC, hexanes:ethyl acetate (1:1), indicated that only one product remains. IR-120, ion exchange resin was then added to the stirred solution (10 min), and then filtered. Filtrate was then concentrated, dissolved in water (30 mL), and transferred to a separatory funnel. The water layer was then extracted with dichloromethane (3 x 20 mL). The organic extracts were dried ($MgSO_4$), and concentrated to give the title

compound (10.62g, 88%) as a white solid: $[\alpha]^{25}_{D}$ +52.7° (*c* 1.2, $CHCl_3$); lit.[187] $[\alpha]_D$ +49.5° (*c* 0.18, $CHCl_3$).

5.3.20 Phenyl 2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (22)

Tert-butyldimethylsilyl chloride (0.702 g, 4.66 mmol) in dry pyridine (15 mL) was added dropwise (3 mL / 20 min) via a syringe pipet to a stirred solution of compound **21** (1.558 g, 3.88 mmol) in dry pyridine (30 mL) and the reaction mixture was stirred 12 h, then poured into water (200 mL). The mixture was extracted with diethyl ether (5 x 40 mL). The combined extracts were dried ($MgSO_4$), concentrated, and the residue purified by flash chromatography on silica gel using hexanes:ethyl acetate (1:2) to give the title compound (R_f = 0.66) as a colorless syrup (1.402 g, 70%), crystallized from ethanol-water to give very fine colorless needles: mp 68-70 °C; $[\alpha]_D$ +26.1° (*c* 2.1, CH_2Cl_2); ^{1}H NMR δ 0.11 (d, 2 x 3H, $Si(CH_3)_2$), 0.91 (d, 9H,$SiC(CH_3)_3$, , 3.58 (broad m, 2H, H-4, H-5), 3.93 (dq, 2H, H-6a,b), 4.18(t,1H, $J_{1,2}$ = $J_{2,3}$ =10.4 Hz, H-2) 4.35 (broad t, 1H, $J_{3,4}$ = $J_{2,3}$ = 7.9 Hz, H-3), 5.60 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 7.2-7.6 (4m, 9H, S*Ph*, N*Phth*): ^{13}C NMR δ -5.4 ($SiC(CH_3)_3$), 18.2 ($SiC(CH_3)_3$), 25.8 ($SiC(CH_3)_3$), 55.2 (C-2), 64.7 (C-6), 72.7, 74.3 (C-3, C-4), 78.0 (C-5), 83.6 (C-1), 127.8-134.2 (S*Ph*, N*Phth*), 210.6 (2 x *C*O, N*Phth*); EIMS m/z, 515 (M^+ not observed), 406 (10%, M - SPh), 388 (72%, 406 - H_2O); Anal. Calcd for $C_{26}H_{33}NO_6SSi.H_2O$: C, 58.51; H, 6.61; N, 2.62. Found: C, 58.69; H, 6.54; N, 2.55.

5.3.21 Phenyl 2-phthalimido-3,4-di-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-1-thio-β-D-glucopyranoside (23)

Compound **22** (0.100 g, 0.194 mmol) was dissolved in dry benzyl chloride (10 mL, 71.8 mmol). Sodium hydride (60% in mineral oil, 0.017 g, 0.423 mmol) was then added and the reaction was allowed to proceed under nitrogen at 70 °C for 12 h. The reaction mixture was then poured into water (20 mL) and extracted with dichloromethane (3 x 30 mL). The combined extracts were dried ($MgSO_4$), concentrated, and the residue purified by flash chromatography using hexanes:ethyl acetate (2:1)as eluent to give the title compound (R_f = 0.65) as a colorless syrup (0.068 g, 50%): $[\alpha]_D$ +22.3° (*c* 0.9, CH_2Cl_2); ^{1}H NMR δ 0.11 (d, 2 x 3H, $Si(CH_3)_2$), 0.91 (d, 9H, $SiC(CH_3)_3$), 3.52 (broad dt, 1H, $J_{4,5}$ = 10.4, $J_{5,6}$ = $J_{5,6'}$ = 2.6 Hz, H-5), 3.78 (broad t, 1 H, $J_{3,4}$ = $J_{4,5}$ = 8.5 Hz, H-4), 3.92 (broad s, 2H, H-6a,b), 5.16 (t,1H, $J_{1,2}$ = $J_{2,3}$ =10.2 Hz, H-2) 4.35 (t, 1H, $J_{3,4}$ = $J_{2,3}$ = 8.8 Hz, H-3), 4.43, 4.78 (AB 2d, 2H, J_{AB} = -12.0 Hz, OCH_2Ph), 4.77, 4.86 (AB 2d, 2H, J_{AB} = -11.0 Hz, OCH_2Ph), 5.51 (d, 1H, $J_{1,2}$ = 10.2 Hz, H-1), 7.2-7.6 (4m, 19H, 2 x CH_2Ph, S*Ph*, N*Phth*); ^{13}C NMR δ -4.9 ($SiC(CH_3)_3$) 26.0 ($SiC(CH_3)_3$), 29.7 ($SiC(CH_3)_3$), 54.9 (C-2), 61.5 (C-3), 62.1 (C-6), 72.1, 75.0 (2 x OCH_2Ph), 79.0 (C-4), 80.2 (C-5), 83.1 (C-1), 127.4-132.7, (2 x CH_2Ph, S*Ph*, N*Phth*), 138.2, 138.4 (2 x *C*O, N*Phth*).

5.3.22 Phenyl 4,6-di-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (24)

To a solution of compound 21 (0.186 g, 0.463 mmol) in dry DMF (20 mL) at 60°C was added *p*-toluenesulfonic acid (29 mg) and benzaldehyde dimethyl acetal (0.07 mL, 1.0

eq.). The reaction was allowed to proceed until the starting material was consumed (2 h) and a new spot appeared on TLC, hexanes:ethyl acetate (2:1) with R_f = 0.43. A solution containing $NaHCO_3$ (0.25 g) in water (25 mL) was then added to the reaction mixture and the solution allowed to cool to room temperature. The reaction mixture was then extracted with CH_2Cl_2 (3 x 25 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated. The resulting residue was purified by flash chromatography using hexanes:ethyl acetate (2:1) as eluent to afford compound **24** (0.147 g, 65%). $[\alpha]^{25}_D$ +35.2° (*c* 1.1, $CHCl_3$); lit.[187] $[\alpha]_D$ +36.3° (*c* 0.7, $CHCl_3$); ^{1}H NMR δ 3.56 (t, 1 H, $J_{3,4}$ = 9.2, H-4), 3.70 (m, 1H, H-5), 3.80 (t,1H, $J_{4,5}$ = 9.6 H-6a), 4.30 (t, 1H, $J_{1,2}$ = 10.4, $J_{2,3}$ = 10.2, H-2), 4.36 (dd, 1H, $J_{4,5}$ = 10.1, $J_{5,6b}$ = 4.3 H-6b), 4.60 (broad t, 1H, $J_{3,4}$ = 9.2, H-3), 5.54 (s, 1H, O_2CHPh) 5.66 (d, 1H, $J_{1,2}$ = 10.5, H-1), 7.20-7.90 (14H, S*Ph*, N*Phth*, O_2CHPh); ^{13}C NMR δ 55.7 (C-3), 68.7 (C-6), 69.8 (C-2), 70.4 (C-5), 82.0 (C-4), 84.4 (C-1), 102.1 (O_2CHPh), 123.5-137.1 (N*Phth*, O_2CHPh, S*Ph*).

5.3.23 Phenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (25)

To a solution of compound **24**, (15.38 g, 30.6 mmol) in dry pyridine (250 mL) at room temperature was added benzoyl chloride (5.33 mL, 4.83 mmol, 1.5 eq.). The reaction was allowed to proceed overnight where a new spot (R_f = 0.50) appeared on TLC, hexanes:ethyl acetate (3:1). The reaction mixture was then poured into cold water (200 mL) and extracted with CH_2Cl_2 (3 x 150 mL). The combined organic extracts were washed with saturated sodium hydrogen carbonate (100 mL) followed by water (100 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography using hexanes:ethyl acetate

(3:1) to give compound **25** (18.01g, 99%): ^{1}H NMR: δ 7.90-7.20 (19H, S*Ph*, N*Phth*, O_2CH*Ph*, CO*Ph*), 6.21 (t, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 9.0 Hz, H-3), 5.91 (d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), 5.56 (s, 1H, O_2C*H*Ph), 4.56 (t,1H, $J_{1,2}$ = 10.2 Hz, H-2), 4.45 (m, 1H, H-6'), 3.89 (m, 3H, H-4, H-5, H-6); ^{13}C NMR: δ 165.8 (*C*OPh), 136.9-123.9 (N*Phth*, O_2CH*Ph*, S*Ph*, CO*Ph*) 101.7 (O_2*C*HPh), 84.3 (C-1), 79.5 (C-4), 71.1 (C-3), 70.8 (C-5), 68.8 (C-6), 54.4 (C-2).

5.3.24 Attempt to prepare ethyl 6-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyloxy)hexanoate (26)

To a solution of glycosyl donor **20** (0.539 g, 1.02 mmol), glycosyl acceptor **8** (0.400 g, 0.680 mmol) and NIS (0.345 g) in dichloromethane (10 mL) was added freshly flame-dried powdered 4 Å molecular sieves (0.50 g). After 30 min, AgOTf (0.393 g) in dry toluene (2 mL) was added. The reaction mixture was monitored by TLC for disappearance of the starting materials. After 30 min, the reaction mixture was filtered over celite and washed successively with saturated sodium hydrogen carbonate solutions (3 x 30 mL) and water (30 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated and the residue separated by flash chromatography using hexanes:ethyl acetate (1:1) as eluent. The first product (R_f = 0.90) was compound **8**, (0.101 g, 25%). The second product (R_f = 0.50) was 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**9**, 0.082 g, 17%).

The last fraction (R_f = 0.35) was 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl succinimide (**27**, 0.085 g, 16%) ^{1}H NMR δ 7.90-7.50 (2m, 4H, N*Phth*-H), 5.73 (dd, 1H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 9.3 Hz, H-3), 5.56 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 4.94 (t, 1H,

$J_{4,5}$ = 9.7 Hz, H-4), 4.20 (dd, 1H, $J_{1,2}$ = 8.7 Hz, $J_{2,3}$ = 10.8 Hz, H-2), 4.15-3.90 (m, 2H, H-6a,b), 3.77 (m, 1H, H-5) 2.12, 2.04, 1.97, 1.94 (3s, 9H, 3 x C*H*$_3$CO), 1.82 (s, 4H, 2 x NCOC*H*$_2$); ^{13}C NMR δ 170.8, 170.1, 169.6 (3 x CH$_3$*C*O), 167.8 (2 x N*C*O), 134.2, 131.5, 123.5 (N*Phth*), 97.2 (C-1), 71.7 (C-5), 70.3 (C-3), 68.5 (C-4), 61.7 (C-6), 54.0 (C-2), 20.9, 20.9, 20.5 (3 x *C*H$_3$COO), 14.2 (NCO*C*H$_2$).

5.3.25 Attempt to prepare 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-pivaloyl-β-D-galactopyranosyl fluoride (28)

To a solution of glycosyl donor **20** (0.096 g, 0.183 mmol) and glycosyl acceptor **14** (0.049 g, 0.122 mmol) and NIS (0.032 g) in dichloromethane (10 mL) and was added dried powdered 4 Å molecular sieves (0.50 g). The mixture was stirred for 30 min then treated with a solution of AgOTf (0.032 g) in dry toluene (2 mL). The reaction was monitored by TLC for disappearance of the starting materials. After 3 h, the reaction mixture was filtered over celite and washed successively with saturated sodium hydrogen carbonate solutions (3 x 20 mL) and water (20 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated to a residue that was fractionated by flash chromatography using hexanes:ethyl acetate (2:3) as eluent. The first product (R_f = 0.80) was compound **14**, (0.017 g, 35%). The second product (R_f = 0.70) was 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**9**, 0.012 g, 14%).

The last fraction (R_f = 0.50) was *N*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl) succinimide (**27**, 0.027 g, 29%).

5.3.26 Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-1-phenylsulfinyl-2-phthalimido-β-D-glucopyranoside (29)

A solution of compound **10** (0.827 g, 1.57 mmol) in dry dichloromethane (40 mL) was cooled in an ethyl acetate/liquid nitrogen bath at -78 °C for 10 min followed by dropwise addition of 85% *m*-chloroperoxybenzoic acid (0.35 g, 1.72 mmol, 1.1 eq.) in dry dichloromethane (2 mL) over a 10 min period. The reaction mixture was stirred for 20 min, warmed to room temperature and quenched by washing with saturated sodium hydrogen carbonate solutions (20 mL) and water (20 mL). The organic layer was then dried ($MgSO_4$) and concentrated to give a bright yellowish solid which was purified by flash chromatography using dichloromethane:acetone (19:1) as eluent to afford **29a** and **29b** as a mixture of diasteromers (0.814 g, 96%). A portion of each diastereomer was separated by flash cromatography for NMR analysis. The first fraction (colorless crystals, $R_f = 0.68$) contained the isomer present to the larger extent as observed on TLC. This isomer was previously identified by X-ray crystallography as the (*S*)-sulfoxide isomer **29a**: ^{1}H NMR (400 MHz):[169] δ 7.68-7.13 (m, 9H, N*Phth*, S*Ph*), 5.77 (t, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.40 (d, 1H, $J_{1,2} = 10.1$ Hz, H-1), 5.16 (t, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 4.89 (t, 1H, $J_{1,2} = 10.1$ Hz, H-2), 4.24 (AB of ABX pattern, ddd, 2H, $J_{5,6} = 4.9$ Hz, $J_{6a,6b} = -12.3$ Hz, H-6a,b), 3.91 (X of ABX system, m, 1H, H-5), 2.09, 2.05, 1.85 (3s, 9H, 3 x CH_3CO); ^{13}C NMR: δ 170.1, 169.8, 168.9 (3 x CH_3CO), 167.6 (2 x N*C*O), 138.8- 123.9 (Ar-C), 89.1 (C-1), 76.0 (C-5), 71.1 (C-3), 67.6 (C-4), 61.4 (C-6), 47.4 (C-2), 20.4, 20.2, 20.0 (3 x CH_3CO).

The second fraction (colorless crystals, $R_f = 0.54$) contained the (*R*)-sulfoxide isomer **29b**. ^{1}H NMR (400 MHz):[169] δ 7.90-7.58 (m, 9H, N*Phth*, S*Ph*), 5.81 (t, 1H, $J_{2,3} = J_{3,4} = 9.7$

Hz, H-3), 5.51 (d, 1H, $J_{1,2}$ 10.6 Hz, H-1), 5.02 (t, 1H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4), 4.62 (t, 1H, H-2), 4.17 (AB of ABX pattern, ddd, 2H, $J_{5,6}$ = 4.8 Hz, $J_{6a,6b}$ = -12.8 Hz, H-6a,b), 3.88 (X of ABX system, m, 1H, H-5), 2.01, 2.00, 1.99 (3s, 9H, 3 x CH_3CO); ^{13}C NMR: δ 170.0, 169.8, 168.9 (3 x CH_3CO), 167.9 (2 x NCO), 137.9-123.4 (Ar-C), 86.1 (C-1), 76.3 (C-5), 71.2 (C-3), 67.8 (C-4), 61.4 (C-6), 49.4 (C-2), 20.3, 20.2, 20.0 (3 x CH_3CO).

5.3.27 Phenyl 3-O-benzoyl-4,6-*O*-benzylidene-2-deoxy-1-phenylsulfinyl-2-phthalimido-β-D-glucopyranoside (30)

A solution of compound **25** (0.252 g, 0.42 mmol) in dry dichloromethane (40 mL) was cooled in an ethyl acetate/liquid nitrogen bath at -78 °C for 10 min followed by dropwise addition of 85% *m*-chloroperoxybenzoic acid (0.100 g, 0.51 mmol, 1.2 eq.) in dry dichloromethane (2 mL) over a 10 min period. The reaction mixture was warmed to room temperature and quenched by washing with saturated sodium hydrogen carbonate solutions (25 mL) and the aqueous layer back extracted with CH_2Cl_2 (3 x 25 mL). The organic layers were then combined, dried ($MgSO_4$) and concentrated to give a bright yellowish solid which was purified by flash chromatography using hexanes:ethyl acetate (1:1) as eluent to afford **30a** and **30b** as a mixture of diasteromers (0.230 g, 89%) and **30c** as a minor byproduct. A portion of each diastereomer was separated for NMR analysis.

The first fraction (R_f = 0.65) contained phenyl 3-O-benzoyl-4,6-*O*-benzylidene-2-deoxy-1-phenylsulfonyl-2-phthalimido-β-D-glucopyranoside (**30c**); ^{1}H NMR (250 MHz): δ 8.00-7.20 (19H, N*Phth*, SO_2*Ph*, O_2CH*Ph*, CO*Ph*), 6.19 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3), 5.68 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 5.50, (s, 1H, O_2C*H*Ph) 4.91 (t, 1H, $J_{1,2} = J_{2,3}$ = 10.1 Hz, H-2), 4.32

(dd, 1H, $J_{5,6a} = J_{5,6b} = 8.8$ Hz, $J_{6a,6b} = 2.9$ Hz, H-6a), 3.80 (m, 3H, H-4, H-5, H-6b); ^{13}C NMR δ 171.4 (*C*OPh), 165.8 (2 x N*C*O), 136.6-124.0 (Ar-C), 101.8 (O_2*C*HPh), 86.2 (C-1), 78.7 (C-4), 70.9 (C-3), 70.6 (C-5), 60.5 (C-6), 49.7 (C-2).

The second fraction ($R_f = 0.60$) was tentatively assigned as the (*S*)-sulfoxide isomer **30a**; ^{1}H NMR (250 MHz) δ 8.00-7.10 (19H, N*Phth*, SO*Ph*, O_2CH*Ph*, CO*Ph*), 6.15 (t, 1H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3), 5.52, (s, 1H, O_2C*H*Ph), 5.45 (d, 1H, $J_{1,2} = 10.5$ Hz, H-1), 5.00 (t, 1H, $J_{1,2} = J_{2,3} = 9.9$ Hz, H-2), 4.24 (dd, 1H, $J_{5,6a} = J_{5,6b} = 8.8$ Hz, $J_{6a,6b} = 3.2$ Hz, H-6a), 3.96 (t, 1H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4) 3.81 (m, 3H, H-5, H-6b); ^{13}C NMR δ 166.0 (*C*OPh), 163.0 (2 x N*C*O), 138.8-124.0 (Ar-C), 101.8 (O_2*C*HPh), 88.6 (C-1), 78.9 (C-4), 71.3 (C-3), 68.3 (C-5), 61.0 (C-6), 50.39 (C-2).

The third fraction ($R_f = 0.55$) was tentatively assigned as the (R)-sulfoxide **30b**: ^{1}H NMR (250 MHz): δ 8.00-7.10 (19H, N*Phth*, SO*Ph*, O_2CH*Ph*, CO*Ph*), 6.12 (t, 1H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3), 5.68 (d, 1H, $J_{1,2} = 10.4$ Hz, H-1), 5.57 (s, 1H, O_2C*H*Ph), 5.15 (t, 1H, $J_{1,2} = J_{2,3} = 9.8$ Hz, H-2), 4.49 (broad d, 1H, $J_{5,6a} = J_{5,6b} = 6.7$ Hz, H-6a), 3.96 (m, 3H, H-4, H-5, H-6b); ^{13}C NMR δ 165.9 (*C*OPh), 163.0 (2 x N*C*O), 138.9-124.0 (Ar-C), 101.7 (O_2*C*HPh), 90.8 (C-1), 78.5 (C-4), 70.9 (C-3), 68.3 (C-5), 60.5 (C-6), 53.9 (C-2).

5.3.28 Attempt to prepare phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-pivaloyl-1-thio-β-D-galactopyranoside (31)

To a solution of compounds **29a,b** (0.355 g, 0.610 mmol), 2,6-di- *tert*-butyl-4-methylpyridine (0.166 g, 0.820 mmol) and compound **13** (0.200 g, 0.410 mmol) in dichloromethane (10 mL) under nitrogen at -78°C, was added triflic anhydride (0.127 g, 80

μL) dropwise. The reaction mixture was gradually warmed to room temperature and quenched with a saturated sodium hydrogen carbonate solution (10 mL). The organic layer was then separated and the aqueous layer back extracted with CH_2Cl_2 (2 x 20 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated for flash chromatography using hexanes:ethyl acetate (1:1) as eluent. The first product (R_f = 0.89) was recovered glycosyl acceptor (0.095 g, 48%). The second product (R_f = 0.46) was 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**9**) in low yield. The third product isolated (R_f = 0.40) was 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl succinimide (**27**), in low yield.

The last fraction (R_f = 0.24) was 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (0.115 g, 43%); ^{1}H NMR δ 7.90-7.72 (2m, 4H, N*Phth*), 5.75 (dd, 1H, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 9.0 Hz, H-3), 5.55 (t, 1H, $J_{1,2}$ = 8.5, Hz, H-1), 4.94 (t, 1H, $J_{4,5}$ = 10.4 Hz, H-4), 4.20 (t, 1H, $J_{1,2}$ = 9.2 Hz, $J_{2,3}$ = 11.0 Hz, H-2), 3.97 (dd, $J_{5,6a}$ = -12.1 Hz, $J_{6a,6b}$ = 4.9 Hz, H-6a), 3.81-3.71 (m, 2H, H-5, H-6b), 2.04, 1.97, 1.95 (3s, 9H, 3 x C*H*$_3$CO); ^{13}C NMR: δ 170.8, 170.1, 169.6 (3 x CH$_3$*C*O), 167.8 (2 x N*C*O), 134.3, 131.3, 123.6 (N*Phth*-C), 92.6 (C-1), 72.0 (C-5), 70.5 (C-3), 68.9 (C-4), 62.0 (C-6), 56.0 (C-2), 20.7, 20.6, 20.4 (3 x *C*H$_3$CO).

5.3.29 Attempt to prepare 3-O-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-pivaloyl-1-thio-β-D-galactopyranoside (33)

Triflic anhydride (0.117 g, 70 μL) was added dropwise to a solution of compounds **30a,b** (0.230 g, 0.380 mmol), 2,6-DTBMP (0.155 g, 0.750 mmol) and compound **13** (0.132 g, 0.250 mmol) in dichloromethane (10 mL) under nitrogen at -78°C. The reaction mixture

was gradually warmed to room temperature and quenched with a saturated sodium hydrogen carbonate solution (20 mL). The organic layer was then separated and the aqueous layer back extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated. The residue was separated by flash chromatography using hexanes:ethyl acetate (1:1) as eluent into five fractions. The first product (R_f = 0.95) was compound **13**.

The second and third products (R_f = 0.90, 0.85) were an α/β mixture of glycosyl donor hydrolysis products, **34α** and **34β**. This mixture was enriched in **34β**: ^{1}H NMR (250 MHz) δ 8.00-7.10 (19H, N*Phth*-H, S*Ph*, O_2CH*Ph*, CO*Ph*), 6.23 (t, 1H, $J_{1,2}$ = 10.4 Hz, $J_{1,OH}$ = 8.8 Hz, H-1) 5.76 (t, 1H, $J_{2,3} = J_{3,4}$ = 7.5 Hz, H-3), 5.57 (s, 1H, O_2C*H*Ph), 4.34 (m, 2H, H-2, H-6a), 3.95 (m, 3H, H-4, H-5, H-6b).

The final two products were present in an insignificant amount and were therefore not isolated or characterized.

5.3.30 Ethyl 3-O-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (35)

Triflic anhydride (0.117 g, 70 μL) was added dropwise to a solution of compounds **30a,b** (0.272 g, 0.446 mmol), in dichloromethane (10 mL) under nitrogen at -78 °C, was added triflic anhydride (0.16 mL) dropwise. The solution was allowed to warm to -60 °C whereupon a solution of 2,6-DTBMP (0.376 g, 1.83 mmol) and ethanol (18 μL, 22.7 mmol) in dichloromethane (2 mL) was added. The reaction mixture was gradually warmed to -30 °C and quenched with a saturated sodium hydrogen carbonate solution (20 mL). The organic

layer was separated and the aqueous layer was back extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated for flash chromatography using hexanes:ethyl acetate (1:1) as eluent. The first fraction (R_f = 0.85) contained recovered **30a,b** (0.124 g, 46%). The second fraction contained the desired product ethyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**35**), (0.087 g, 64% based on conversion of **30a,b**): ^{1}H NMR (250 MHz) δ 8.00-7.20 (14H, N*Phth*, O_2CH*Ph*, CO*Ph*), 6.18 (t, 1H, $J_{2,3} = J_{3,4}$ = 10.4 Hz, H-3), 5.57, (s, 1H, O_2C*H*Ph), 5.53 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 4.46 (m, 1H, H-2, H-6a), 3.90 (m, 4H, H-4, H-5, H-6b, one OC*H*$_2CH_3$), 3.60 (m, 1H, OC*H*$_2CH_3$), 1.07 (t, 1H, J = 7.0 Hz, OCH_2C*H*$_3$); ^{13}C NMR δ 165.7 (*C*OPh), 163.0 (2 x N*C*O), 137.0-123.7 (Ar-C), 101.7 (O_2*C*HPh), 98.6 (C-1), 79.8 (C-4), 70.3 (C-3), 68.9 (C-6), 66.5 (C-5), 65.8 (OCH_2CH_3), 55.5 (C-2), 15.2 (OCH_2CH_3).

5.3.31 Attempt to prepare 3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-1-thio-β-D-galactopyranoside (36)

Triflic anhydride (0.139 g, 83 μL) was added dropwise to a solution of compounds **30a,b** (0.150 g, 0.246 mmol), in dichloromethane (10 mL) under nitrogen at -78 °C. The solution was allowed to warm to -60 °C whereupon a solution of 2,6-DTBMP (0.376 g, 1.83 mmol) and compound **17** (0.096 g, 0.164 mmol) in dichloromethane (2 mL) were added. The reaction mixture was gradually warmed to -30 °C and quenched with a saturated sodium hydrogen carbonate solution (20 mL). The organic layer was then separated and the aqueous layer back extracted with CH_2Cl_2 (3 x 20 mL). TLC analysis of the crude mixture revealed the presence of seven distinct products and was not purified further.

5.3.32 5-Allyloxy-1-pentanol (38)

1,5-pentanediol (**37**) (4.00 g, 38.4 mmol) and dibutyltin oxide (9.56 g, 38.4 mmol, 1 eq.) were refluxed for 12 h in toluene (200 mL) with azeotropic removal of water. The solvent was removed from the trap and the resulting solution treated with freshly distilled allyl bromide (7.03 g, 5.03 mL) and tetrabutylammonium bromide (4.95 g, 15.4 mmol, 0.4 eq.). The reaction mixture was allowed to reflux for 12 h when TLC [hexanes:ethyl acetate (1:1)] indicated that the starting material had been consumed and a new product (R_f = 0.60) had appeared. The solution was then concentrated under reduced pressure and purified by flash chromatography using hexanes:ethyl acetate (1:1) as eluent to afford the title compound as a colorless oil (5.54 g, 56%): ^{1}H NMR δ 5.90 (8 lines, 1H, J = 5.5 Hz, J = 10.6 Hz, J = 16.5 Hz, CH_2=C*H*CH_2O), 5.23 (two overlapping signals, 8 lines, 2H, C*H*$_2$=$CHCH_2O$), 3.95 (dd, 2H, J = 1.4 Hz, J = 5.6 Hz, CH_2=$CHCH_2O$) 3.59 (t, 1H, J = 6.5 Hz, C*H*$_2$OH), 3.43 (t, 2H, J = 6.4 Hz, $CH_2OCH_2CH_2$), 1.60 (m, 4H, $CH_2OCH_2CH_2CH_2CH_2$), 1.45 (m, 2H, $OCH_2CH_2CH_2$); ^{13}C NMR δ 135.0 (CH_2=*C*HCH_2O), 117.0 (*C*H_2=$CHCH_2O$), 71.9 ($CH_2OCH_2CH_2$), 70.4 ($CH_2OCH_2CH_2$), 62.6 (*C*H_2OH), 32.5 ($HOCH_2CH_2CH_2$), 29.5 ($CH_2OCH_2CH_2$), 22.5 ($OCH_2CH_2CH_2$).

5.3.33 5-*O*-Allyloxypentyl toluenesulfonate (39)

To a solution of compound **38** (3.06 g, 21.2 mmol) in dry pyridine (70 mL) was added freshly recrystallized toluenesulfonyl chloride (6.07 g, 31.8 mmol, 1.5 eq.). The reaction mixture was then stirred at 0 °C for 90 min, then poured onto ice water (150 mL). The aqueous mixture was extracted with dichloromethane (3 x 100 mL). The combined organic

layers were washed with hydrochloric acid solutions (1.0 M, 3 x 75 mL) and water (2 x 100 mL), then dried ($MgSO_4$) and concentrated. The residue was fractionated by flash chromatography using hexanes:ethyl acetate (4:1) as eluent. The title compound ($R_f = 0.37$), **39**, was obtained as a colorless oil (2.77 g, 46%): ^{1}H NMR δ 7.78, 7.34 (2d, 4H, *Ts-H*), 5.83 (8 lines, 1H, J = 5.5 Hz, J = 10.6 Hz, J = 16.5 Hz, CH_2=C*H*CH_2O), 5.24 (two overlapping signals, 8 lines, 2H, C*H*$_2$=$CHCH_2O$), 4.02 (t, 1H, J = 6.4 Hz, TsOC*H*$_2CH_2$), 3.93 (dd, 2H, J = 1.4 Hz, J = 7.0 Hz, CH_2=CHC*H*$_2O$) 3.37 (t, 2H, J = 6.3 Hz, $CH_2OCH_2CH_2$), 2.44 (s, 3H, PhC*H*$_3$), 1.66 (m, 2H, $CH_2OCH_2CH_2CH_2$), 1.50 (m, 2H, $HOCH_2CH_2CH_2$), 1.38 (m, 2H, $OCH_2CH_2CH_3$); ^{13}C NMR δ 144.8-127.9 (Ar-C), 135.0 (CH_2=*C*HCH_2O), 116.8 (*C*H_2=$CHCH_2O$), 71.9 ($CH_2OCH_2CH_2$), 70.6 ($CH_2OCH_2CH_2$), 69.9 (*C*H_2OTs), 29.1 ($HOCH_2CH_2CH_2$), 28.7 ($CH_2OCH_2CH_2$), 22.2, (PhC*H*$_3$), 22.1 ($OCH_2CH_2CH_3$).

5.3.34 1-Allyloxy-5-azidopentane (40)

To a solution of compound **39** (2.77 g, 9.3 mmol) in dry DMF (70 mL) was added sodium azide (0.72 g, 11.1 mmol, 1.2 eq) and the reaction stirred at room temperature for 2.5 h. The reaction mixture was poured into water (50 mL) and extracted with ethyl acetate (2 x 100 mL). The combined organic layers were then washed with brine (2 x 100 mL) then dried ($MgSO_4$) and concentrated. The residue purified by flash chromatography using hexanes:ethyl acetate (4:1) as eluent to afford the title compound (R_f = 0.70), **40**, as a colorless oil (1.38 g, 88%): ^{1}H NMR δ 5.90 (8 lines, 1H, CH_2=C*H*CH_2O), 5.22 (two overlapping signals, 4 lines, 2H, C*H*$_2$=$CHCH_2O$), 3.96 (dd, 2H, J = 1.2 Hz, J = 5.5 Hz, CH_2=CHC*H*$_2O$), 3.43 (t, 1H, J = 6.1 Hz, $CH_2OCH_2CH_2$), 3.26 (t, 2H, J = 6.7 Hz, C*H*$_2N_3$), 1.59

(m, 4H, $OCH_2CH_2CH_2CH_2$), 1.44 (m, 2H, $OCH_2CH_2CH_2$); ^{13}C NMR δ 135.1 (CH_2=$CHCH_2O$), 116.9 (CH_2=$CHCH_2O$), 72.0 ($CH_2OCH_2CH_2$), 70.1 ($CH_2OCH_2CH_2$), 51.5 (CH_2N_3), 29.4 ($CH_2OCH_2CH_2$), 28.8 ($CH_2CH_2N_3$), 23.6 ($OCH_2CH_2CH_2$).

5.3.35 Synthesis of 5-azido-1-pentanol (41) using palladium(II) chloride

To a solution of compound **40** (0.367 g, 2.17 mmol) in acetic acid:water (20:1, v:v, 21 mL) was added sodium acetate (0.480 g, 5.86 mmol) and pallaium(II) chloride (0.077 g, 0.434 mmol). The reaction mixture was stirred at room temperature for 5 d until the majority of the starting material had been consumed. It was then diluted with ethyl acetate (30 mL), filtered and washed with saturated sodium hydrogen carbonate solutions (3 x 25 mL) and water (3 x 25 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was fractionated by flash chromatography using hexanes:ethyl acetate (4:1) as eluent to afford three fractions. The first fraction contained recovered starting material **40** (23 mg, 6%). The second fraction contained 5-acetoxy-1-azidopentane, compound **42** (11.7 mg, 4%): ^1H NMR δ 4.07 (t, 1H, J = 6.1 Hz, CH_2OAc), 3.29 (t, 2H, J = 6.7 Hz, CH_2N_3), 2.05 (s, 3H, CH_3CO), 1.63 (m, 4H, $OCH_2CH_2CH_2CH_2$), 1.43 (m, 2H, $OCH_2CH_2CH_2$); ^{13}C NMR δ 171.2 (CH_3CO), 63.4 ($AcOCH_2CH_2$), 51.5 (CH_2N_3), 29.4 ($CH_2OCH_2CH_2$), 28.8 ($CH_2CH_2N_3$), 23.6 ($OCH_2CH_2CH_2$).

The third fraction contained the desired product, 5-azido-1-pentanol **41** (R_f = 0.20), compound **41**, in 10% yield: ^1H NMR δ 3.66 (t, 1H, J = 6.7 Hz, CH_2OH), 3.29 (t, 2H, J = 6.7 Hz, CH_2N_3), 1.63 (m, 4H, $OCH_2CH_2CH_2CH_2$), 1.43 (m, 2H, $OCH_2CH_2CH_2$); ^{13}C NMR δ 62.8 ($HOCH_2CH_2$), 51.6 (CH_2N_3), 32.8 ($HOCH_2CH_2$), 28.8 ($CH_2CH_2N_3$), 23.2 ($OCH_2CH_2CH_2$).

5.3.36 Synthesis of 5-azido-1-pentanol (41) using light catalysed α-bromination

Compound **40** (0.138 g, 0.82 mmol) was dissolved in carbon tetrachloride (10 mL) and water (5 mL). NBS (0.350 g, 1.96 mmol, 2.4 eq) was then added, followed by calcium carbonate (0.330 g, 3.26 mmol, 4.0 eq.). The solution was then purged with nitrogen for one hour. Using a 350 W incandescent light, the reaction mixture was stirred and irradiated for 25 min while monitoring by TLC. The mixture was then poured into water (20 mL) and extracted with dichloromethane (3 x 20 mL). The combined extracts were dried ($MgSO_4$), concentrated, and the residue purified by flash chromatography using hexanes:ethyl acetate (4:1) as eluent, to give the title compound **41** (R_f = 0.20, 0.023 g, 22%) as a colorless syrup. The ^1H and ^{13}C NMR recorded were in good agreement with those reported earlier.

5.3.37 5-Iodo-1-pentanol (43)

To a solution of compound **37** (2.51 g, 25.1 mmol) in acetonitrile (100 mL) was added cerium trichloride heptahydrate (14.0 g, 37.6 mmol) and sodium iodide (4.51 g, 30.1 mmol). The reaction was heated to reflux and stirred for 48 h. The reaction mixture was then cooled and diluted with ether (50 mL) and the organic layer washed with a hydrochloric acid solution (1.0 M, 100 mL), a saturated sodium hydrogen carbonate solution (2 x 50 mL) and water (2 x 50 mL). The organic layer was dried ($MgSO_4$), concentrated and the residue fractionated by flash chromatography using hexanes:ethyl acetate (1:1) as eluent to the afford the title compound (R_f = 0.60), **43**, as a colorless oil (0.403 g, 8%); ^1H NMR[319] δ 3.64 (t, 1H, J = 6.1 Hz, CH_2OH), 3.20 (t, 2H, J = 6.7 Hz, CH_2I), 2.25 (s, 1H, O*H*), 1.83 (m, 2H, OCH_2CH_2), 1.65-1.40 (m, 4H, $ICH_2CH_2CH_2$).

5.3.38 5-Bromo-1-pentanol (44)

To a mixture of compound **37** (69.0 g, 0.663 mol) in toluene (700 mL) was added hydrobromic acid (9 M, 48% in H_2O, 88 mL, 0.795 mol).[282] The reaction was heated to reflux and allowed to stir for 24 h. The reaction mixture was then cooled and washed with sodium hydroxide (1.0 M, 2 x 100 mL) and water (2 x 100 mL). The organic layer was dried ($MgSO_4$), concentrated and the residue applied to a flash chromatography column using hexanes:ethyl acetate (1:1) as elutent to give the title compound (R_f = 0.40), **44**, as an oil (26.05 g, 24%): ^{1}H NMR δ 3.65 (t, 1H, J = 6.1 Hz, C*H*$_2$OH), 3.42 (t, 2H, J = 6.7 Hz, C*H*$_2$Br), 2.60 (s, 1H, O*H*), 1.89 (m, 2H, OCH$_2$C*H*$_2$), 1.63-1.48 (m, 4H, BrCH$_2$C*H*$_2$C*H*$_2$); ^{13}C NMR δ 62.7 (*C*H$_2$OH), 33.9 (*C*H$_2$Br), 32.6 (*C*H$_2$CH$_2$OH), 31.8 (*C*H$_2$CH$_2$Br), 24.6 (OCH$_2$CH$_2$*C*H$_2$).

5.3.39 Synthesis of 5-azido-1-pentanol (41) using sodium azide in DMF

Sodium azide (8.60 g, 0.132 mol, 2.0 eq.) was added to a stirred solution of compound **43** (11.05 g, 66.0 mmol) in dry DMF (200 mL). After 1 h, the reaction mixture was poured into water (100 mL) and the aqueous mixture extracted with ethyl acetate (5 x 100 mL). The combined organic layers were then dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography using hexanes:ethyl acetate (2:1) as eluent to afford the title compound (R_f = 0.35), **41**, as a colorless oil (6.82 g, 80%).

5.3.40 Phenyl 3-*O*-allyl-1-thio-β-D-galactopyranoside (45)

Dibutyltin oxide (69.94 g, 0.281 mol) and phenyl 1-thio-β-D-galactopyranoside (**4**, 76.68 g, 0.281 mmol) were refluxed in toluene (600 mL) overnight using a Dean Stark

apparatus for the azeotropic removal of water. Most of the solvent was removed by distillation using the Dean Stark apparatus. The last traces of solvent were removed under vacuum. Cesium fluoride (44.0 g, 0.425 mol, 1.5 eq) was added, followed by a solution of distilled allyl bromide (44.28 g, 31.7 mL, 0.366 mol, 1.3 eq.) in dry DMF (400 mL). The reaction was stirred at room temperature until TLC indicated that the starting material had disappeared (3.5 h). The solution was concentrated, redissolved in methanol (500 mL) to remove the inorganic residues, and filtered using a glass sintered funnel. The filtrate was concentrated to a yellowish syrup which was purified by flash chromatography using hexanes:ethyl acetate (1:3) as eluent to yield the title compound (45.86 g, 52%) as a clear colorless syrup that crystallized from hexanes:ethyl acetate (1:2) as fine white needles: mp 120-121 °C; $[\alpha]^{25}_{D}$ -20.0° (*c* 0.5, $CHCl_3$); ^{1}H NMR: δ 7.55-7.29 (2m, 5H, Ar-H), 5.92 (8 lines, 1H, J = 5.8 Hz, J = 10.4 Hz, J = 16.2 Hz, CH_2=C*H*CH_2O), 5.27 (two overlapping signals, 8 lines, 2H, J = 17.1 Hz, J = 10.4 Hz, J = 1.4 Hz, C*H*$_2$=CHCH_2O), 4.54 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 4.21 (dd, 2H, J = 1.2 Hz, J = 5.8 Hz, CH_2=CHC*H*$_2$O), 4.08 (t, 1H, H-4), 4.99 (m, 1H, H-6a), 3.83 (m, 1H, H-6b), 3.75 (dd, 1H, $J_{1,2}$ = 9.5 Hz, H-2), 3.58 (m, 1H, H-5), 3.41 (dd, 1H, $J_{2,3}$ = 7.2 Hz, $J_{3,4}$ = 3.2 Hz, H-3), 2.60 (br s, 2H, 2OH), 2.27 (br s, 1H, OH); ^{13}C NMR δ 134.2 (CH_2=*C*HCH_2O), 132.5, 132.0, 129.0, 128.0 (Ar-C), 118.2 (*C*H$_2$=CHCH_2O), 88.5 (C-1), 81.1 (C-3), 78.3 (C-5), 71.1 (CH_2=CH*C*H$_2$O), 68.7 (C-2), 67.2 (C-4), 62.7 (C-6).

Anal. Calcd for $C_{15}H_{20}O_5S$: C, 57.67; H, 6.45; S, 10.26. Found: C, 57.56; H, 6.41; S, 9.55.

5.3.41 Phenyl 3-*O*-allyl-2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (46)

Phenyl 3-*O*-allyl-1-thio-β-D-galactopyranoside (**45**, 5.64 g, 18.0 mmol) was stirred with dry benzyl chloride (45.71 g, 41.55 mL, 0.362 mol) and powdered KOH (10.23 g, 0.181 mol) for 8 h at 100 °C. The reaction was cooled to room temperature and chloroform (70 mL) and water (70 mL) were added. The organic layer was washed with water (3 x 70 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure to yield a bright yellowish syrup which was purified by flash chromatography using hexanes:ethyl acetate (4:1) as eluent to give the title compound (9.64 g, 92%). The product was crystallized from methanol to obtain thin colorless needles: mp 63-65 °C; $[\alpha]^{25}_{D}$ -26.8° (*c* 1.1, $CHCl_3$); ^{1}H NMR δ 7.33-7.13 (m, 20H, Ar-H), 5.87 (8 lines, 1H, J = 5.2 Hz, J = 10.7 Hz, J = 17.4 Hz, CH_2=C*H*CH_2O), 5.20 (two overlapping signals, 8 lines, 2H, J = 17.4 Hz, J = 10.4 Hz, J =1.5 Hz, C*H*$_2$=CHCH_2O), 4.94 (d, 1H, $J_{6a,6b}$ = -11.6 Hz, H-6a), 4.73 (AB q, 2H, $J_{A,B}$ = -10.4 Hz, OC*H*$_2$Ph), 4.70-4.52 (m, 2H, H-1, H-6b), 4.41 (AB q, 2H, $J_{A,B}$ = -11.6 Hz, OC*H*$_2$Ph), 4.14 (d, 2H, J = 5.5 Hz, CH_2=CHC*H*$_2$O), 3.96-3.87 (m, 2H, H-2, H-4), 3.71-3.60 (m, 3H, OC*H*$_2$Ph, H-5), 3.47 (dd, 1H, $J_{2,3}$ = 6.4 Hz, $J_{3,4}$ = 2.8 Hz, H-3); ^{13}C NMR δ 134.6 (CH_2=*C*HCH_2O), 138.6-126.8 (Ar-C), 116.6 (*C*H$_2$=CHCH_2O), 87.5 (C-1), 83.6 (C-3), 77.1 (C-2 and C-5), 75.5 (O*C*H$_2$Ph), 74.2 (C-6), 73.4 (O*C*H$_2$Ph), 73.2 (C-4), 71.3 (CH_2=CH*C*H$_2$O), 68.6 (OCH_2Ph).

Anal. Calcd for $C_{36}H_{38}O_5S$: C, 74.20; H, 6.57; S, 5.50. Found: C, 74.08; H, 6.39; S, 5.56.

5.3.42 Phenyl 2,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (47)

Compound **46** (1.01 g, 1.73 mmol) was dissolved in DMSO (50 mL) and potassium *tert*-butoxide (0.28 g, 0.05 M, 0.87 mmol) was added. The solution was heated at 100 °C

under an argon atmosphere. The solution turned orange-red within 5 min. The isomerization of the allyl ether was completed in 20 min as indicated by TLC, (R_f prop-1-enyl ether = 0.59, R_f **46** = 0.49) in hexanes:ethyl acetate (5.5:1). The reaction mixture was cooled, then diluted with water (50 mL). The aqueous layer was then saturated with K_2CO_3 and the product separated by extraction with hexanes (3 x 100 mL). The hexane layers were combined, dried ($MgSO_4$) and concentrated under reduced pressure to yield an orange syrup. This syrup was refluxed with hydrochloric acid (0.1 M, 25 mL) in acetone-water (9:1, v:v, 250 mL) for 6 h, then cooled and neutralized with a saturated sodium hydrogen carbonate solution (20 mL). The aqueous solution was then extracted with CH_2Cl_2 (3 x 100 mL) and the organic layers combined, dried ($MgSO_4$) and concentrated. Crystallization from 5% ethyl acetate in hexanes afforded small clear needle shaped crystals (0.735 g, 84%): mp 93-94 °C; lit.[165] mp 94.5-95 °C; $[\alpha]^{25}_D$ +4.0° (*c* 0.50, $CHCl_3$); lit.[165] $[\alpha]_D$ = +6.4°; ^{1}H NMR δ 7.58-7.20 (m, 20H, Ar-H), 4.89, 4.64 (AB 2d, 2H, $J_{A,B}$ = -11.0 Hz, OC*H*$_2$Ph), 4.74, 4.64 (AB 2d, 2H, $J_{A,B}$ = -11.5 Hz, OC*H*$_2$Ph), 4.62 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 4.49 (AB q, 2H, $J_{A,B}$ = -10.7 Hz, OC*H*$_2$Ph), 3.92 (m, 1H, H-3), 3.68 (broad s, 5H, H-2, H-4, H-5, H-6a,b), 2.20 (s, 1H, 3-OH); ^{13}C NMR δ 138.4, 138.0, 137.7 (3 x Bn-ipso C), 134.1-127.1 (Ar-C), 87.4 (C-1), 78.2, 77.3 (C-2, C-5), 76.0 (C-3), 75.7 (C-4), 75.2, 74.9, 73.5 (3 O*C*H$_2$Ph), 68.6 (C-6).

5.3.43 5-Azidopentyl 2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (48β)

To a solution of glycosyl donor **47** (0270 g, 0.497 mmol), 5-azido-1-pentanol (**41**) (0.150 g, 1.24 mmol, 2.5 eq.) and NIS (0.167g, 1.5 eq.) in acetonitrile (100 mL) was added freshly flame-dried powdered 4 Å molecular sieves (1.0 g). The solution was allowed to stir

for 30 minutes at room temperature and then cooled to -35°C in a bath of liquid nitrogen:acetonitrile. To the suspension was then added a solution of AgOTf (0.256 g, 1.5 eq.) in dry acetonitrile (3 mL). The reaction mixture was monitored by TLC for disappearance of the starting material. After 1 hour, the reaction was warmed to room temperature, filtered over celite and concentrated. The resulting residue was then redissolved in CH_2Cl_2 (100 mL) and washed successively with a saturated sodium hydrogen sulfite solution (50 mL) and water (50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated. Fractionation using flash chromatography with toluene:ethyl acetate (5:1) gave the title compound as a colorless syrup (0.205 g, 73%) and a minor amount of the α anomer (31.2 mg, 11%). Characterization data for the β-anomer **48β** are: $[\alpha]^{25}_D$ +2.7° (*c* 3.4, $CHCl_3$), lit.[264] $[\alpha]^{25}_D$ +2.3° (*c* 0.6, $CHCl_3$), R_f = 0.50 (toluene:ethyl acetate 5:1);. ESMS m/z: 584 (M^+ + Na), 560 (M^+, not observed), Exact mass (ESMS) calcd for $C_{32}H_{39}N_3O_6$ + Na: calcd 584.2737. Found, 584.2737.

Characterization data for the α-anomer **48α** are: $[\alpha]^{25}_D$ = +34.2° (*c* 4.3, $CHCl_3$), lit.[264] $[\alpha]^{25}_D$ +46.0° (*c* 0.3, $CHCl_3$), R_f = 0.45 (toluene:ethyl acetate, 5:1); ESMS m/z: 584 (M^+ + Na), 560 (M^+, not observed); Exact mass (ESMS) calcd for $C_{32}H_{39}N_3O_6$ + Na: calcd 584.2737. Found, 584.2719.

5.3.44 Synthesis of 5-azidopentyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (49) using NIS TfOH method

To a solution of compounds **20** (0.233 g, 0.443 mmol, 1.5 eq.), **48β** (0.166 g, 0.295 mmol) and NIS (0.160 g, 2.4 eq.) in dichloromethane (10 mL) was added freshly flame-dried, powdered 4 Å molecular sieves (0.50 g). The solution was allowed to stir for 30 min at 0°C and then treated with TfOH (1 μL). The reaction mixture was monitored by TLC for disappearance of the starting material. After 30 min, the reaction was filtered over celite and washed successively with saturated sodium hydrogen sulfite solution (50 mL) and water (50 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated for flash chromatography using hexanes:ethyl acetate (1:1) as eluent to give the title compound (R_f = 0.71) as a colorless syrup (0.108 g, 37%): $[\alpha]^{25}_D$ - 1.7 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.52 (br s, 4H, N*Phth*-H), 7.18-7.40 (m, 15H, Ar-H), 5.85 (t, 1H, $J_{2',3'}$ = 10.7, $J_{3',4'}$ = 9.0 Hz, H-3'), 5.71 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 5.17 (t, 1H, $J_{3',4'}$ = 9.0 Hz, $J_{4',5'}$ = 10.2 Hz, H-4'), 4.90, 4.54 (AB 2d, 2H, $J_{A,B}$ = -11.7 Hz, OC*H*$_2$Ph), 4.54, 4.15 (AB 2d, 2H, $J_{A,B}$ = -11.8 Hz, OC*H*$_2$Ph), 4.44-4.38 (AB q, 2H, OC*H*$_2$Ph, H-2'), 4.24 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.21 (dd, 1H, $J_{5',6a'}$ = 5.0 Hz, $J_{6a',6b'}$ = -12.2 Hz, H-6a') 4.18 (br s, 1H, H-6b'), 3.98 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4), 3.80 (m, 2H, one OC*H*$_2$(CH$_2$)$_4$, H-3, H-5'), 3.59-3.48 (m, 4H, H-2, H-5, H-6a,b), 3.33 (m, 1H, one OC*H*$_2$(CH$_2$)$_4$), 2.98 (t, 2H, J = 7.1 Hz, C*H*$_2$N$_3$), 2.04, 2.02, 1.85 (3s, 9H, 3 Ac), 1.48-1.35 (m, 4H, OCH$_2$C*H*$_2$CH$_2$C*H*$_2$), 1.25-1.16 (m, 2H, O(CH$_2$)$_2$C*H*$_2$); ^{13}C NMR δ 170.7, 170.2, 169.7 (3 x CH$_3$*C*OO), 167.6 (2 x NH*C*O), 139.0, 138.9, 138.1 (3 x Bn-ipso C), 134.2-123.4 (Ar-C, NPhth-C), 104.0 (C-1), 99.4 (C-1'), 82.1 (C-5'), 78.4 (C-2 or C-5), 76.0 (C-4), 74.8, 73.8, 73.5

(3 x OCH_2Ph), 73.6 (C-2 or C-5), 71.8 (C-3), 70.7 (C-3'), 69.6 ($OCH_2(CH_2)_4$), 69.2 (C-4'), 69.0 (C-6), 62.2 (C-6'), 55.2 (C-2'), 51.2 (CH_2N_3), 29.2 (OCH_2CH_2), 28.6 ($CH_2CH_2N_3$), 23.3 ($OCH_2CH_2CH_2$), 20.8, 20.7 (3 x CH_3COO); ESMS m/z: 1001 (M^+ + Na); Exact mass (ESMS) calcd for $C_{52}H_{58}N_4O_{15}$ + Na: 1001.3796. Found, 1001.3789.

5.3.45 Synthesis of 5-azidopentyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (49) using NIS AgOTf method

To a solution of compounds **20** (0.336 g, 0.637 mmol, 2 eq.), **48β** (0.179 g, 0.319 mmol) and NIS (0.215 g, 3 eq.) in dichloromethane (10 mL) was added freshly flame-dried, powdered 4 Å molecular sieves (0.50 g). The solution was allowed to stir for 30 min at 0 °C and treated with a solution of AgOTf (0.245g, 3 eq.) in dry acetonitrile (2 mL). The reaction mixture was monitored by TLC for disappearance of the starting materials. After 30 min. the reaction was filtered over a celite and concentrated. The resulting residue was then taken up in dichloromethane (20 mL) and washed successively with a saturated sodium hydrogen sulfite solution (50 mL) and water (50 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated and fractionated by flash chromatography using hexanes:ethyl acetate (1:1) as eluent to give the title compound (R_f = 0.71), **49**, as a colorless syrup (0.266 g, 85%).

5.3.46 5-Azidopentyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (50)

Compound **49** (0.340 g, 0.347 mmol) was dissolved in a mixture of methanol:4-aminobutane (5:3, 80 mL) and refluxed for 48 h. The reaction was then concentrated to give a yellowish solid which was dissolved in a mixture of methanol:acetic anhydride (10:1, v:v, 11 mL) and stirred at room temperature (24 h). The solution was concentrated and the residue purified by flash chromatography using ethyl acetate:acetone (4:1) as eluent to yield the title compound (0.206 g, 77%) as a clear colorless syrup: $[\alpha]^{25}_{D}$ -10.3° (*c* 1.4, $CHCl_3$); R_f 0.20 (ethyl acetate:acetone 4:1); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.45-7.20 (m, 15H, Ar-H), 6.85 (d, 1H, J = 8.2 Hz, AcN*H*), 5.08, 4.57 (AB 2d, 2H, $J_{A,B}$ = -11.3 Hz, OCH_2Ph), 4.89, 4.82 (AB 2d, 2H, $J_{A,B}$ = -11.73 Hz, OCH_2Ph), 4.89 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1') 4.50 (AB 2d, 2H, $J_{A,B}$ = -12.0 Hz, OCH_2Ph), 4.40 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.08 (d, 1H, $J_{3,4}$ = 2.5 Hz, H-4), 3.95-3.87 (m, 2H, H-3, H-6a'), 3.84-3.79 (m, 1H, one OCH_2(CH$_2$)$_4$), 3.79- 3.69 (m, 3H, H-2', H-5, H-6b'), 3.67-3.55 (m, 4H, H-3', H-2, H-6a,b), 3.49-3.42 (m, 1H, one OCH_2(CH$_2$)$_4$), 3.45 (t, 1H, $J_{3',4'}$ = $J_{4',5'}$ = 8.9 Hz, H-4'), 3.36 (m, 1H, H-5'), 3.17 (t, 2H, J = 7.0 Hz, CH_2N$_3$), 1.70 (s, 3H, CH_3CONH), 1.58-1.42 (m, 4H, OCH$_2$CH_2CH$_2$CH_2), 1.40-1.27 (m, 2H, O(CH$_2$)$_2$CH_2); ^{13}C NMR (acetone-d_6) δ 172.0 (Me*C*ONH), 141.4-128.54 (Ar-C), 105.2 (C-1), 104.4 (C-1'), 83.2 (C-3), 80.8, 76.8, 70.9 (C-2, C-3', C-6), 78.7 (C-4), 78.1 (C-5'), 76.1, 75.5, 74.4 (3 x OCH$_2$Ph), 74.9 (C-5), 73.3 (C-4'), 70.4 (OCH$_2$(CH$_2$)$_4$), 63.9 (C-6'), 58.8 (C-2'), 52.6 (*C*H$_2$N$_3$), 30.1 (OCH$_2$*C*H$_2$), 29.3 (*C*H$_2$CH$_2$N$_3$), 24.8 (OCH$_2$CH$_2$*C*H$_2$), 24.1 (*C*H$_3$CONH). ESMS m/z: 787 (M^+ + Na), 766 (M^+); Exact mass (ESMS) calcd for $C_{40}H_{52}N_4O_{11}$ + Na: 787.3531. Found, 787.3553.

5.3.47 5-Aminopentyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (51)

To a solution of **50** (50 mg, 65.3 μmol) in methanol:acetic acid (4:1, v:v, 5 mL) was added 10% Pd/C (50 mg) and the mixture stirred at room temperature for 21 h under an atmosphere of hydrogen (1 atm). The reaction mixture was then filtered over celite and washed with methanol (2 x 25 mL). The combined washings were concentrated to dryness to obtain the title compound as a colorless crystalline product (24.2 mg, 79%). ^{1}H NMR (water-d_2, 400 MHz) δ 4.70 (under H_2O peak, 1H, H-1), 4.43 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1'), 4.30 (m, 2H), 4.11 (dd, 1H, $J_{2,3}$ = 8.8 Hz, $J_{3,4}$ = 4.1 Hz, H-3), 4.07 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4), 4.02 (m, 1H, H-6a), 3.96-3.88 (m, 1H, one $OCH_2(CH_2)_4$), 3.83 (m, 1H, H-6a'), 3.78- 3.64 (m, 3H), 3.60 (t, 1H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 9.7 Hz, H-2'), 3.00 (t, 2H, J = 7.4 Hz, CH_2NH_2), 1.97 (s, 3H, CH_3CONH), 1.71-1.63 (m, 4H, $OCH_2CH_2CH_2CH_2$), 1.49-1.43 (m, 2H, $O(CH_2)_2CH_2$); ^{13}C NMR δ 174.8 (MeCONH), 109.2 (C-1), 103.1 (C-1'), 82.0, 81.9, 75.4, 74.3, 70.6 (OCH_2CH_2), 70.4 (C-2'), 70.2 (C-4), 68.9 (C-3), 64.0 (C-6), 63.3, 61.5 (C-6'), 40.0 (CH_2NH_2), 28.8 (OCH_2CH_2), 27.0 ($CH_2CH_2NH_2$), 22.7 ($OCH_2CH_2CH_2$), 22.4 (CH_3CONH). ESMS m/z: 491 (M^+ + Na), 469 (M^+ + H). Exact mass (ESMS) calcd for $C_{19}H_{36}N_2O_{11}$ + Na calcd 491.2217. Found, 491.2218.

5.3.48 5-Azidopentyl 3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (52)

To a solution of glycosyl donor **23** (0.358 g, 0.510 mmol, 1.7 eq.), **48β** (0.166 g, 0.300 mmol) and NIS (0.175 g, 2.6 eq.) in CH_2Cl_2 (10 mL) was added freshly flame dried powdered

4 Å molecular sieves (0.50 g). The mixture was stirred for 30 min at room temperature and then treated with a solution of AgOTf (0.200 g, 3 eq.) in dry acetonitrile (2 mL). The reaction was monitored by TLC for disappearance of the starting materials. After 2.5 h, the reaction was filtered over celite and concentrated. The resulting residue was then taken up in CH_2Cl_2 (20 mL) and washed successively with a saturated sodium hydrogen sulfite solution (50 mL) and water (50 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated and the residue separated by flash chromatography using hexanes:ethyl acetate (4:1) as eluent. Three fractions were isolated from the crude mixture. The first, (R_f = 0.51), was the title compound (**52**) as a colorless syrup (0.088 g, 26%): $[\alpha]^{25}_D$ +7.2° (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.74-6.77 (m, 29H, Ar-H), 5.50 (d, 1H, $J_{1',2'}$ = 8.2 Hz, H-1'), 5.03, 4.82 (AB 2d, 2H, $J_{A,B}$ = -11.4 Hz, OC*H*$_2$Ph), 4.89, 4.79 (AB 2d, 2H, $J_{A,B}$ = -10.9 Hz, OC*H*$_2$Ph), 4.55, 4.08 (AB 2d, 2H, $J_{A,B}$ = -11.6 Hz, OC*H*$_2$Ph), 4.55-4.42 (m, 5H, 2 x OC*H*$_2$, H-3'), 4.28 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 4.24 (t, 1H, $J_{1',2'}$ = 7.5 Hz, $J_{2',3'}$ = 7.3 Hz, H-2') 3.95 (m, 3H, H-6a',b', H-3) 3.81 (m, 3H, one OC*H*$_2$(CH$_2$)$_4$, H-4, H-4'), 3.62-3.47 (m, 6H, H-2, H-3, H5, H5', H-6a,b), 3.33 (m , 1H, one OC*H*$_2$(CH$_2$)$_4$), 3.00 (t, 2H, J = 7.0 Hz, C*H*$_2$N$_3$), 1.48-1.35 (m, 4H, OCH$_2$C*H*$_2$CH$_2$C*H*$_2$), 1.25-1.16 (m, 2H, O(CH$_2$)$_2$C*H*$_2$), 0.92 (d, 9H,SiC(C*H*$_3$)$_3$, 0.08, 0.06 (2s, 2 x 3H, Si(C*H*$_3$)$_2$); ^{13}C NMR: δ 139.3-123.3 (Ar-C), 104.1 (C-1), 99.5 (C-1'), 80.6, 79.6 (C-4, C-4'), 79.4 (C-3'), 78.8, 75.2, 73.4 (C-2, C-5, C-5'), 76.0 (C-3), 75.2, 75.1, 74.6, 73.8, 73.7, 76.9 (5 O*C*H$_2$Ph), 69.5 (O*C*H$_2$(CH$_2$)$_4$), 69.4 (C-6), 62.1 (C-6'), 55.6 (C-2'), 51.3 (*C*H$_2$N$_3$), 29.3 (Si*C*(CH$_3$)$_3$), 28.7 (OCH$_2$*C*H$_2$), 28.6 (*C*H$_2$CH$_2$N$_3$), 26.0 (SiC(*C*H$_3$)$_3$) 23.3 (OCH$_2$CH$_2$*C*H$_2$), -4.9, -5.2 (2 x SiC(*C*H$_3$)$_3$);. ESMS m/z: 1185 (M^+ + K), 1147 (M^+, not observed); Exact mass (ESMS) calcd for $C_{66}H_{78}N_4O_{12}Si$ + K; 1185.502. Found, 1185.539.

The second fraction contained compound **48β** (0.086 g, 53%). The third fraction contained an unidentified byproduct (0.125 g).

5.3.49 5-Azidopentyl 3,4-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (53)

To a solution of compound **52** (88 mg, 0.077 mmol) in tetrahydrofuran (15 mL) and acetic acid (0.1 mL) was added tetrabutylammonium fluoride (1.0 M, 0.76 mL, 10 eq). The reaction mixture was stirred for 3.5 h where TLC [hexanes:ethyl acetate (3:1)] indicated the complete conversion of **52** into a slower moving product. The reaction mixture was then diluted with dichloromethane (30 mL) and the organic layer washed with brine (30 mL) and water (30 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated and the residue separated by flash chromatography using hexanes:ethyl acetate (1:1) as eluent. Fractionation of the mixture afforded the title compound, **53** (R_f = 0.76), as a colorless oil (6.82 g, 80%): $[\alpha]^{25}_D$ +3.1° (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.74-6.77 (m, 29H, Ar-H), 5.49 (d, 1H, $J_{1',2'}$ = 8.5 Hz, H-1'), 4.88, 4.71 (AB 2d, 2H, $J_{A,B}$ = -11.8 Hz, OCH_2Ph), 4.78, 4.41 (AB 2d, 2H, $J_{A,B}$ = -12.1 Hz, OCH_2Ph), 4.48, 4.07 (AB 2d, 2H, $J_{A,B}$ = -11.7 Hz, OCH_2Ph), 4.48-4.38 (m, 3H, OCH_2, H-3'), 4.22 (t, 1H, $J_{1',2'}$ = 8.4 Hz, $J_{2',3'}$ = 10.8 Hz, H-2'), 4.20 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.24-3.95 (m, 2H, H-6a', H-3), 3.79-3.68 (m, 4H, one OCH_2(CH$_2$)$_4$, H-4, H-4', H-6b'), 3.57-3.47 (m, 6H, H-2, H-3, H5, H5', H-6a,b), 3.28 (m , 1H, one OCH_2(CH$_2$)$_4$), 2.96 (t, 2H, J = 7.1 Hz, CH_2N_3), 2.46 (br t, 1H, O*H*), 1.48-1.35 (m, 4H, OCH$_2$CH_2CH$_2$CH_2), 1.25-1.16 (m, 2H, O(CH_2)$_2$CH$_2$); ^{13}C NMR: δ 139.1-123.3 (Ar-C), 104.2 (C-1), 99.6 (C-1'), 81.2, 79.4 (C-4, C-4'), 79.3 (C-3'), 78.7, 75.3, 73.5 (C-2, C-5, C-5'), 76.0 (C-3), 75.2, 75.1, 74.6, 73.9, 73.7 (5

OCH_2Ph), 69.6 ($OCH_2(CH_2)_4$), 69.1 (C-6), 61.9 (C-6'), 56.7 (C-2'), 51.3 (CH_2N_3), 29.2 (OCH_2CH_2), 28.7 ($CH_2CH_2N_3$), 23.4 ($OCH_2CH_2CH_2$).

5.3.50 Phenyl 3-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (54)

Compound **25**, (1.34 g, 2.25 mmol) was refluxed in acetic acid (70%, 150 mL) for 2 h where TLC indicated the complete conversion of **25** into a slower moving product [hexanes:ethyl acetate(1:1)] with $R_f = 0.34$. The reaction mixture was then cooled to room temperature and the solvent removed under reduced pressure. The resulting residue was purified by flash chromatography using hexanes:ethyl acetate (2:3) as eluent to give compound **54** as an amorphous solid that was used without further purification (0.881 g, 77%): ^{1}H NMR δ 7.90-7.10 (14H, S*Ph*, N*Phth*, CO*Ph*), 5.95 (t, 1H, $J_{2,3} = J_{3,4} = 10.1$ Hz, H-3), 5.87 (d, 1H, $J_{1,2} = 10.5$ Hz, H-1), 4.49 (t,1H, $J_{1,2} = 10.4$ Hz, H-2), 4.01-3.85 (m, 3H, H-4, H-6, H-6'), 3.74 (m, 1H, H-5); ^{13}C NMR δ 168.8, 167.2 (2 x N*C*O) 134.5-123.8 (Ar-C), 83.4 (C-1), 80.1 (C-5), 76.7 (C-4), 75.4 (C-3), 62.6 (C-6), 53.8 (C-2).

5.3.51 Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (56)

Sodium hydride (60% in mineral oil, 0.82 g, 20.5 mmol) was added to a solution of compound **24** (4.02 g, 8.21 mmol) in dry DMF (50 mL) and the reaction mixture was stirred under nitrogen for 10 min. To this mixture was then added benzyl bromide (3.51 g, 20.5 mmol, 2.44 mL) and the reaction monitored by TLC [hexanes ethyl acetate (2:1)]. After 20 min, TLC indicated the complete conversion of **24** into a faster moving compound (R_f =

0.45). The reaction mixture was then quenched with saturated ammonium chloride (10 mL) and extracted with dichloromethane (3 x 30 mL). The combined extracts were dried ($MgSO_4$), concentrated, and a portion of the residue purified by flash chromatography using hexanes ethyl acetate (2:1) as eluent, to give the title compound as a colorless syrup (0.068 g, 50%): $[\alpha]_D$ +68.7° (*c* 1.4, $CHCl_3$); lit.[289] $[\alpha]_D$ +73.2°(*c* 4.2, $CHCl_3$); ^{1}H NMR δ 7.20-7.90 (19H, S*Ph*, N*Phth*, O_2CHPh, CH_2Ph), 5.62 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 5.61 (s, 1H, O_2CHPh), 4.77, 4.50 (AB dd, 2H, $J_{A,B}$ = -12.2 Hz, OCH_2Ph), 4.44 (m, 2H, H-3, H-6a), 4.30 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 10.4 Hz, H-2), 3.88-3.69 (m, 3H, H-4, H-5, H-6b). ^{13}C NMR δ 168.3, 167.5 (2 x NCO),137.8-123.5 (S*Ph*, N*Phth*, O_2CHPh, CH_2Ph) 101.4 (O_2CHPh), 84.2 (C-1), 82.9, 70.5 (C-4, C-5) 75.6 (C-3), 74.3 (CH_2Ph), 68.8 (C-6), 54.9 (C-2).

5.3.52 Phenyl 3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (57)

Crude compound **56** (4.63 g, 8.0 mmol) was refluxed in acetic acid (70%, 250 mL) for 2 h where TLC indicated the complete conversion of **25** into a slower moving product [toluene:ethyl acetate (2:1)] with R_f = 0.34. The reaction mixture was then cooled to room temperature and the solvent removed under reduced pressure. The resulting residue was purified by flash chromatography using toluene:ethyl acetate (2:1) as eluent to give compound **57** as a colorless syrup (2.31 g, 59%, from **24**): $[\alpha]_D$ +59.6° (*c* 1.4, $CHCl_3$); ^{1}H NMR δ 7.20-7.90 (19H, S*Ph*, N*Phth*, CH_2Ph), 5.60 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 4.73, 4.53 ((AB 2d, 2H, $J_{A,B}$ = -12.2 Hz, OCH_2Ph), 4.26 (m, 2H, H-2, H-3), 3.89 (m, 3H, H-4, H-6a,b), 3.56 (m, 1H, H-5); ^{13}C NMR δ 168.3, 167.5 (2 x *C*O, N*Phth*), 137.8-123.5 (S*Ph*, N*Phth*, CH_2Ph), 83.8 (C-1), 80.2 (C-3), 79.6 (C-5), 74.8 (CH_2Ph), 72.1 (C-4), 62.5 (C-6), 54.8 (C-2).

EIMS m/z: 491 (M^+, not observed), 382 (19.8% M - SPh) HRMS calcd for $C_{21}H_{20}NO_6$: 382.1291. Found, 382.1299.

5.3.53 Phenyl 3-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (58)

To a stirred solution of phenyl 3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**57**) (2.10 g, 4.29 mmol) in dry pyridine (50 mL) under nitrogen atmosphere was added *tert*-butyldimethylsilyl chloride (0.776 g, 5.15 mmol). The reaction mixture stirred for 12 h where TLC [hexanes:ethyl acetate (2:1)] revealed the conversion of **57** into a faster moving product (R_f = 0.65). The mixture was then quenched with water (50 mL) and diluted with dichloromethane (50 mL). The organic layer was then washed with hydrochloric acid (2 x 50 mL) and water (50 mL) dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography using hexanes:ethyl acetate (3:1) to give the title compound as a colorless syrup (2.10 g, 81%): $[\alpha]_D$ +65.9° (*c* 2.8, $CHCl_3$); ^{1}H NMR δ 7.90-6.80 (19H, S*Ph*, N*Phth*, CH_2*Ph*) 5.57 (d, 1H, $J_{1,2}$ = 9.2 Hz, H-1), 4.78, 4.53 (AB 2d, 2H, $J_{A,B}$ = -12.2 Hz, OC*H*$_2$Ph) 4.25 (m, 2H, H-2, H-3), 3.94 (m, 2H, H-6a,b), 3.83 (m, 1H, $J_{3,4}$ = 9.8 Hz, H-4), 3.58 (m, 1H, H-5);. ^{13}C NMR δ 168.1, 167.4 (2 x CO, N*Phth*), 138.1-123.4 (S*Ph*, N*Phth*, CH_2*Ph*), 83.6 (C-1), 79.7 (C-3), 78.4 (C-5), 74.8 (C-4), 74.6 (*C*H$_2$Ph), 64.7 (C-6), 54.4 (C-2), 26.0 (SiC(*C*H$_3$)$_3$), 18.3 (Si*C*(CH$_3$)$_3$), -5.4 (2 x SiC(*C*H$_3$)$_2$). EIMS m/z: 605 (M^+, not observed), 496 (19.8% M - SPh). HRMS calcd for $C_{27}H_{34}NO_6Si$: 496.2155. Found, 496.2153.

5.3.54 Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (59)

Sodium hydride (60% in mineral oil, 2.4 g, 62.6 mmol) was added to a solution of compound **4** (4.09 g, 15.0 mmol) in dry DMF (100 mL). The reaction was allowed to stir under nitrogen for 10 min and then treated with benzyl bromide (12.94 g, 75.7 mmol, 9.0 mL) and monitored by TLC [hexanes ethyl acetate (4:1)]. After 20 min TLC indicated the complete conversion of **4** into a faster moving compound ($R_f = 0.51$). The reaction mixture was then quenched with saturated ammonium chloride (50 mL) and extracted with dichloromethane (2 x 100 mL). The combined extracts were dried ($MgSO_4$), concentrated, and the residue purified by flash chromatography using hexanes;ethyl acetate (6:1) as eluent, to give the title compound as a colorless syrup (6.94 g, 73%). A portion of the isolated product was crystallized from ether:hexanes to give fine white needles: mp 87-88 °C, lit.[290] mp 88-89 °C; $[\alpha]_D$ +1.4° (*c* 1.4, $CHCl_3$), lit.[290] $[\alpha]_D$ +1°.

5.3.55 Attempt to prepare phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (60)

To a solution of glycosyl donor **59** (0.252 g, 0.398 mmol, 1.5 eq.), glycosyl acceptor **58** (0.160 g, 0.265 mmol) and NIS (0.345 g) in acetonitrile (50 ml) was added freshly flame dried powdered 4 Å molecular sieves (0.40g). The reaction mixture was allowed to stir (30 min) and then cooled to -35 °C. The cooled solution was then treated with a solution of AgOTf (0.393 g) in dry acetonitrile (2 mL). The reaction mixture was monitored by TLC for disappearance of the glycosyl donor. After 12 h, the reaction was quenched with water (2

mL), filtered over celite and concentrated. The resulting residue was then taken up in dichloromethane (50 mL) and the dichloromethane solution washed successively with a saturated sodium hydrogen carbonate solution (25 mL) and water (25 mL). TLC analysis of the crude mixture revealed the presence of ten distinct products and was not purified further.

5.3.56 Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (61)

Compound **61**, prepared as a colorless syrup from compound **2** (0.660 g, 1.69 mmol) by the procedure described by Ferrier *et al.*,[185] and was purified by flash chromatography using toluene:ethyl acetate (2:1) as eluent (R_f = 0.56) to give the title compound (0.630 g, 95%): $[\alpha]_D$ -7.3° (*c* 1.1, $CHCl_3$), lit.[292] $[\alpha]_D$ -8.0°(*c* 2.1, $CHCl_3$); ^{1}H NMR δ 5.4 (br d, 1H, $J_{3,4}$ = 3.0 Hz, H-4), 5.24 (t, 1H, $J_{1,2}$ = 9.8, Hz, $J_{2,3}$ = 9.1 Hz, H-2), 5.05 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.7 Hz, H-3), 4.51 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1), 4.14 (m, 2H, H-6a,b), 3.96 (broad t, 1H, $J_{5,6}$ = 6.1 Hz, H-5), 2.73 (m, 2H, CH_2CH_3), 2.16, 2.07, 2.05, 1.99 (4s, 12H, 4 x CH_3CO), 1.29 (t, 3H, J = 7.3 Hz, CH_2CH_3); ^{13}C NMR δ 170.4, 170.2, 170.1, 169.6 (4 x CH_3CO), 84.1 (C-1), 74.4 (C-5), 72.0 (C-3), 67.4 (C-4), 67.3 (C-2), 61.6 (C-6), 24.4 (CH_2CH_3), 20.9, 20.7, 20.6 (4 x CH_3CO), 14.9 (CH_2CH_3).

5.3.57 Attempt to prepare phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (62)

To a solution of glycosyl donor **61** (0.800 g, 2.04 mmol, 5.0 eq.), glycosyl acceptor **58** (0.247 g, 0.407 mmol) and NIS (0.412 g) in dichloromethane (30 ml) was added freshly

flame-dried powdered 4 Å molecular sieves (0.50 g). The solution was allowed to stir for 20 min at 0 °C and then treated with a solution of AgOTf (0.471 g) in dry acetonitrile (2 mL). The reaction was monitored by TLC for disappearance of the glycosyl donor. After 10 min the reaction was quenched with water (2 mL), filtered over celite and concentrated. The resulting residue was taken up in dichloromethane (50 mL) and washed successively with saturated sodium hydrogen carbonate solutions (50 mL) and water (50 mL). The organic layer was then dried ($MgSO_4$), concentrated and purified by flash chromatography using toluene:ethyl acetate (2:1) as eluent to afford four fractions.

The first fraction (R_f = 0.90) contained a small amount of compound **58**.

The second fraction (R_f = 0.70) contained a cross coupled disaccharide product that was tentatively assigned as phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranosyl-(1→3)-3-*O*-benzyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**63**); ^{1}H NMR δ 7.76-7.65 (S*Ph*), 7.20-6.83 (N*Phth*), 5.51 (s, 1H, H-1), 5.41 (d, 1H, H-4'), 5.30 (t, 1H, H-2'), 5.06 (dd, 1H, H-3'), 4.72, 4.50 (2d, 2H, ABq, OCH_2Ph), 4.70 (d, 1H, H-1'), 4.61 (m, 1H, H-6a), 4.10, 3.99 (2m, 6H, H-3, H-4, H-5, H-5', H-6a,b'), 3.85 (m, 2H, H-2, H-6b), 2.15, 2.13, 2.04, 2.00 (4s, 12H, 4 x CH_3CO); ^{13}C NMR δ 170.6, 169.5, 167.6 (CH_3*C*O), 138.0-123.6 (Ar-*C*), 102.3, 99.5 (C-1'', C-1'), 84.4 (C-1), 73.7, 71.2, 69.2, 67.6, 67.2, 61.4 (C-6), 56.4 (C-2), 21.0, 20.8, 20.8 (4s, 12H, 4 x *C*H_3CO).

The third fraction (R_f = 0.67) was tentatively assigned as the non-reducing disaccharide, compound **64**; ^{1}H NMR δ 6.32 (d, 1H, H-1), 5.43 (m, 2H, H-4, H-4'), 5.35-5.29 (m, 2H, H-2, H-3), 5.10 (t, 1H, H-2'), 4.96 (dd, 1H, H-3'), 4.57 (d, 1H, H-1'), 4.25 (br t, 1H, H-4), 4.17-4.04 9 (m, H-5', H-6a,b, H-6a,b'), 3.92 (t, 1H, H-5), 2.35, (s, 1H, O*H*), 2.18, 2.16,

2.16, 2.07, 2.04, 2.03, 2.00, 1.97 (8s, 24H, 8 x C*H*$_3$CO); ^{13}C NMR δ 170.0, 169.7, 168.9 (CH$_3$*C*O), 101.5 (C-1'), 90.7 (C-1), 72.4, 70.7, 69.2, 69.1, 68.8, 68.1, 67.6, 66.7, (C-2, C-2', C-3, C3', C-4, C-4', C-5, C-5'), 61.1, 61.0 (C-6, C-6'), 20.7, 20.7, 20.5 (*C*H$_3$CO).

The fourth fraction (R_f= 0.25) was 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**65**): ^{1}H NMR δ 6.30 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 5.45 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4), 5.18 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 4.28 (t, 1H, J = 6.7 Hz. H-5), 4.17 (dd, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.4 Hz, H-2), 4.13 (m, 2H, H-6a,b), 2.18, 2.15, 2.06, 2.04 (4s, 12H, 4 x C*H*$_3$CO); ^{13}C NMR δ 171.0, 170.6, 170.3, 169.6 (4 x CH$_3$*C*O), 92.1 (C-1), 70.5 (C-5), 68.8 (C-3), 67.7 (C-4), 66.1 (C-2), 61.5 (C-6), 24.4 (*C*H$_2$CH$_3$), 21.1, 20.9, 20.8, 20.8 (4 x *C*H$_3$CO).

5.3.58 2,3,4,6-Tetra-*O*-acetyl-1-thio-α-D-galactopyranosyl bromide (66)

Bromine (0.318 g, 0.110 mL, 1.0 eq.) was added to a stirred solution of compound **61** (0.780 g, 1.44 mmol) in dichloromethane (40 mL) at 0 °C and stirring was continued at room temperature for 20 min. TLC [hexanes:ethyl acetate (1:1)] indicated the complete conversion of **61** into two products, (R_f = 0.70) and (R_f = 0.20), the same as compound **65**. The reaction mixture was then washed with saturated sodium hydrogen sulfite solution (30 mL) and water (30 mL), dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography using hexanes:ethyl acetate 1:1 as eluent to afford the title compound, **66**, as an amorphous solid (0.210 g, 26%); ^{1}H NMR[299] δ 6.71 (d, 1H, $J_{1,2}$ = 4.3 Hz, H-1), 5.52 (d, 1H, $J_{3,4}$ = 3.7 Hz, H-4) 5.24 (dd, 1H, $J_{1,2}$ = 3.0, Hz, $J_{2,3}$ = 10.4 Hz, H-2), 5.05 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.7 Hz, H-3), 4.50 (broad t, 1H, $J_{5,6}$ 6.1 Hz, H-5), 4.16 (m, 2H, H-6a,b), 2.16, 2.12, 2.06, 2.02 (4s,

12H, 4 x CH_3CO); ^{13}C NMR δ 170.3, 170.0, 169.9, 169.7 (4 x CH_3CO), 88.3 (C-1), 71.1 (C-5), 68.0 (C-3), 67.8 (C-4), 67.0 (C-2), 60.8 (C-6), 20.7, 20.6, 20.6, 20.5 (4 x CH_3CO).

5.3.59 Attempt to prepare phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzyl-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (62)

A mixture of glycosyl donor **66** (0.210 g, 0.511 mmol, 2.0 eq.), glycosyl acceptor **58** (0.161 g, 0.265 mmol) and 2,6-DTBMP (0.131 g, 0.638 mmol) in dichloromethane (20 mL) with freshly flame-dried powdered 4 Å molecular sieves (0.50 g) was stirred for 30 min at 0 °C and then treated with a solution of AgOTf (0.131 g) in dry acetonitrile (3 mL). The reaction was monitored by TLC for disappearance of the glycosyl donor. After 2 h, the reaction was warmed to room temperature and stirred for an additional 1 h. The reaction was then quenched with tetraethylammonium chloride (0.250 g), filtered over celite and washed successively with a saturated sodium hydrogen carbonate solution (25 mL) and water (25 mL). The organic layer was then dried ($MgSO_4$), concentrated and purified by flash chromatography using hexanes:ethyl acetate (3:1) as eluent to afford four fractions.

The first fraction contained compound **58** (0.160 g, R_f = 0.50, 99%). The second fraction contained compound **66** (66 mg, R_f = 0.30, 32%). The third fraction contained a minor amount of compound **65** (R_f = 0.10).

5.3.60 Attempt to prepare phenyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→4)-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (67)

A mixture of glycosyl donor **59** (0.309 g, 0.489 mmol, 1.2 eq.), glycosyl acceptor **22** (0.210 g, 0.407 mmol) and NIS (0.110 g, 0. 490 mmol) in dry butyronitrile (30 mL) with freshly flame-dried powdered 4 Å molecular sieves (0.40 g) was stirred for 30 min and then cooled to -63 °C. To the mixture was then added AgOTf (0.126 g) in dry acetonitrile (2 mL). The reaction mixture was monitored by TLC for disappearance of the glycosyl donor. After 90 min, the reaction was warmed to -30 °C and stirred for an additional 5 h. The reaction was quenched with water (2 mL), filtered over celite and concentrated. The resulting residue was then taken up in dichloromethane (100 mL) and washed successively with a saturated sodium hydrogen carbonate solution (100 mL) and water (100 mL). TLC analysis of the crude mixture revealed the presence of seven distinct products and was not purified further.

5.3.61 Attempt to prepare phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (68)

A mixture of glycosyl donor **61** (0.209 g, 0.532 mmol, 1.4 eq.), glycosyl acceptor **22** (0.196 g, 0.311 mmol) and NIS (0.120 g) in dry dichloromethane (15 mL) with freshly flame-dried powdered 4 Å molecular sieves (0.50 g) was stirred for 20 min at 0 °C and then treated with a solution of AgOTf (0.137 g) in dry acetonitrile (2 mL). The reaction mixture was monitored by TLC for disappearance of the glycosyl donor. After 2 h the reaction was

quenched with water (1 mL), filtered over celite and concentrated. The resulting residue was taken up in dichloromethane (150 mL) and washed successively with a saturated sodium hydrogen carbonate solution (100 mL) and water (100 mL). The organic layer was then dried ($MgSO_4$), and concentrated, TLC, hexanes:ethyl acetate (1:2) revealed the presence of seven products. The major product (R_f = 0.55) was purified by flash chromatography and determined to be 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-deoxy-2-phthalimido-β-D-glucopyranoside (**69**) (0.020 g, 6%); ^{1}H NMR δ 7.76-7.65 (S*Ph*), 8.20-7.80 (N*Phth*), 6.53 (d, 1H, H-1), 5.63 (m, 1H, H-4'), 5.31 (t, 1H, H-2'), 5.16 (dd, 1H, H-3'), 4.79 (d, 1H, H-1'), 4.53-3.70 (series of multiplets, H-6a,b, H-3, H-4, H-5, H-5', H-6a,b'), 2.23, 2.21, 2.20, 2.17 (4 x C*H*$_3$CO); ^{13}C NMR: δ 170.2, 170.1, 169.9, 169.2, 169.1, 167.8 (4 x CH_3CO, 2 x N*C*O), 134.2, 132.1, 123.5 (N*Phth-C*), 101.7 (C-1'), 90.9 (C-1), (C-1), 72.6, 70.8, 69.3, 68.9, 68.5, 68.3, 66.9, 66.8, 61.2, 61.1, (C-2, C-3, C-4, C-5, C-6, C-2', C-3', C-4', C-5', C-6'), 25.9 (SiC(*C*H$_3$)$_3$), 20.9, 20.8, 20.8, 20.7 (4 x *C*H$_3$CO), 18.3 (Si*C*(CH$_3$)$_3$), -5.2 (2 x SiC(*C*H$_3$)$_2$).

5.3.62 Attempt to prepare phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (62)

A mixture of glycosyl donor **66** (0.085 g, 0.165 mmol, 1.0 eq.), glycosyl acceptor **22** (0.068 g, 0.165 mmol) and 2,6-DTBMP (0.040 g, 0.154 mmol) in dichloromethane:acetonitrile (10 mL, 1:1 v:v) with freshly flame-dried powdered 4 Å molecular sieves (0.20 g) was stirred for 1 h at 0 °C and then treated with a solution of AgOTf (0.050 g) in dry acetonitrile (1 mL). The reaction mixture was then stirred at for 30 min at

-45 °C and warmed to 0 °C. After stirring at this temperature for 3 h, the reaction was quenched with tetraethylammonium chloride (0.250 g), filtered over celite and concentrated. The residue was purified by flash chromatography using hexanes:ethyl acetate (1:1) as eluent to afford one fraction that was determined to be recovered glycosyl acceptor (0.025 g, R_f = 0.50, 29%).

5.3.63 2,3,4,6-Tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (71)

Compound **71** was prepared from D-galactose (**1**) (5.06 g, 28.1 mmol) in a two step procedure reported by Fletcher[307] to give compound **71** (13.65 g, 74%): ^{1}H NMR[308] δ 8.2-6.9 (m, 20H, CO*Ph*), 7.00 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 6.16 (d, 1H, $J_{3,4}$ = 3.7 Hz, H-4) 6.08 (dd, 1H, $J_{1,2}$ = 3.0, Hz, $J_{2,3}$ = 10.4 Hz, H-2), 5.70 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 4.94 (broad t, 1H, $J_{5,6}$ = 6.7 Hz, H-5), 4.66 (dd, 1H, $J_{5,6a}$ = 6.7 Hz, $J_{6a,6b}$ = -11.6 Hz, H-6a), 4.48 (dd, 1H, $J_{5,6b}$ = 6.7 Hz, $J_{6a,6b}$ = -11.6 Hz, H-6b); ^{13}C NMR δ 166.1, 165.7, 165.5, 162.5 (4 x Ph*C*O), 134.7-128.5 (Ar-C), 88.5 (C-1), 72.0 (C-5), 69.1 (C-3), 68.8 (C-4), 68.3 (C-2), 61.8 (C-6).

5.3.64 Attempt to prepare phenyl (2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-deoxy-2-phthalimido-6-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-glucopyranoside (72)

To a solution of glycosyl donor **71** (0.403 g, 0.611 mmol, 1.4 eq.) and glycosyl acceptor **22** (0.224 g, 0.436 mmol) in dichloromethane (15 mL) was added freshly flame-dried powdered 4 Å molecular sieves (1.0 g). The mixture was allowed to stir for 30 min at room temperature, cooled to 0 °C and treated with a solution of AgOTf (0.224 g) in dry

acetonitrile (1 mL). The mixture was stirred at 0 °C for 30 min and then warmed to room temperature. After stirring at this temperature for 1 h the reaction was quenched with triethylamine (0.50 mL), filtered over celite and concentrated. The residue was purified by flash chromatography using hexanes:ethyl acetate (2:1) as eluent to afford the major disaccharide component (0.026 g, R_f = 0.20, 6%) as a colorless oil that was determined to be 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→6)-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**73**): ^{1}H NMR δ 8.2-7.2 (m, 29H, CO*Ph*, S*Ph*, N*Phth*), 5.99 (d, 1H, $J_{3',4'}$ = 3.0 Hz, H-4'), 5.76 (dd, 1H, $J_{1',2'}$ = 7.9, Hz, $J_{2,3}$ = 10.4 Hz, H-2'), 5.56 (m, 2H, H-1, H-3'), 4.95 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.66 (m, 1H, H-6a'), 4.49 (m, 1H, H-6b'), 4.30-4.15 (m, 3H, H-3, H-5', H-6a), 4.14 (t, 1H, $J_{1,2}$ = $J_{2,3}$ = 10.4 Hz, H-2), 4.01 (m, 1H - H-6b), 3.64 (m, 1H, H-5), 3.51 (t, 1H, $J_{3,4}$ = 8.5 Hz, H-4); ^{13}C NMR δ 165.8, 165.8, 165.8, 165.7 (4 x PhCO), 134.4-123.7 (Ar-C), 101.7 (C-1'), 83.8 (C-1), 78.8 (C-5), 72.9 (C-3), 72.8 (C-4), 71.8 (C-5'), 71.7 (C-3'), 70.0 (C-2'), 69.4 (C-6'), 68.4 (C-4'), 62.3 (C-6), 55.5 (C-2).

5.3.65 Phenyl 2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (74)

Phenyl 2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**21**)[205] (10.18 g, 0.0254 mol) and dibutyltin oxide (6.94 g, 0.0297 mol, 1.1 mol eq.) were refluxed overnight in toluene (400 mL) with azeotropic removal of water. To the refluxing solution was then added tetrabutylammonium iodide (4.68 g, 0.0127 mol, 0.50 eq) followed by 2-(bromomethyl) naphthylene (8.41 g, 0.0380 mol, 1.5 eq). The reaction mixture was stirred at reflux for 18 h where TLC (hexanes:ethyl acetate 1:3) indicated the complete conversion of **21** into a faster

moving product (R_f = 0.60). The reaction was then cooled and washed with water (2 x 200 mL) and the organic layer dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography using hexanes:ethyl acetate (1:3) as eluent to give the title compound as a colorless oil (8.16 g, 64%): ^{1}H NMR δ 7.8-6.9 (16H, S*Ph*, N*Phth*, OCH_2*NAP*), 5.58 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 4.68 (m, 2H, OCH_2NAP), 4.33 (broad t, 1H, $J_{3,4} = J_{2,3}$ = 10.1 Hz, H-3), 4.20 (t,1H, $J_{1,2} = J_{2,3}$ =10.7 Hz, H-2), 3.77 (broad m, 2H, H-4, H-5), 3.60 (m, 2H, H-6a,b), lit.[302] was very similar; ^{13}C NMR δ 133.1-125.8 (Ar-C), 83.7 (C-1), 78.6 (C-5), 73.8 (OCH_2NAP), 72.9, 72.9 (C-4, C-4), 70.1 (C-6), 55.7 (C-2).

5.3.66 Phenyl 3,4-di-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (75)

To a solution of compound **74**, (0.584 g, 1.16 mmol) in dry pyridine (25 mL) at room temperature was added benzoyl chloride (1.08 mL, 9.29 mmol, 8 eq.). The reaction was allowed to proceed overnight where a new spot appeared on TLC, hexanes:ethyl acetate (2:1) with R_f = 0.40. The reaction mixture was then diluted with CH_2Cl_2 (50 mL) and poured into cold a hydrochloric acid solution (1.0 M, 50 mL). The organic layer was separated and washed successively with hydrochloric acid solutions (1.0 M, 2 x 50 mL) and water (50 mL). The organic layer was then dried over $MgSO_4$, filtered and concentrated to yield an oily residue that was purified by flash chromatography using hexanes:ethyl acetate (2:1) to give compound **75** (0.549 g, 66%): $[\alpha]^{25}_D$ + 58.8° (*c* 2.4, $CHCl_3$); ^{1}H NMR δ 8.10-7.10 (26H, Ar-H), 6.27 (t, 1H, $J_{3,4} = J_{2,3}$ = 9.8 Hz, H-3), 5.93 (d, 1H, $J_{1,2}$ = 10.4 Hz, H-1), 5.53 (t, 1H, $J_{3,4}$ = 9.8 Hz, H-4), 4.69 (m, 2H, CH_2NAP), 4.63 (t, 1H, $J_{1,2}$ = 10.4 Hz, H-2), 4.19 (dt, 1 H, H-5),

3.78 (broad m, 2H, H6a,b);[13]C NMR δ 168.2, 167.1(2 x N*C*O) 165.9, 165.4 (2 x *C*OPh), 135.7-123.8 (Ar-*C*), 83.6 (C-1), 78.2 (C-5), 73.8 (*C*H_2NAP), 72.4 (C-3), 70.3 (C-4), 69.4 (C-6), 54.1 (C-2); FABMS m/z: 749 (M^+), 639 (M - SPh); HRMS (FABMS) calcd for $C_{45}H_{35}NO_8S$ + H: 750.2250. Found, 750.2242.

5.3.67 Attempt to prepare phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→4)-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (76)

To a solution of glycosyl donor **71** (0.293 g, 0.445 mmol, 1.3 eq.), glycosyl acceptor **74** (0.172 g, 0.342 mmol) and 2,6-DTBMP (0.137 g, 0.667 mmol) in dichloromethane (10 mL) was added freshly flame-dried powdered 4 Å molecular sieves (0.50 g). The solution was allowed to stir for 1 h at room temperature, cooled to 0 °C and treated with a solution of AgOTf (0.171 g) in dry acetonitrile (2 mL). The reaction mixture was stirred at this temperature for 20 min and then warmed to room temperature. Te reaction mixture was stirred at this temperature for 12 h, then quenched with triethylamine (0.50 mL), filtered over celite and concentrated. The residue was purified by flash chromatography using hexanes:ethyl acetate (3:1) as eluent to afford three identifiable fractions.

The first fraction contained 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranoside (**77**) in 59% yield.

The first fraction contained the major disaccharide component (0.084 g, R_f = 0.37, 22%) as a colorless oil that was determined to be an ortho-ester product, compound **78**: ^{1}H NMR δ 8.0-7.1 (m, 36H, 4 x CO*Ph*, S*Ph*, N*Phth*, CH_2*NAP*), 6.12 (d, 1H, $J_{1',2'}$ = 4.9 Hz, H-1'), 5.67 (d, 1H, $J_{1,2}$ = 10.4 Hz), 5.59 (m, 1H, H-4'), 5.03 (dd, 1H, $J_{2',3'}$ = 6.7 Hz, $J_{3,4}$ = 3.0 Hz, H-

3'), 4.81 (dd, 1H, $J_{1',2'}$ = 4.9, Hz, $J_{2,3}$ = 6.7 Hz, H-2'), 4.78 (s, 2H, OC*H*$_2$NAP), 4.56 (m, 2H, H-6a,b'), 4.38-4.27 (m, 2H, H-5', H-6a), 4.14 (m, 2H, H-2, H-6b), 3.98-3.93 (m, 1H, H-5), 3.84-3.77 (m, 2H,H-3, H-4); ^{13}C NMR δ 165.8, 165.8, 165.8, (3 x Ph*C*OO), 137.9-125.3 (Ar-C), 121.1 (O_3CPh), 99.0 (C-1'), 83.4 (C-1), 80.0 (C-5), 76.2 , 74.9, 73.8 (O*C*H$_2$NAP), 70.1, 70.1, 69.3 (C-6'), 66.7, 62.1 (C-6), 54.1 (C-2).

The third fraction isolated was compound **74** (0.082 g, 48%).

5.3.68 Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (76)

To a solution of the glycosyl donor **71** (3.93 g, 5.96 mmol, 1.5 equiv.) and **74** (1.98 g, 3.94 mmol) in dichloromethane (50 mL) was added freshly flame dried powdered 4 Å molecular sieves (1.00 g). The solution was allowed to stir for 30 min and treated with solid AgOTf (2.32 g, 8.94 mmol) under N_2 atmosphere. The reaction mixture was monitored by TLC for disappearance of the starting materials. After 2 h, the reaction was quenched with triethylamine (1 mL), filtered over celite and concentrated for MPLC using hexanes:ethyl acetate (2:1) as eluent to give the title compound (R_f = 0.33) as an amorphous solid (2.85 g, 65%): $[\alpha]^{25}_D$ +102.9° (*c* 6.8, $CHCl_3$); ^{1}H NMR δ 8.2-6.9 (m, 36H, Ar-*H*), 5.91 (d, 1H, $J_{3',4'}$ = 3.2 Hz, H-4'), 5.85 (dd, 1H, $J_{1',2'}$ = 8.1, Hz, $J_{2',3'}$ = 10.3 Hz, H-2'), 5.61 (d, 1H, $J_{1,2}$ = 10.5 Hz, H-1), 5.56 (dd, 1H, $J_{2',3'}$ = 10.5 Hz, $J_{3,4}$ = 3.4 Hz, H-3'), 4.90 (d, 1H, 4.9, $J_{1',2'}$ = 8.1 Hz, H-1') 4.64 (m, 2H, H-3, H-6a') 4.43-4.26 (broad m, 4H, H-2, H-6b', *C*H$_2$NAP), 4.22 (dd, 1H, $J_{5',6a'}$ = 8.5 Hz, H-5'), 3.86 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 8.3 Hz, H-4), 3.72 (m, 1H, H-5), 3.55 (s, 2H, H-6a,b); ^{13}C NMR δ 168.3, 167.6 (2 x N*C*O) 166.2, 165.5, 165.5, 165.1 (4 x *C*OPh), 135.8-123.4 (Ar-

C), 102.1, (C-1'), 83.6 (C-1), 82.7 (C-4), 78.0 (C-5), 73.2 (CH_2NAP), 72.4 (C-5'), 71.5 (C-3'), 71.1 (C-3), 69.7 (C-2'), 68.2 (C-4'), 68.2 (C-6), 62.7 (C-6'), 55.2 (C-2).

5.3.69 Phenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (79)

To a solution of compound **76**, (1.156 g, 1.03 mmol) in dry pyridine (50 mL) at 0°C was added benzoyl chloride (0.36 mL, 3.1 mmol, 3 eq.) and the reaction mixture warmed to room temperature. After stirring overnight, a new spot appeared on TLC [toluene:ethyl acetate (10:1)] (R_f = 0.50) and the reaction mixture was poured into cold H_2O (100 mL) and extracted with CH_2Cl_2 (100 mL). The organic layer was then washed successively with a hydrochloric acid solution (1.0 M, 2 x 100 mL) and water (100 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography using toluene:ethyl acetate (10:1) as eluent to give the title compound, **79**, as an amorphous solid (1.126 g, 89%): $[\alpha]^{25}_D$ +42.1° (c 4.2, $CHCl_3$); ^{1}H NMR δ 8.2-6.9 (m, 41H, Ar-*H*), 6.18 (t, 1H, $J_{2,3}$ = 10.4, $J_{3,4}$ = 9.2 Hz, H-3), 5.81 (d, 1H, $J_{1,2}$ = 10.6 Hz, H-1), 5.67 (d, 1H, $J_{3',4'}$ = 3.23 Hz, H-4'), 5.64 (dd, 1H, $J_{1',2'}$ = 7.8, Hz, $J_{2',3'}$ = 10.1 Hz, H-2'), 5.38 (dd, 1H, $J_{2',3'}$ = 10.1 Hz, $J_{3,4}$ = 3.23 Hz, H-3'), 4.91 (d, 1H, 4.94, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.90, 4.63 (AB 2d, 2H, $J_{A,B}$ = -12.2 Hz, OC*H*$_2$NAP), 4.62 (t, 1H, $J_{2,3}$ = 10.6 Hz, H-2), 4.36 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4) 3.82-3.52 (broad m, 6H, H-5, H-5', H-6a,b, H-6a',b'); ^{13}C NMR: δ 168.0, 167.3 (2 x N*C*O) 165.7, 165.6, 165.5, 165.4, 165.8 (5 x *C*OPh), 138.0-123.7 (Ar-C), 100.7, (C-1'), 83.6 (C-1), 78.9 (C-5), 75.6 (C-4), 73.8 (CH_2NAP), 72.4 (C-3), 71.9 (C-3'), 71.1 (C-5'), 70.2 (C-2'), 67.8 (C-4'), 67.7 (C-6), 61.0 (C-

6'), 54.1 (C-2);. ESMS m/z: 1246 (M^+ + Na); Exact mass (ESMS) calcd for $C_{72}H_{57}N_2O_{16}S$ + Na: 1246.3296. Found, 1246.3294.

5.3.70 5-Azidopentyl 3,4-di-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (80)

To a solution of glycosyl donor **75** (0.747 g, 0.996 mmol, 1.6 eq.), glycosyl acceptor **48β** (0.358 g, 0.634 mmol) and NIS (0.337 g, 1.494 mmol) in dichloromethane (10 mL) was added freshly flame-dried powdered 4 Å molecular sieves (0.50 g). The solution was allowed to stir for 30 min at 0°C and then treated with a solution of AgOTf (0.384 g) in dry acetonitrile (2 mL). The reaction mixture was then warmed to room temperature and after 2 h was filtered over celite and concentrated. The residue was then taken up in CH_2Cl_2 (50 mL) and washed successively with saturated sodium hydrogen sulfite solutions (2 x 50 mL) and water (50 mL). The organic layer was dried ($MgSO_4$), filtered, concentrated, and the residue purified by flash chromatography using toluene:ethyl acetate (8:1) as eluent, to give the title compound, **80**, (R_f = 0.45) as a colorless syrup (0.569 g, 74%): $[\alpha]^{25}_D$ - 0.7° (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.88-7.05 (m, 36H, Ar-H), 6.30 (t, 1H, $J_{3,4}$ = 9.3 Hz, $J_{2,3}$ = 10.9 Hz, H-3'), 5.88 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1'), 5.63 (t,1H, $J_{3,4}$ = 10.0 Hz, H-4'), 4.97, 4.57 (AB 2d, 2H, $J_{A,B}$ = -11.6 Hz, OC*H*$_2$Ph), 4.69-4.60 (m, 4H, H-2', C*H*$_2$NAP, one OC*H*$_2$Ph), 4.40, 4.37 (AB 2d, 2H, $J_{A,B}$ = -11.8 Hz, OC*H*$_2$Ph), 4.24 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.19 (d, 1H, one OC*H*$_2$Ph), 4.06 (d, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 4.04 (m, 1H under H-4, H-5'), 3.89 (dd, 1H, $J_{2,3}$ = 9.7 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 3.80 (m, 1H, one OC*H*$_2$(CH$_2$)$_4$), 3.73 (m, 2H, H-6a',b'), 3.59 (t, 1H, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 10.0 Hz, H-2), 3.58-3.49 (broad m, 4H, H-2, H-5, H6a,b), 3.32 (m,

1H, one OCH_2(CH_2)$_4$), 3.00 (t, 2H, J = 7.0 Hz, CH_2N_3), 1.48-1.35 (m, 4H, $OCH_2CH_2CH_2CH_2$), 1.25-1.16 (m, 2H, O(CH_2)$_2$$CH_2$); ^{13}C NMR δ 168.2, 167.1(2 x N*C*O) 165.9, 165.4 (2 x *C*OPh), 139.1, 139.0, 138.2, 138.1 (4 x Bn-ipso C), 133.7-123.3 (Ar-C), 104.1 (C-1), 99.5 (C-1'), 81.7 (C-3). 78.7 (C-2), 76.2 (C-4), 75.0, 74.0, 73.7 (3 x O*C*H_2Ph), 74.0 (*C*H_2NAP), 73.6, 73.6 (C-5', C-5), 71.3 (C-3'), 70.7 (C-4'), 69.7 (O*C*H_2(CH_2)$_4$), 69.5 (C-6'), 69.3 (C-6), 55.6 (C-2'), 51.4 (*C*H_2N_3), 29.3 (OCH_2CH_2), 28.7 (*C*$H_2CH_2N_3$), 23.4 ($OCH_2CH_2CH_2$); ESMS m/z: 1223 (M^+ + Na), Exact mass (ESMS) calcd for $C_{71}H_{68}N_4O_{14}$ + Na: 1223.4630. Found, 1223.4635.

5.3.71 5-Azidopentyl 3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (81)

DDQ (63 mg, 0.280 mmol) was added to a stirred solution of **80** (112 mg, .093 mmol) in dichloromethane:methanol (4:1, 12.5 mL). The reaction mixture was stirred for 17 h until the starting material had been consumed and a new spot (R_f = 0.35) appeared on TLC [toluene:ethyl acetate (5:1)]. The mixture was then concentrated under reduced pressure and the residue taken up in dichloromethane (30 mL). The organic layer was then washed successively with saturated sodium hydrogen carbonate solutions (2 x 25 mL) and water (25 mL), dried ($MgSO_4$), filtered, and concentrated. Fractionation by flash chromatography using toluene:ethyl acetate (5:1) as eluent, afforded the title compound as a colorless syrup (58.3 mg, 59%): $[\alpha]^{25}_D$ +0.7° (*c* 1.5, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.97-7.14 (m, 29H, Ar-H), 6.40 (t, 1H, $J_{3',4'}$ = 9.5 Hz, $J_{2,3}$ = 10.7 Hz, H-3'), 5.89 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 5.51 (t,1H, $J_{3',4'}$ = 9.5 Hz, H-4'), 4.93 (A of AB, d, 1H, $J_{A,B}$ = -11.6 Hz, OCH_2Ph), 4.65 (m, 3H, H-2', one OCH_2Ph, one OCH_2Ph), 4.45, 4.39 (AB 2d, 2H, $J_{A,B}$ = -11.8 Hz, OCH_2Ph), 4.28 (d, 1H, $J_{1,2}$

= 7.6 Hz, H-1), 4.19 (d, 1H, one OC*H*$_2$Ph), 4.00 (d, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 3.90 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 3.80 (m, 2H, one OC*H*$_2$(CH$_2$)$_4$, H-6a'), 3.71 (m, 2H, H-6b', H-5), 3.61 (t, 1H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.59-3.48 (broad m, 3H, H-5', H6a,b), 3.35 (m, 1H, one OC*H*$_2$(CH$_2$)$_4$), 3.01 (t, 2H, J = 7.2 Hz, CH_2N_3), 1.48-1.35 (m, 4H, $OCH_2CH_2CH_2CH_2$), 1.25-1.16 (m, 2H, O(C*H*$_2$)$_2$CH$_2$); ^{13}C NMR δ 168.2, 167.1 (2 x NCO), 165.9, 165.4 (2 x *C*OPh), 135.0-123.6 (Ar-C), 104.1 (C-1), 99.2 (C-1'), 81.2 (C-3). 78.9 (C-2), 76.0 (C-4), 74.8, 74.0, 73.7 (3 x O*C*H$_2$Ph) 74.5 (C-5), 73.5, (C-5'), 70.8 (C-3'), 70.4 (C-4',) 69.7 (O*C*H$_2$(CH$_2$)$_4$), 69.1 (C-6), 61.3 (C-6'), 55.6 (C-2'), 51.4 (*C*H$_2$N$_3$), 29.3 (OCH$_2$*C*H$_2$), 28.7 (*C*H$_2$CH$_2$N$_3$), 23.4 (OCH$_2$CH$_2$*C*H$_2$).

5.3.72 Sodium 5-azidopentyl 3,4-di-*O*-benzoyl-2-deoxy-2-phthalimido-6-*O*-sulfonate-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (82)

Sulfur trioxide/trimethylamine complex (43 mg, 0.308 mmol) was added to a stirred solution of **81** (82 mg, .077 mmol) in dry DMF (5 mL) at 60°C. The mixture stirred at 60°C for 14 h until the starting material had been consumed and a new spot (R_f = 0.50) appeared on TLC [dichloromethane:methanol (9:1)]. The mixture was then concentrated under reduced pressure and taken up in methanol (20 mL). To the solution was then added IR-120 Na^+ cation exchange resin and stirred for 20 min. The mixture was then filtered, concentrated and fractionated by flash chromatography dichloromethane:methanol (9:1) as eluent, to afford the title compound as a colorless syrup (65.3 mg, 73%): $[\alpha]^{25}_D$ +1.4° (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.91-6.92 (m, 29H, Ar-H), 6.24 (t, 1H, $J_{3',4'}$ = 9.3 Hz, $J_{2',3'}$ = 10.7 Hz, H-3'), 5.88 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 5.49 (t,1H, $J_{3',4'}$ = 9.3 Hz, H-4'), 5.00, 4.67 (AB 2d, 2H, $J_{A,B}$ = -

11.6 Hz, OCH_2Ph), 4.54 (m, 3H, $J_{1',2'}$ = 8.4 Hz, $J_{2',3'}$ = 10.7 Hz, H-2'), 4.49 (s, 2H, OCH_2Ph), 4.46, 4.16 (AB 2d, 2H, $J_{A,B}$ = -12.3 Hz, OCH_2Ph), 4.34 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.32-4.20 (m, 4H, H-4, H-5', H-6a',b'), 3.98 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 3.76 (m, 2H, one OCH_2(CH_2)$_4$, H-5), 3.60 (m, 2H, H-6a,b), 3.46 (t, 1H, $J_{1,2}$ = 7.7 Hz, $J_{2,3}$ = 9.7 Hz, H-2), 3.35 (m, 1H, one OCH_2(CH_2)$_4$), 2.94 (t, 2H, J = 7.0 Hz, CH_2N_3), 1.48-1.35 (m, 4H, OCH$_2$CH_2CH$_2$CH_2), 1.25-1.16 (m, 2H, O(CH_2)$_2$CH$_2$); ^{13}C NMR δ 168.2, 167.1 (2 x NCO), 165.9, 165.4 (2 x COPh), 135.0-123.6 (Ar-C), 105.1 (C-1), 101.2 (C-1'), 84.1 (C-3), 79.6 (C-2), 78.7 (C-4), 76.3, 74.8, 74.0 (3 x OCH$_2$Ph), 75.0 (C-5), 74.0, (C-5'), 72.9 (C-3'), 71.6 (C-4'), 71.0 (C-6), 70.7 (OCH$_2$(CH_2)$_4$), 67.8 (C-6'), 52.4 (C-2'), 51.4 (CH_2N_3), 30.4 (OCH$_2$CH$_2$), 29.7 ($CH_2CH_2N_3$), 24.5 (OCH$_2$CH$_2$CH$_2$).

5.3.73 Sodium 5-azidopentyl 2-acetamido-2-deoxy-6-*O*-sulfonate-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (83)

Compound **82** (0.117 g, 0.100 mmol) was dissolved in a mixture of 1-aminobutane:methanol (3:5, v:v, 24 mL) and refluxed for 48 h. By that time, TLC gave no evidence of the starting material. The reaction was concentrated to give a yellowish solid which was dissolved in a mixture of methanol:acetic anhydride (10:1 v:v, 11 mL) and stirred at room temperature. After 16 h, the mixture was concentrated and fractionated by flash chromatography using dichloromethane:methanol (9:1) as eluent to yield the title compound (**83**) as a colorless syrup (71 mg, 82%): $[\alpha]^{25}_D$ -11.7° (*c* 0.9, $CHCl_3$); ^{1}H NMR (methanol-d_4, 400 MHz) δ 7.45-7.20 (m, 15H, Ar-H), 5.02, 4.60 (AB 2d, 2H, $J_{A,B}$ = -11.8 Hz, OCH_2Ph), 4.89 (m, AB, 2H, OCH_2Ph), 4.72 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1'), 4.50 (AB q, 2H, OCH_2Ph), 4.38

(m, 2H, H-1, H-6a'), 4.20 (m, 2H, H-4, H-6b'), 3.85-3.78 (m, 3H, H-2', H-3, one OCH_2CH_2), 3.74-3.69 (m, 1H), 3.64-3.36 (m, 6H), 3.31 (m, 1H, OH), 3.06 (t, 2H, J = 6.7 Hz, CH_2N_3), 1.71 (s, 3H, CH_3CONH), 1.53-1.43 (m, 4H, $OCH_2CH_2CH_2CH_2$), 1.35-1.25 (m, 2H, $O(CH_2)_2CH_2$); ^{13}C NMR (Acetone-d_6) δ 172.0 (Me*C*ONH), 140.6, 140.5, 139.6 (3 x Bn-ipso C), 129.6-127.9 (Ar-C), 104.8 (C-1), 104.5 (C-1'), 84.2 (C-3), 79.9, 78.5 (C-4), 75.8, 75.8, 75.3 (OCH_2Ph), 74.9, 74.2 (OCH_2Ph), 72.1, 71.1 (C-6), 70.5 (OCH_2CH_2), 68.4 (C-6'), 57.8 (C-2'), 52.2 (CH_2N_3), 30.3 (OCH_2CH_2), 29.6 ($CH_2CH_2N_3$), 24.4 ($OCH_2CH_2CH_2$), 23.2 (CH_3CONH).

5.3.74 Sodium 5-aminopentyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside 6'-sulfonate (84)

To a solution of **83** (55 mg, 63.0 μmol) in methanol:acetic acid (4:1, 10 mL) was added 10% Pd/C (110 mg) and the mixture stirred at room temperature for 28 h under hydrogen atmosphere (1 atm). The reaction mixture was then filtered over celite and washed with methanol (2 x 25 mL). The combined washings were concentrated to dryness to obtain the title compound as a colorless crystalline product (36 mg, 100%): ^1H NMR (water-d_2, 400 MHz) δ 4.72 (d, 1H, H-1'), 4.38 (m, 2H, H-1, H-6a'), 4.20 (m, 2H, H-4, H-6b'), 3.91 (m, 1H, one $OCH_2(CH_2)_4$), 3.80-3.47 (m, 10H), 3.00 (t, 2H, J = 6.9 Hz, CH_2NH_2), 2.00 (s, 3H, CH_3CONH), 1.72-1.57 (m, 4H, $OCH_2CH_2CH_2CH_2$), 1.49-1.43 (m, 2H, $O(CH_2)_2CH_2$); ^{13}C NMR δ 174.8 (Me*C*ONH), 103.5 (C-1), 103.4 (C-1'), 83.2, 75.5, 74.2, 70.6, 70.6 (OCH_2CH_2), 70.4 (C-2'), 70.3 (C-4), 69.0 (C-3), 67.9 (C-6'), 61.8 (C-6), 56.3 (C-2'), 40.0 (CH_2NH_2), 28.8

(OCH_2CH_2), 27.0 ($CH_2CH_2NH_2$), 22.8 ($OCH_2CH_2CH_2$), 22.6 (CH_3CONH). ESMS m/z: 571 (M^+ + Na), Exact mass (ESMS) calcd for $C_{19}H_{36}N_2O_{14}S$ + Na: 571.1785. Found, 571.1784.

4.3.75 5-Azidopentyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (85) using NIS-AgOTf as promotor

To a solution of glycosyl donor **79** (0.316 g, 0.259 mmol, 1.5 eq.), **48β** (0.096 g, 0.173 mmol) and NIS (0.172 g, 4.5 eq.) in dichloromethane (10 mL) was added freshly flame dried powdered 4 Å molecular sieves (0.50 g). The solution was stirred for 20 min and then treated with AgOTf (0.200 g, 4.5 eq.) under nitrogen atmosphere. The reaction mixture turned a light pink color which was maintained for 3 d. Monitoring the reaction by TLC for this time period showed the disappearance of the glycosyl donor. After 3 d, the reaction was filtered over celite and the organic layer washed successively with a saturated sodium hydrogen sulfite solution (50 mL) and water (50 mL), dried ($MgSO_4$), and concentrated. Fractionation by flash chromatography using toluene:ethyl acetate (10:1) as eluent afforded four fractions. The first and second fractions were 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-α-D-glucopyranoside (**86α**) and 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranoside (**86β**), (R_f = 0.50, 0.40).

The third fraction, (R_f = 0.33) contained the title compound **85** isolated as a colorless syrup (0.060 g, 22%): $[\alpha]^{25}_D$ +18.7° (*c* 2.0, $CHCl_3$); ^{1}H NMR (400 MHz) δ 8.05-7.05 (m, 36H, Ar-H), 6.14 (t, 1H, $J_{3',4'} = J_{2',3'}$ = 10.0 Hz, H-3'), 5.74 (d, 1H, $J_{1',2'}$ = 8.5 Hz, H-1'), 5.60 (m, 2H,

H-2'', H-4''), 5.29 (dd, 1H, $J_{2'',3''}$ = 10.2 Hz, $J_{3'',4''}$ = 3.3 Hz, H-3''), 4.96 (d, 1H, $J_{A,B}$ = -11.5 Hz, one OCH_2Ph), 4.81 (d, 2H, H-1', one OCH_2Ph), 4.57-4.46 (m, 5H, 4 x OCH_2Ph, H-2'), 4.41-4.28 (m, 3H, OCH_2NAP, H-4'), 4.21-4.17 (m, H-1, one OCH_2Ph), 3.98 (d, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 3.79-3.71 (m, 4H), 3.61 (m, 2H), 3.56-3.48 (m, 6H), 3.31 (m, 1H, one OC$H_2$$CH_2$), 2.99 (t, 2H, J = 6.7 Hz, C$H_2$$N_3$), 1.48-1.35 (m, 4H, $OCH_2CH_2CH_2CH_2$), 1.25-1.16 (m, 2H, $O(CH_2)_2CH_2$); ^{13}C NMR δ 178.5, 177.8 (2 x N*C*O), 165.9, 165.8, 165.6, 165.6, 165.0 (5 x Ph*C*O) 139.3, 139.1, 138.5 (3 x Bn-ipso C), 135.7-127.1 (Ar-C), 104.2 (C-1), 100.8 (C-1''), 99.3 (C-1'), 81.5 (C-3). 78.8 (C-2), 76.4 (C-4), 75.2, 73.7, 73.7 (3 x O*C*H_2Ph), 76.0, 74.0 (*C*H_2NAP), 73.6, 73.6, 72.1 (C-5''), 71.3 (C-3'), 71.2, 70.4 (C-4'), 69.7 ($OCH_2(CH_2)_4$), 69.3 (C-6'), 68.0 (C-6), 61.1 (C-6''), 55.7 (C-2'), 51.4 (*C*$H_2$$N_3$), 29.3 ($OCH_2CH_2$), 28.7 (*C*$H_2$$CH_2N_3$), 23.4 ($OCH_2CH_2CH_2$). ESMS m/z: 1698.6 ($M^+$ + Na).

The fourth fraction (R_f = 0.25) was compound **48β** (0.031 g, 32%).

5.3.76 5-Azidopentyl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-3-*O*-benzoyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (85) using NIS-TfOH as promotor

To a solution of glycosyl donor **79** (0.340 g, 0.278 mmol, 2.0 eq.), **48β** (0.080 g, 0.142 mmol) and NIS (0.094 g, 3.0 eq.) in dichloromethane (10 mL) was added freshly flame dried powdered 4 Å molecular sieves (0.50 g). The solution was allowed to stir for 20 min and then treated with TfOH (2.3 μL, 0.2 eq.). The reaction mixture turned a dark purple color which was maintained for 3 d. Monitoring the reaction by TLC for this time period showed the disappearance of the glycosyl donor. After 3 d the reaction was filtered over celite and the

organic layer washed successively with saturated sodium hydrogen sulfite solutions (2 x 50 mL), a saturated sodium hydrogen carbonate solution (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$), concentrated and fractionated by flash chromatography using toluene:ethyl acetate (10:1) as eluent to afford three fractions. The first and second fractions were **86α** and **86β**, (R_f = 0.50, 0.40). The third fraction, (R_f = 0.33), was the title compound (**85**) isolated as a colorless syrup (0.023 g, 10%).

5.3.77 Phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-1-thio-β-D-glucopyranoside (87)

Compound **79** (0.673g, 0.550 mmol) was dissolved in dry methanol (50 mL) and treated with a solution of sodium methoxide (0.24 g Na in 1.0 mL methanol) and the reaction allowed to for 1 h until all the starting material was converted into a lower spot as indicated by TLC. To the reaction mixture was then added, ion exchange resin (IR-120) and stirring continued for an additional 10 min. The mixture was then filtered, concentrated and taken up in dry pyridine (10 mL). The mixture was then treated with acetic anhydride (5 mL) and stirred for 2 h. The volatiles were then evaporated and the residue fractionated by flash chromatography using hexanes:ethyl acetate (1:1) as eluent to afford the title compound, **87**, (0.468 g, 93%) as an amorphous white solid: $[\alpha]^{25}_{D}$ +11.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR and ^{13}C NMR consistent with that reported by Xia et al.[302]

5.3.78 5-Azidopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-6-*O*-(2-naphthylmethyl)-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (88)

To a solution of glycosyl donor **87** (0.407 g, 0.445 mmol, 2.5 eq.), **48β** (0.100 g, 0.178 mmol) and NIS (0.150 g, 3.75 eq.) in dichloromethane (10 mL) was added freshly flame dried powdered 4 Å molecular sieves (0.50 g). The solution was stirred for 30 min and then treated with AgOTf (0.172 g, 3.75 eq.) under nitrogen atmosphere. The reaction mixture turned a light pink color which disappeared after 20 min. The reaction mixture was then filtered over celite and the organic layer washed successively with a saturated sodium hydrogen sulfite solution (50 mL) and brine (50 mL). The organic layer was dried ($MgSO_4$), concentrated and fractionated by flash chromatography, using hexanes:ethyl acetate (1:1) as eluent to give the title compound, **88**, (R_f = 0.50) as a colorless syrup (0.150 g, 61%): $[\alpha]^{25}_D$ +6.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz) δ 8.05-7.05 (m, 26H, Ar-H), 6.07 (t, 1H, $J_{2',3'}$ = 10.8 Hz, $J_{3',4'}$ = 9.0 Hz, H-3'), 5.79 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 5.03 (d, 1H, $J_{3'',4''}$ = 3.1 Hz, H-4''), 4.95 (m, 4H, H-2'', OCH_2Ph, one OCH_2Ph), 4.72 (dd, $J_{2'',3''}$ = 10.4 Hz, $J_{3'',4''}$ = 3.5 Hz, H-3''), 4.62-4.49 (m, 5H, H-1'', H-2', OCH_2Ph, one OCH_2Ph), 4.44, 4.37 (AB 2d, 2H, $J_{A,B}$ = -11.8 Hz, OCH_2NAP), 4.24-4.17 (m, 3H, H-1, H-4', one OCH_2Ph), 4.03 (d, 1H, $J_{3,4}$ = 2.7 Hz, H-4), 3.87-3.76 (m, 4H), 3.68 (m, 1H, H-5), 3.59-3.52 (m, 3H), 3.48 (m, 1H, one OCH_2CH$_2$), 3.39-3.31 (m, 3H), 3.26 (t, 1H, J = 7.0 Hz, H-5'), 2.99 (t, 2H, J = 7.0 Hz, CH_2N_3), 2.17, 2.05, 1.91, 1.91, 1.90 (5s, 15H, 5 x CH_3CO), 1.48-1.35 (m, 4H, OCH$_2$CH_2CH$_2$CH_2), 1.25-1.16 (m, 2H, O(CH_2)$_2$CH$_2$); ^{13}C NMR δ 178.5, 177.8 (2 x NCO), 170.5, 170.4, 170.3, 170.2, 169.1 (5 x CH_3CO) 139.3, 139.1, 138.5 (3 x Bn-ipso C), 135.7-127.1 (Ar-C), 104.2 (C-1), 100.4 (C-1''),

99.3 (C-1'), 81.3 (C-3), 78.8 (C-2), 76.4 (C-4), 75.2, 74.2, 73.7 (3 x O*C*H_2Ph), 74.5 (C-5), 74.0 (*C*H_2NAP), 73.5, 71.2, 70.5, (C-4'), 69.7 (O*C*H_2(CH_2)$_4$), 69.5, 69.1 (C-6'), 67.9 (C-6), 60.6 (C-6''), 55.6 (C-2'), 51.4 (*C*H_2N_3), 29.3 (OCH_2CH_2), 28.7 (*C*$H_2CH_2N_3$), 23.4 ($OCH_2CH_2CH_2$), 21.3, 20.8, 20.8, 20.7, 20.7, (5 x *C*H_3CO); FABMS m/z: 1388 (M^-); HRMS (FABMS) calcd for $C_{73}H_{81}N_4O_{22}$ + Na: 1388.5185. Found, 1388.5190.

5.3.79 5-Azidopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (89)

DDQ (49 mg, 0.216 mmol) was added to a stirred solution of **88** (90 mg, 0.065 mmol) in dichloromethane:methanol (4:1, 12.5 mL). The mixture was stirred for 24 h until TLC [hexanes:ethyl acetate (1:1)] indicated the conversion of the starting material into a new spot (R_f = 0.30). The reaction mixture was concentrated under reduced pressure, taken up in dichloromethane (20 mL), and washed successively with saturated sodium hydrogen carbonate solutions (2 x 20 mL) and water (20 mL). The organic layer was then dried ($MgSO_4$), filtered, concentrated and fractionated by flash chromatography using hexanes:ethyl acetate (5:1) as eluent, to give the title compound as a colorless syrup (66 mg, 81%): $[\alpha]^{25}_D$ -2.0° (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz) δ 8.05-7.05 (m, 26H, Ar-H), 5.78 (t, 1H, $J_{2',3'} = J_{3',4'}$ = 8.7 Hz, H-3'), 5.73 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 5.33 (d, 1H, $J_{3'',4''}$ = 3.1 Hz, H-4''), 5.12 (t, 1H, $J_{1',2'}$ = 7.9 Hz, $J_{2'',3''}$ = 10.7 Hz, H-2''), 5.00 (dd, $J_{2'',3''}$ = 10.5 Hz, $J_{3'',4''}$ = 3.5 Hz, H-3'') 4.80 (d, 1H, J = -11.6 Hz, one O*CH*$_2$Ph), 4.65 (d, 1H, $J_{1'',2''}$ = 7.9 Hz, H-1''), 4.58, 4.52 (AB 2d, 2H, $J_{A,B}$ = -11.6 Hz, O*CH*$_2$Ph), 4.50-4.41 (m, 3H, H-5', O*CH*$_2$Ph), 4.28-4.19 (m,

3H, H-1, H-2', one OCH_2Ph), 4.16-4.04 (m, 2H), 3.99 (t, 1H, $J_{3',4'}$ = 9.7 Hz, H-4'), 3.90-3.75 (m, 6H), 3.60-3.44 (m, 5H), 3.33 (m, 1H, one OCH_2CH$_2$), 3.00 (t, 2H, J = 7.0 Hz, CH_2N_3), 2.14, 2.07, 2.04, 1.97, 1.89 (5s, 15H, 5 x CH_3CO), 1.48-1.35 (m, 4H, OCH$_2$CH_2CH$_2$CH_2), 1.25-1.16 (m, 2H, O(CH_2)$_2$CH$_2$); ^{13}C NMR δ 173.2, 173.1 (2 x NCO), 170.5, 170.4, 170.3, 170.2, 169.8 (5 x CH$_3$CO), 138.8, 138.8, 138.0 (3 x Bn-ipso C), 128.6-126.9 (Ar-C), 104.1 (C-1), 101.1 (C-1''), 98.9 (C-1'), 81.1 (C-3), 78.7 (C-2), 76.0 (C-4), 74.7, 73.9, 73.7 (3 x OCH$_2$Ph), 75.6 (C-4'), 74.7 (C-5), 73.3, 71.3 (C-3'), 71.2 (C-3''), 70.7, 69.7 (OCH$_2$(CH$_2$)$_4$), 69.4 (C-2''), 68.8 (C-6), 66.9 (C-4''), 61.1 (C-6'), 60.6 (C-6''), 55.6 (C-2'), 51.3 (CH$_2$N$_3$), 29.3 (OCH$_2$CH$_2$), 28.7 (CH$_2$CH$_2$N$_3$), 23.4 (OCH$_2$CH$_2$CH$_2$), 20.9, 20.9, 20.9, 20.8, 20.8, (5 x CH$_3$CO); FABMS m/z: 1255 (M$^+$); HRMS (FABMS) calcd for $C_{63}H_{73}N_2O_{23}$: 1225.3988. Found, 1225.4044.

5.3.80 5-Aminopentyl β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (90)

Compound **89** (0.140 g, 0.114 mmol) was dissolved in a mixture of 1-aminobutane:methanol (3:5 v:v, 24 mL) and refluxed for 48 h. By that time, TLC gave no evidence of the starting material. The reaction was concentrated to give a yellowish solid which was dissolved in a mixture of methanol:acetic anhydride (10:1 v:v, 11 mL) and stirred at room temperature for 16 h. The mixture was then concentrated and the residue taken up in methanol:acetic acid (4:1, 10 mL). To the crude mixture was then added 10% Pd/C (100 mg) and the mixture stirred at room temperature for 48 h under hydrogen atmosphere (1 atm). The reaction mixture was then filtered over celite and washed with methanol (2 x 25 mL).

The combined washings were concentrated to dryness to obtain the title compound as a white solid (47.0 mg, 67%); ^{1}H NMR (D_2O, 250 MHz): δ 4.70 (under H_2O peak, H-1'), 4.48 (d, 1H, J = 7.9 Hz, H-1), 4.38 (d, 1H, J = 7.5 Hz, H-1''), 4.12 (d, 1H, J = 3.3 Hz), 3.22 (m, one OCH_2CH_2), 3.02 (m, 2H, CH_2NH_2), 2.02 (s, 3H, CH_3CONH), 1.72-1.57 (m, 4H, OCH_2CH_2, CH_2CH_2), 1.49-1.43 (m, 2H, $O(CH_2)_2CH_2$)

5.3.81 Sodium 5-azidopentyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3-*O*-acetyl-2-deoxy-2-phthalimido-6-*O*-sulfonate-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzyl-β-D-galactopyranoside (91)

Sulfur trioxide/Trimethylamine complex (49 mg, 0.356 mmol) was added to a stirred solution of **89** (110 mg, .089 mmol) in dry DMF (5 mL) at 60°C. The mixture stirred at 60°C for 16 h until the starting material had been consumed and a new spot (R_f = 0.30) appeared on TLC [dichloromethane:methanol (9:1)]. The reaction mixture was then concentrated under reduced pressure, taken up in methanol (20 mL), and stirred with ion exchange resin (IR-120) for 20 min. The mixture was then filtered, concentrated and fractionated by flash chromatography dichloromethane:methanol (9:1) as eluent, to give the title compound as a colorless syrup (95.4 mg, 80%): $[\alpha]^{25}_D$ -6.9° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, methanol-d_4) δ 8.05-7.05 (m, 26H, Ar-H), 5.72 (m, 2H, H-1'', H-3'), 5.37 (d, 1H, $J_{3'',4''}$ = 3.2 Hz, H-4''), 5.16, (m, 2H, H-1', H-2''), 5.08-5.01 (m, 2H, H-3'', one OCH_2Ph), 4.77 (d, 1H, J_{AB} = -12.0 Hz, one OCH_2Ph), 4.62-4.46 (m, 4H, OCH_2Ph, one OCH_2Ph, one OCH_2Ph), 4.41 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1), 4.36-4.18 (m, 6H), 4.12 (m, 2H), 4.04 (t, 1H, $J_{3,4}$ = 9.7 Hz, H-4'), 3.96-3.88 (m, 2H), 3.82 (broad m, 1H), 3.72 (m, 1H, one OCH_2CH_2), 3.68-3.52 (m, 5H), 3.45 (m, 1H), 3.32

(m, 1H, one OC*H*$_2$CH$_2$), 3.18 (t, 2H, J = 7.0 Hz, CH$_2$N$_3$), 2.15, 2.04, 1.98, 1.90, 1.86 (5s, 15H, 5 x C*H*$_3$CO), 1.48-1.35 (m, 4H, OCH$_2$C*H*$_2$CH$_2$C*H*$_2$), 1.25-1.16 (m, 2H, O(C*H*$_2$)$_2$CH$_2$); ^{13}C NMR δ 173.2, 173.1 (2 x N*C*O), 171.3, 171.3, 171.2, 170.9, 170.8 (5 x CH$_3$*C*O), 141.2, 140.4, 140.3 (3 x Bn-ipso C), 130.6-124.6 (Ar-C), 103.9 (C-1), 101.8 (C-1''), 100.6 (C-1'), 83.5, 79.9, 78.4, 78.4, 77.6, 76.1, 74.9, 74.3 (3 x O*C*H$_2$Ph), 75.4, 75.1, 74.9, 72.7 (C-3'), 72.5 (C-2''), 71.7 (C-6), 70.9 (C-3''), 70.3 (O*C*H$_2$(CH$_2$)$_4$), 68.9 (C-4''), 66.1 (C-6'), 62.5 (C-6''), 57.0 (C-2'), 52.4 (*C*H$_2$N$_3$), 30.6 (OCH$_2$*C*H$_2$), 29.8 (*C*H$_2$CH$_2$N$_3$), 24.6 (OCH$_2$CH$_2$*C*H$_2$), 21.7, 21.4, 21.4, 21.3, 21.3, (5 x *C*H$_3$CO).

5.3.82 Sodium 5-aminopentyl β-D-galactopyranosyl-(1→4)-2-deoxy-2-acetamido-6-*O*-sulfonate-β-D-glucopyranosyl-(1→3)-β-D-galactopyranoside (92)

Compound **91** (0.090 g, 0.068 mmol) was refluxed in a mixture of 1-aminobutane:methanol (3:5 v:v, 24 mL) for 48 h. By that time, TLC gave no evidence of the starting material. The reaction was concentrated to give a yellowish solid which was dissolved in a mixture of methanol:acetic anhydride (10:1 v:v, 11 mL) and stirred at room temperature for 24 h. The solution was concentrated and the residue taken up in methanol:acetic acid (4:1, 10 mL). To the solution was then added 10% Pd/C (50 mg) and the mixture stirred at room temperature for 48 h under hydrogen atmosphere (1 atm). The reaction mixture was then filtered over celite and washed with methanol (2 x 25 mL). The combined washings were concentrated to dryness to obtain the title compound (**92**) as a white solid (23.5 mg, 48%); ^{1}H NMR (400 MHz, water-d$_2$) δ 4.70 (under H$_2$O peak, H-1'), 4.53 (d, 1H, J = 7.3 Hz, H-1''), 4.47 (d, 2H, J = 7.5 Hz, H-1), 4.08, (t, 1H, J = 6.3 Hz), 3.95 (d, J

= 3.1 Hz), 3.17 (t, 2H, J = 7.1 Hz, C*H*$_2$NH$_2$), 1.98 (s, 3H, C*H*$_3$CONH), 1.72-1.57 (m, 4H, OCH$_2$C*H*$_2$ CH$_2$C*H*$_2$), 1.49-1.43 (m, 2H, O(CH$_2$)$_2$C*H*$_2$); ^{13}C NMR (H_2O-d_2): δ 174.8 (Me*C*ONH), 102.8 (C-1''), 102.8 (C-1), 102.0 (C-1'), 82.7, 77.9, 72.7, 72.6, 71.7 (OC*H*$_2$CH$_2$), 71.2, 69.9, 68.8, 66.7 (C-6') 61.2, 60.6 (C-6''), (C-6), 55.4 (C-2'), 39.3 (*C*H$_2$NH$_2$), 28.6 (OCH$_2$*C*H$_2$), 28.3 (*C*H$_2$CH$_2$NH$_2$), 22.3 (*C*H$_3$CONH), 21.2 (OCH$_2$CH$_2$*C*H$_2$); FABMS m/z: 733 (M^+); HRMS (FABMS) calcd for $C_{25}H_{45}N_2O_{19}S$ + Na: calcd 733.2312. Found, 733.5511.

6. References

(1) Alexander, N. J. *Reprod. Fert. Dev.* **1994**, *6*, 417-419.

(2) Alexander, N. J.; Bialy, G. *Reprod. Fert. Dev.* **1994**, *6*, 273-280.

(3) Aldhous, P. *Science* **1994**, *266*, 1484-1486.

(4) Dell'Omo, D.; Palmery, M. *Contraception* **2002**, *65*, 273-275.

(5) Tuyttens, F. A. M.; MacDonald, D. W. *Animal Welfare* **2002**, *7*, 339-364.

(6) Anderson, I.; Nowak, R. *New Scientist* **1997**, *153*, 34-37.

(7) Turner, J. W.; Liu, I. K. M.; Flanagan, D. R.; Bynum, K. S.; Rutberg, A. T. *Reproduction* **2002**, *60*, 177-186.

(8) Naugle, R. E.; Rutberg, A. T.; Underwood, H. B.; Turner, J. W.; Liu, I. K. M. *Reproduction* **2002**, 143-153.

(9) Barr, J. J. F.; Lurz, P. W. W.; Shirley, M. D. F.; Rushton, S. P. *Environ. Manag.* **2002**, *30*, 342-351.

(10) Delsink, A. K.; van Altena, J. J.; Kirkpatrick, J.; Grobler, D.; Fayrer-Hosken, R. A. *Reproduction* **2002**, *60*, 117-124.

(11) Hammill, M. O.; Stenson, G. B.; Myers, R. A.; Stobo, W. T. *Can. J. Fish. Aquat. Sci.* **1998**, *55*, 423-430.

(12) Eldridge, D. J.; Myers, C. A. *J. Arid. Envir.* **2001**, *47*, 325-337.

(13) Bell, A. C.; Byrne, P. M.; Watson, S. *Ann. Appl. Biology* **1998**, *133*, 431-442.

(14) Kovaliski, J. *J. Wildlife Dis.* **1998**, *34*, 421-428.

(15) Hone, J. *Biological Conserv.* **2002**, *105*, 231-242.

(16) Boulton, W. J.; Freeland, W. J. *Wildlife Res.* **1991**, *18*, 63-73.

(17) Choquenot, D. *J. Mammology* **1990**, *71*, 151-155.

(18) Pople, A. R.; Grigg, G. C.; Cairns, S. C.; Alexander, P.; Beard, L. A.; Henzell, R. P. *Wildlife Res.* **1996**, *23*, 687-696.

(19) Bayne, P.; Harden, B.; Pines, K.; Taylor, U. *Wildlife Res.* **2000**, *27*, 517-523.

(20) Lesage, V.; Hammill, M. O. *Can. Field-Nat.* **2001**, *115*, 653-662.

(21) Stobo, W. T.; Fannning, L. P.; Beck, B.; Fowler, G. M. *Can. J. Zoology* **2002**, *80*, 442-449.

(22) Wassarman, P. M. *Theriogenology* **1994**, *41*, 31-44.

(23) Epifano, O.; Dean, J. *Reprod. Fert. Dev.* **1994**, *6*, 319-330.

(24) Hardy, D. M.; Garbers, D. L. *J. Biol. Chem.* **1994**, *269*, 19000-19004.

(25) Brown, R. G.; Bowen, W. D.; Eddington, J. D.; Kimmins, W. C.; Mezei, M.; Parsons, J. L.; Pohajdak, B. *J. Reprod. Immun.* **1997**, *35*, 43-51.

(26) Brown, R. G.; Kimmins, W. C.; Mezei, M.; Parsons, J.; Pohajdak, B.; Bowen, W. D. *Nature* **1996**, *379*, 30-31.

(27) Gorman, S. P.; Levy, J. K.; Hampton, A. L.; Collante, W. R.; Harris, A. L.; Brown, R. G. *Theriogenology* **2002**, *58*, 135-149.

(28) Harris, J. D.; Hibler, D. W.; Fontenot, G. K.; Hsu, K. T.; Yurewicz, E. C.; Sacco, A. G. *J. Sequence. Mapping* **1995**, *4*, 361-393.

(29) Hasegawa, A.; Tsubamoto, H.; Hamada, Y.; Koyama, K. *Amer. J. Reprod. Immun.* **2000**, *44*, 59-64.

(30) Binkley, R. W. Definitions; In *Modern Carbohydrate Synthesis*; Fennema, O. R., Sanderson, G. W., Walstra, P., Karel, M., Tannenmaum, S. R., Whitaker, J. R., eds. Marcell Dekker, Inc.: New York, 1988; pp 7-12.

(31) Greenwell, P. *Glycoconjugate J.* **1997**, *14*, 159-173.

(32) Maegawa, M.; Kamada, M.; Yamamoto, S.; Yamano, S.; Irahara, M.; Kido, H.; Aono, T. *J. Reprod. Immun.* **2002**, *53*, 79-89.

(33) Dunbar, B. S.; Timmons, T. M.; Skinner, S. M.; Prasad, S. V. *Biol. Reprod.* **2001**, *65*, 951-960.

(34) Kuby, J. Antigen-Antibody Interactions; In *Immunology*; W.H. Freeman and Company: New York, 1994; pp 135-154.

(35) Kuby, J. Antigen Processing and Presentation; In *Immunology*; W.H. Freeman and Company: New York, 1996; pp 239-250.

(36) Kuby, J. T-Cell Activation, Maturation, and Differentiation; In *Immunology*; W.H. Freeman and Company: New York, 1996; pp 274-295.

(37) Kuby, J. Generation of the Humoral Immune Response; In *Immunology*; W.H. Freeman and Company: New York, 1996; pp 324-345.

(38) Primakoff, P.; Myles, D. G. *Science* **2002**, *296*, 2183-2185.

(39) Wassarman, P. M.; Litscher, E. S. *Cell Tiss. & Org.* **2001**, *168*, 36-45.

(40) Abou-Haila, A.; Tulsiani, D. R. P. *Arch. Biochem. Biophys.* **2000**, *379*, 173-182.

(41) Wassarman, P. M. *Mount Sinai J. Med.* **2002**, *69*, 148-155.

(42) Nixon B.; Lu, Q. X.; Wassler, M. J.; Foote, C. I.; Ensslin, M. A.; Shur, B. D. *Cell Tiss. & Org.* **2001**, *168*, 46-57.

(43) Shi, X. D.; Amindari, S.; Paruchuru, K.; Skalla, D.; Burkin, H.; Shur, B. D.; Miller, D. J. *Development* **2001**, *128*, 645-654.

(44) Aitken, R. J.; Paterson, M.; Van Duin, M. *Amer. J. Reprod. Immun.* **1996**, *35*, 175-180.

(45) Skinner, S. M.; Prasad, S. V.; Ndolo, T. M.; Dunbar, B. S. *Amer. J. Reprod. Immun.* **1996**, *35*, 163-174.

(46) Turner, J. W.; Liu, I. K. M.; Flanagan, D. R.; Rutberg, A. T.; Kirkpatrick, J. F. *J. Wildlife Management* **2001**, *65*, 235-241.

(47) Miller, L. A.; Crane, K.; Gaddis, S.; Killian, G. J. *J. Wildlife Management* **2001**, *65*, 941-945.

(48) de Kretser, D. *Reprod. Fert. Dev.* **1994**, *6*, 357-368.

(49) Epifano, O.; Liang, L.-F.; Familari, M.; Moos, M. C., Jr.; Dean, J. *Development* **1995**, *121*, 1947-1956.

(50) Cooper, D. W.; Herbert, C. A. *Reprod. Fert. Dev.* **2002**, *13* , 451-458.

(51) Srivastava, N.; Santanham, R.; Sheela, P.; Mukund, S.; Thakral, S. S.; Malik, B. S.; Gupta, S. K. *Reproduction* **2002**, *123*, 847-857.

(52) Koyama, K.; Hasegawa, A.; Gupta, S. K. *Amer. J. Reprod. Immun.* **2002**, *47*, 303-310.

(53) Parks, C. L.; Wang, H. P.; Kovacs, G. R.; Vasilakis, N.; Kowalski, J.; Nowak, R. M.; Lerch, W. A.; Walpita, P.; Sidhu, M. S.; Udem, S. A. *Virus Res.* **2002**, *83*, 131-147.

(54) Zhang, G. G.; Rodrigues, L.; Rovinski, B.; White, K. A. *Molec. Biotech.* **2002**, *20*, 131-136.

(55) Pech, R.; Hood, G. M.; McIlroy, J.; Saunders, G. *Reprod. Fert. Dev.* **1997**, *9*, 41-50.

(56) Bradley, M. P.; Hinds, L. A.; Bird, P. H. *Reprod. Fert. Dev.* **1997**, *9*, 111-116.

(57) DeNicola, A. J.; Kesler, D. J.; Swihart, R. K. *Wildlife Society Bulletin* **1996**, *24*, 301-305.

(58) Easton, R. L.; Patankar, M. S.; Lattanzio, F. A.; Leaven, T. H.; Morris, H. R.; Clark, G. F.; Dell, A. *J. Biol. Chem.* **2000**, *275*, 7731-7742.

(59) Schachter, H.; Brockhausen, I. The biosynthesis of Serine (Threonine)-*N*-acetylgalactosamine-linked carbohydrate moieties; In *Glycoconjugates Composition, Structure, and Function*; Allen, H. J., Kisailus, E. C., eds. Marcel Dekker Inc.: New York, 1992; pp 263-332.

(60) Mori, E.; Takasaki, S.; Hedrick, J. L.; Wardrip, N. J.; Mori, T.; Kobata, A. *Biochemistry* **1991**, *30*, 2078-2087.

(61) Cummings, R. D. Synthesis of Asparagine-linked oligosaccharides: pathways, genetics, and metabolic regulation; In *Glycoconjugates Composition, Structure, and Function*; Allen, H. J., Kisailus, E. C., eds. Marcel Dekker Inc.: New York, 1992; pp 333-360.

(62) Bleil, J. D.; Wassarman, P. M. *Proc. Nat. Acad. Sci.* **1988**, *85*, 6778-6782.

(63) Litscher, E. S.; Juntunen, K.; Seppo, A.; Penttilä, L.; Niemelä, R.; Renkonen, O.; Wassarman, P. M. *Biochemistry* **1995**, *34*, 4662-4669.

(64) Wassarman, P. M. *Development* **1990**, *108*, 1-17.

(65) Florman, H. M.; Wassarman, P. M. *Cell* **1985**, *41*, 313-324.

(66) Bleil, J. D.; Wassarman, P. M. *Proc. Nat. Acad. Sci.* **1990**, *87*, 5563-5567.

(67) Yurewicz, E. C.; Pack, B. A.; Sacco, A. G. *Mol. Reprod. Dev.* **1991**, *30*, 126-134.

(68) Noguchi, S.; Hatanaka, Y.; Tobita, T.; Nakano, M. *Eur. J. Biochem.* **1992**, *204*, 1089-1100.

(69) Benoff, S. *Mol. Human Reprod.* **1997**, *3*, 599-637.

(70) Shalgi, R.; Raz, T. *Histol. Histopathol.* **1997**, *12*, 813-822.

(71) Dell, A.; Morris, H. R.; Easton, R. L.; Patankar, M.; Clark, G. F. *Biochim. Biophys. Acta Gen.* **1999**, *1473*, 196-205.

(72) Avilés, M.; Okinaga, T.; Shur, B. D.; Ballesta, J. *Mol. Reprod. Dev.* **2000**, *57*, 296-308.

(73) Nagdas, S. K.; Araki, Y.; Chayko, C. A.; Orgebin-Crist, M. C.; Tulsiani, D. R. P. *Biol. Reprod.* **1994**, *51*, 262-272.

(74) Howes, L.; Jones, R. *J. Reprod. Immun.* **2002**, *53*, 181-192.

(75) Kerr, C. L.; Hanna, W. F.; Shaper, J. H.; Wright, W. W. *Biol. Reprod.* **2002**, *66*, 1585-1595.

(76) Liu, C.; Litscher, E. S.; Wassarman, P. M. *J. Cell Science* **1997**, *110*, 745-752.

(77) Chen, J.; Litscher, E. S.; Wassarman, P. M. *Proc. Nat. Acad. Sci.* **1998**, *95*, 6193-6197.

(78) Thall, A. D.; Maly, P.; Lowe, J. B. *J. Biol. Chem.* **1995**, *270*, 21437-21440.

(79) Avilés, M.; Jaber, L.; Castells, M. T.; Ballesta, J.; Kan, F. W. K. *Biol. Reprod.* **1997**, *57*, 1155-1163.

(80) Miller, D. J.; Macek, M. B.; Shur, B. D. *Nature* **1992**, *357*, 589-593.

(81) Johnston, D. S.; Wright, W. W.; Shaper, J. H.; Hokke, C. H.; Van den Eijnden, D. H.; Joziasse, D. H. *J. Biol. Chem.* **1998**, *273*, 1888-1895.

(82) Urch, U. A.; Patel, H. *Development* **1991**, *111*, 1165-1172.

(83) Jones, R. *Development* **1991**, *111*, 1155-1163.

(84) Powell, L. D.; Sgroi, D.; Sjoberg, E. R.; Stamenkovic, I.; Varki, A. *J. Biol. Chem.* **1993**, *268*, 7019-7027.

(85) Takasaki, S.; Mori, E.; Mori, T. *Biochim. Biophys. Acta Gen.* **1999**, *1473*, 206-215.

(86) Cohen, N.; Wassarman, P. M. *Int. J. Dev. Biol.* **2002**, *45*, 569-576.

(87) Lu, Q. X.; Shur, B. D. *Development* **1997**, *124*, 4121-4131.

(88) Shur, B. D. *Biochem. Biophys. Res. Comm.* **1998**, *250*, 537-543.

(89) Dunbar, B. S.; Liu, C.; Sammons, D. W. *Biol. Reprod.* **1981**, *24*, 1111-1124.

(90) Hasegawa, A.; Yamasaki, N.; Inoue, M.; Koyama, K.; Isojima, S. *J. Reprod. Fert.* **1995**, *105*, 295-302.

(91) Töpfer-Petersen, E.; Calvete, J. J. *Int. J. Andrology* **1995**, *18*, 20-26.

(92) Noguchi, S.; Nakano, M. *Eur. J. Biochem.* **1992**, *209*, 883-894.

(93) Yonezawa, N.; Aoki, H.; Hatanaka, Y.; Nakano, M. *Eur. J. Biochem.* **1995**, *233*, 35-41.

(94) Mori, E.; Hedrick, J. L.; Wardrip, N. J.; Mori, T.; Takasaki, S. *Glycoconjugate J.* **1998**, *15*, 447-456.

(95) Nakano, M.; Yonezawa, N.; Hatanaka, Y.; Noguchi, S. *J. Reprod. Fert. supplemen* **1996** , *50*, 25-34.

(96) Nakano, M.; Yonezawa, N. *Cell Tiss. & Org.* **2001**, *168*, 65-75.

(97) Hokke, C. H.; Damm, J. B. L.; Penninkhof, B.; Aitken, R. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Eur. J. Biochem.* **1994**, *221*, 491-512.

(98) Spijker, N. M.; Keuning, C. A.; Hooglugt, M.; Veeneman, G. H.; van Boeckel, C. A. A. *Tetrahedron* **1996**, *52*, 5945-5960.

(99) Jansen, S.; Jones, R.; Jenneckens, I.; Marschall, B.; Kriegsmann, B.; Coadwell, J.; Brenig, B. *Mol. Reprod. Dev.* **1998**, *51*, 184-192.

(100) Moreno, R. D.; Barros, C. *Biol. Reprod.* **2000**, *62*, 1536-1542.

(101) Jansen, S.; Quigley, M.; Reik, W.; Jones, R. *Int. J. Dev. Biol.* **1995**, *39*, 501-510.

(102) Urch, U. A. In *Elements of Mammalian Fertilization*; CRC Press: Boca Raton, 1991; pp 233-248.

(103) Töpfer-Petersen, E.; Calvete, J. J.; Sanz, L.; Sinowatz, F. *Andrologia* **1995**, *27*, 303-324.

(104) Carmona, E.; Weerachatyanukul, W.; Soboloff, T.; Fluharty, A. L.; White, D.; Promdee, L.; Ekker, M.; Berger, T.; Buhr, M.; Tanphaichitr, N. *Dev. Biol.* **2002**, *247*, 182-196.

(105) Tantibhedhyangkul, J.; Weerachatyanukul, W.; Carmona, E.; Xu, H. B.; Anupriwan, A.; Michaud, D.; Tanphaichitr, N. *Biol. Reprod.* **2002**, *67* , 212-219.

(106) Lea, I. A.; Widgren, E. E.; O'Rand, M. G. *Vaccine* **2002**, *20*, 1515-1523.

(107) Sivaparapu, N.; Upadhyay, A.; Hasegawa, A.; Koyama, K.; Gupta, S. K. *J. Reprod. Immun.* **2002**, *56*, 77-91.

(108) Hasegawa, A.; Hamada, Y.; Shigeta, M.; Koyama, K. *J. Reprod. Immun.* **2002**, *53*, 91-98.

(109) Hardy, C. M.; ten Have, J. F. M.; Mobbs, K. J.; Hinds, L. A. *Reprod. Fert. Dev.* **2002**, *14*, 151-155.

(110) Paterson, M.; Jennings, Z. A.; Wilson, M. R.; Aitken, R. J. *J. Reprod. Immun.* **2002** , *53*, 99-107.

(111) Paterson, M.; Koothan, P. T.; Morris, K. D.; O'Byrne, K. T.; Braude, P.; Williams, A.; Aitken, R. J. *Biol. Reprod.* **1992**, *46*, 523-534.

(112) Rath, A.; Choudhury, S.; Hasegawa, A.; Koyama, K.; Gupta, S. K. *Mol. Reprod. Dev.* **2002**, *62*, 525-533.

(113) Li, D. J.; Sun, X. X.; Li, C. J.; Cai, L. R.; Meng, Y. *J. Reprod. Immun.* **2002**, *54*, 81-92.

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

(115) Igarashi, K. *Adv. Carbohydr. Chem. Biochem.* **1977**, *34*, 243-283.

(116) Schmidt, R. R. *Angew. Chem. Int. Ed. Eng.* **1986**, *25*, 212-235.

(117) Nicolaou, K. C.; Caulfield, T. J.; Groneberg, X. *Pure Appl. Chem.* **1991**, *63*, 555-560.

(118) Sinaÿ, P. *Pure Appl. Chem.* **1991**, *63*, 519-528.

(119) Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503-1531.

(120) Vasella, A. *Pure Appl. Chem.* **1993**, *65*, 731-752.

(121) Boons, G.-J. *Tetrahedron* **1996**, *52*, 1095-1121.

(122) Haynes, L. J.; Newth, F. H. *Adv. Carbohydr. Chem.* **1955**, *10*, 207-256.

(123) Garegg, P. J. *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 179-200.

(124) Schmidt, R. R.; Machel, J. *Tetrahedron Lett.* **1984**, *25*, 821-824.

(125) Fraser-Reid, B.; Konradsson, P.; Mootoo, D. R.; Udodong, U. E. *J. Chem. Soc. ,Chem. Commun.* **1988**, 823-825.

(126) Halcomb, R. L.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6661-6666.

(127) Danishefsky, S. J.; Bilodeau, M. T. *Angew. Chem. Int. Ed. Eng.* **1996**, *35*, 1380-1419.

(128) Norberg, T. Glycosylation properties and reactivity of thioglycosides, sulfoxides, and other *S*-glycosides: current scope and future prospects; In *Modern Methods in Carbohydrate Synthesis*; Khan, S. H., O'Neill, R. A., eds. Harwood Academic Publishers: Amsterdam, 1995; pp 82-106.

(129) Plante, O. J.; Palmacci, E. R.; Andrade, R. B.; Seeberger, P. H. *J. Am. Chem. Soc.* **2001**, *123*, 9545-9554.

(130) Palmacci, E. R.; Plante, O. J.; Seeberger, P. H. *Eur. J. Org. Chem.* **2002**, 595-606.

(131) Ratner, D. M.; Plante, O. J.; Seeberger, P. H. *Eur. J. Org. Chem.* **2002**, 826-833.

(132) Pinto, B. M.; Mehta, S. *J. Org. Chem.* **1993**, *58*, 3269-3276.

(133) Fraser-Reid, B.; Udodong, U. E.; Wu, Z.; Ottosson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R. *Synlett* **1992**, *12*, 927-942.

(134) Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer-Verlag: New York, 1983; pp 1-49.

(135) Evans, M. E. *Carbohydr. Res.* **1972**, *21*, 473-475.

(136) van Heeswijk, W. A. R.; Goedhart, J. B.; Vliegenthart, J. F. G. *Carbohydr. Res.* **1977**, *58*, 337.

(137) Gigg, J.; Gigg, R. *J. Chem. Soc. C* **1966**, 82-86.

(138) Fletcher, H. G. Formation of Benzyl Ethers with Benzyl Chloride and Powdered KOH at 130-140°; In *Methods in Carbohydrate Chemistry Vol. II*; Whistler, R. L., Wolfrom, M. L., eds. Academic Press: New York, 1963; pp 166-167.

(139) Nakajima, N.; Abe, R.; Yonemitsu, O. *Chem. Pharm. Bull.* **1988**, *36*, 4244.

(140) Xia, J.; Alderfer, J. L.; Piskorz, C. F.; Matta, K. L. *Chem. Eur. J.* **2001**, *7*, 356-367.

(141) Franke, F.; Guthrie, R. D. *Austral. J. Chem.* **1977**, *30*, 639-647.

(142) Hanessian, S.; Lavallee, P. *Can. J. Chem.* **1975**, *53*, 2975-2977.

(143) Jarowicki, K.; Kocienski, P. *J. Chem. Soc. , Perkin Trans. 1* **2000**, 2495-2527.

(144) Tomic-Kulenovic, S.; Keglevic, D. *Carbohydr. Res.* **1980**, *85*, 302-306.

(145) Lemieux, R. U.; Takeda, T.; Chung, B. Y. Synthesis of 2-Amino-2-deoxy-β-D-glucopyranosides; In *Synthetic methods for carbohydrates*; El Khadem, H. S., ed. American Chemical Society: Washington, D.C., 1976; pp 90-115.

(146) Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett.* **1995**, *36*, 5343-5436.

(147) Corey, E. J.; Gras, J.-L.; Ulrich, P. *Tetrahedron Lett.* **1976**, 809-812.

(148) Buskas, T.; Garegg, P. J.; Konradsson, P.; Maloisel, J.-L. *Tetrahedron: Asymmetry* **1994**, *5*, 2187-2194.

(149) Martín-Lomas, M.; Flores-Mosquera, M.; Chiara, J. L. *Eur. J. Org. Chem.* **2000**, 1547-1562.

(150) Mootoo, D. R.; Konradsson, P.; Udodong, U.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988** , *110*, 5583-5584.

(151) Fraser-Reid, B.; Wu, Z.; Udodong, U. E.; Ottosson, H. *J. Org. Chem.* **1990**, *55*, 6068-6070.

(152) Tsuchida, T.; Yoshida, M.; Kurokawa, K.; Nakai, Y.; Achiha, T.; Kiyoi, T.; Kondo, H. *J. Org. Chem* **1997**, *62*, 8676-6881.

(153) Yoshida, M.; Kiyoi, T.; Tsukida, T.; Kondo, H. *J. Carbohydr. Chem.* **1998**, *17*, 673-681.

(154) Geurtsen, R.; Holmes, D. S.; Boons, G.-J. *J. Org. Chem* **1997**, *62*, 8145-8154.

(155) Douglas, N. L.; Ley, S. V.; Lücking, U.; Warriner, S. L. *J. Chem. Soc. , Perkin Trans. 1* **1998**, 51-65.

(156) Cheung, M.-K.; Douglas, N. L.; Hinzen, B.; Ley, S. V.; Pannecoucke, X. *Synlett* **1997**, *3*, 257-260.

(157) Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734-753.

(158) Koeller, K. M.; Wong, C.-H. *Chem. Rev.* **2000**, *100*, 4465-4493.

(159) Choudhury, A. K.; Mukherjee, I.; Mukhopadhyay, B.; Roy, N. *J. Carbohydr. Chem.* **1999**, *18*, 361-367.

(160) Roy, R.; Andersson, F. O.; Letellier, M. *Tetrahedron Lett.* **1992**, *33*, 6053-6056.

(161) Sliedregt, L. A. J. M.; Zegelaar-Jaarsveld, K.; van der Marel, G. A.; van Boom, J. H. *Synlett* **1993**, 335-337.

(162) Cao, S.; Hernandez-Mateo, F.; Roy, R. *J. Carbohydr. Chem.* **1998**, *17*, 609-631.

(163) Kanie, O.; Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1994**, *116*, 12073-12074.

(164) Paulsen, H. *Angew. Chem. Int. Ed. Eng.* **1995**, *34*, 1432-1434.

(165) Kanie, O.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1996**, *37* , 4551-4554.

(166) Yu, H.; Yu, B.; Wu, X.; Hui, Y.; Han, X. *J. Chem. Soc. ,Perkin Trans. 1* **2000**, *9*, 1445-1453.

(167) Poszgay, V.; Jennings, H. J. *J. Org. Chem.* **1988**, *53*, 4042-4052.

(168) Nicolaou, K. C.; Ueno, H. Oligosaccharide synthesis fron Glycosyl Fluorides and Sulfides; In *Preparative Carbohydrate Chemistry*; Hanessian, S., ed. Marcel Dekker: New York, 1997; pp 313-336.

(169) Safatli, M. Synthesis of Neutral and Acidic β-O-linked Glycoconjugates Related to Those Found on the Core Structure of Mammalian Zomna Pellucida. 1998. Dalhousie University.
Ref Type: Thesis/Dissertation

(170) Bock, K.; Pedersen, C. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27-66.

(171) Hall, L. D. *Adv. Carbohydr. Chem.* **1964**, *19*, 51-93.

(172) Bock, K.; Thøgersen, H. *Ann. Rep. NMR Spectrosc.* **1982**, *13*, 1-57.

(173) Ferrier, R. J.; Hay, R. W.; Vethaviyasar, N. *Carbohydr. Res.* **1973**, *27*, 55-61.

(174) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331-1334.

(175) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313-4316.

(176) Basu, S.; Pal, J. N. *Carbohydr. Res.* **1990**, *208*, 241-245.

(177) Lönn, H. *Chem. Commun. Univ. Stockholm* **1984**, 1.

(178) Medgyes, A.; Farkas, E.; Lipták, A.; Pozsgay, V. *Tetrahedron* **1997**, *53*, 4159-4178.

(179) Houdier, S.; Vottero, P. J. A. *Carbohydr. Res.* **1992**, *232* , 349-352.

(180) Fügedi, P.; Garegg, P. J. *Carbohydr. Res.* **1986**, *149*, C9-C12.

(181) Hasegawa, A.; Ando, T.; Kato, M.; Ishida, H.; Kiso, M. *Carbohydr. Res.* **1994**, *257*, 67-80.

(182) Pozsgay, V.; Jennings, H. J. *J. Carbohydr. Chem.* **1990**, *9* , 333-343.

(183) Koenigs, W.; Knorr, E. *Chem. Ber.* **1901**, *34*, 957-981.

(184) Crich, D.; Sun, S. X. *J. Am. Chem. Soc.* **1997**, *119*, 11217-11223.

(185) Ferrier, R. J.; Furneaux, R. H. 1,2-*trans*-1-Thioglycosides; In *Methods in Carbohydrate Chemistry*; Whistler, R. L., Wolfrom, M. L., eds. Academic Press: New York, 1980; pp 251-253.

(186) Shimadate, T.; Chiba, S.; Inouye, K.; IIno, T.; Hosoyama, Y. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3552-3554.

(187) Ogawa, T.; Nakabayashi, S.; Sasajima, K. *Carbohydr. Res.* **1981**, *95*, 308-312.

(188) Nicolaou, K. C.; Seitz, S. P.; Papahatjis, D. P. *J. Am. Chem. Soc.* **1983**, *105*, 2430-2434.

(189) Page, P. C. B.; Graham, A. E.; Bethell, D.; Park, B. K. *Syn. Commun.* **1993**, *23*, 1507-1514.

(190) Yan, L.; Kahne, D. *J. Am. Chem. Soc.* **1996**, *118*, 9239-9248.

(191) Nishihata, K.; Nishio, M. *Tetrahedron Lett.* **1977**, *12*, 1041-1044.

(192) Nishihata, K.; Nishio, M. *J. Chem. Soc. ,Perkin Trans. 2* **1973**, 758-762.

(193) Grindley, T. B. Applications of Stannyl Ethers and Stannylene Acetals to Oligosaccharide Synthesis; In *Synthetic Oligosaccharides: Indispensable Probes for the Life Sciences*; Kovác, P., ed. ACS: Washington, 1994; pp 51-76.

(194) François, G. *Tetrahedron* **1997**, *53*, 13509-13556.

(195) David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643-663.

(196) Grindley, T. B. *Adv. Carbohydr. Chem. Biochem.* **1998**, *53*, 17-142.

(197) Wuts, P. G. M.; Greene, T. W. *Protective Groups in Organic Synthesis*; Wiley: New York, 1999.

(198) Tate, M. E.; Bishop, C. T. *Can. J. Chem.* **1963**, *41*, 1801-1806.

(199) Grice, P.; Ley, S. V.; Pietruszka, J.; Priepke, H. W. M.; Warriner, S. L. *J. Chem. Soc. , Perkin Trans. 1* **1997**, 351-363.

(200) Neises, B.; Steglich, W. *Agnew. Chem. Int. Ed. Engl.* **1978**, *17*, 522-524.

(201) Bergeron, R. J.; Kline, S. J.; Stolowich, N. J.; McGovern, K. A.; Burton, P. S. *J. Org. Chem.* **1981**, *46*, 4524-4529.

(202) Wolfrom, M. L.; Thompson, A. Acetylation; In *Methods in Carbohydrate Chemistry Vol. II*; Whistler, R. L., Wolfrom, M. L., eds. Academic Press: New York, 1963; pp 211-215.

(203) Janaki, N.; Patil, J. R.; Bose, J. L. *Ind. J. Chem.* **1969**, *7*, 227-228.

(204) Zuurmond, H. M.; van der Klein, P. A. M.; de Wildt, J.; van der Marel, G. A.; van Boom, J. H. *J. Carbohydr. Chem.* **1994**, *13*, 323-339.

(205) Malet, C.; Hindsgaul, O. *Carbohydr. Res.* **1997**, *303*, 51-65.

(206) Gallo, C.; Jeroncic, L. O.; Varela, O.; De Lederkremer, R. M. *J. Carbohydr. Chem.* **1993**, *12*, 841-851.

(207) Binkley, R. W. *J. Carbohydr. Chem.* **1990**, *9*, 507-511.

(208) Giuliano, R. M.; Villani, F. J. *J. Org. Chem.* **1995**, *60*, 202-211.

(209) Maryanoff, B. E.; Costanzo, M. J.; Nortey, S. O.; Greco, M. N.; Shank, R. P.; Schupsky, J. J.; Ortegon, M. P.; Vaught, J. L. *J. Med. Chem.* **1998**, *41*, 1315-1343.

(210) Binkley, R. W.; Hehemann, D. G. *J. Org. Chem.* **1990**, *55*, 378-380.

(211) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry 3rd Edition - Part B: Reactions and Synthesis*; Plenum Press: New York, New York, 1990.

(212) Held, G.; Bessent, M. P.; Titmuss, S.; King, D. A. *J. Chem. Phys.* **1996**, *105*, 11305-11312.

(213) Lin, R. F.; Koestner, R. J.; Van Hove, M. A.; Somorjai, G. A. *Surface. Sci.* **1983**, *134*, 161-183.

(214) Gaunt, M. J.; Yu, J. Q.; Spencer, J. B. *J. Org. Chem.* **1998**, *63*, 4172-4173.

(215) Riley, J. G.; Grindley, T. B. *J. Carbohydr. Chem.* **2001**, *20*, 159-169.

(216) Kocienski, P. J. *Protecting Groups*; Thieme: Stuttgart, 1994.

(217) Grindley, T. B. Protecting Groups; In *Modern Methods in Carbohydrate Synthesis*; Khan, S. H., O'Neill, R. A., eds. Harwood Academic Publishers: Amsterdam, 1995.

(218) Ziegler, T. Protecting group strategies for carbohydrates; In *Carbohydrate Chemistry*; Boons, G.-J., ed. Blackie: London, 1998; pp 21-45.

(219) McCloskey, C. M. *Adv. Carbohydr. Chem.* **1957**, *12*, 137-156.

(220) Holick, S. A.; Anderson, L. *Carbohydr. Res.* **1974**, *34*, 208-213.

(221) Hwu, J. R.; Chua, V.; Schroeder, J. E.; Barrans, R. E., Jr.; Khoudary, K. P.; Wang, N.; Wetzel, J. M. *J. Org. Chem.* **1986**, *51*, 4733-4734.

(222) Angyal, S. J.; James, K. *Carbohydr. Res.* **1970**, *12*, 147-149.

(223) Schmidt, W.; Steckhan, E. *Angew. Chem. Int. Ed. Eng.* **1979**, *18*, 801-802.

(224) Schuda, P. F.; Cichowicz, M. B.; Heimann, M. R. *Tetrahedron Lett.* **1983**, *24*, 3829-3830.

(225) Angibeauld, P.; Defaye, J.; Gadelle, A.; Utille, J.-P. *Synthesis* **1999**, 1123-1125.

(226) Akiyama, T.; Hirofuji, H.; Ozaki, S. *Tetrahedron Lett.* **1991**, *32*, 1321-1324.

(227) Hashimoto, H.; Kawa, M.; Saito, Y.; Date, T.; Horito, S.; Yoshimura, J. *Tetrahedron Lett.* **1987**, *28*, 3505-3508.

(228) Mukaiyama, T.; Murai, Y.; Shoda, S.-I. *Chem. Lett.* **1981**, 431-432.

(229) Kunz, H.; Waldmann, H. *J. Chem. Soc. Chem. Commun.* **1985**, 638-640.

(230) Hashimoto, S.; Hayashi, M.; Noyora, R. *Tetrahedron Lett.* **1984**, *25*, 1379-1382.

(231) Nicolaou, K. C.; Caulfield, T. J.; Kataoka, H.; Stylianides, N. A. *J. Am. Chem. Soc.* **1990**, *112*, 3693-3695.

(232) Collins, P. M.; Ferrier, R. J. *Monosaccharides: Their Chemistry and Their Roles in Natural Products*; John Wiley & Sons: Chichester, 1995; pp 1-574.

(233) Bernardi, A.; Raimondi, L. *J. Org. Chem.* **1995**, *60*, 3370-3377.

(234) Baker, B. R.; Joseph, J. P.; Schaub, R. E.; Williams, J. H. *J. Org. Chem.* **1954**, *19*, 1786-1792.

(235) Dasgupta, F.; Garegg, P. J. *J. Carbohydr. Chem.* **1988**, *7*, 701-707.

(236) Jain, R. K.; Matta, K. L. *Carbohydr. Res.* **1992**, *226*, 91-100.

(237) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190-6191.

(238) Boons, G.-J.; Grice, P.; Ley, S. V.; Yeung, L. L. *Tetrahedron Lett.* **1993**, *34*, 8523-8526.

(239) Nicolaou, K. C.; Bockovich, N. J.; Carcanague, D. R.; Hummel, C. W.; Even, L. F. *J. Am. Chem. Soc.* **1992**, *114*, 8701-8702.

(240) Duynstee, H. I.; de Koning, M. C.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1999**, *55*, 9881-9898.

(241) Nicolaou, K. C.; Cho, S. Y.; Hughes, R.; Winssinger, N.; Smethurst, C.; Labischinski, H.; Endermann, R. *Chemistry-A European Journal* **2001**, *7*, 3798-3823.

(242) Crich, D.; Ritchie, T. J. *Carbohydr. Res.* **1990**, *197*, 324-326.

(243) Shekhani, M. S.; Khan, K. M.; Mahmood, K. *Tetrahedron Lett.* **1988**, *29*, 6161-6162.

(244) Ferro, V.; Mocerino, M.; Stick, R. V.; Tilbrook, D. M. G. *Austral. J. Chem.* **1988**, *41*, 813-815.

(245) Wolfrom, M. L.; Diwadkar, A. B.; Gelas, J.; Horton, D. *Carbohydr. Res.* **1974**, *35*, 87-96.

(246) Gelas, J.; Horton, D. *Heterocycles* **1981**, *16*, 1587-1601.

(247) Chládek, S.; Smrt, J. *Coll. Czech. Chem. Commun.* **1963**, *28*, 1301.

(248) Debost, J.-L.; Gelas, J.; Horton, D.; Mols, O. *Carbohydr. Res.* **1984**, *125*, 329-335.

(249) Fernández-Mayoralas, A.; Marra, A.; Trumtel, M.; Veyrières, A.; Sinaÿ, P. *Carbohydr. Res.* **1989**, *188*, 81-95.

(250) Fernández-Mayoralas, A.; Marra, A.; Trumtel, M.; Veyrières, A.; Sinaÿ, P. *Tetrahedron Lett.* **1989**, *30*, 2537-2540.

(251) Evans, M. E.; Parrish, F. W.; Long, L., Jr. *Carbohydr. Res.* **1967**, *3*, 453.

(252) Patroni, J. J.; Stick, R. V.; Skelton, B. W.; White, A. H. *Austral. J. Chem.* **1988**, *41*, 91-102.

(253) Evans, M. E. *Carbohydr. Res.* **1972**, *21*, 473.

(254) Grindley, T. B.; Gulasekharam, V. *Carbohydr. Res.* **1979**, *74*, 7-30.

(255) Watts, J., Jiménez-Barbero, J., Poveda, A., and Grindley, T. B. Control of Disaccharide Formation by pi-stacking. Canadian Journal of Chemistry . 2003. Ref Type: In Press

(256) Kahne, D.; Walker, S.; Cheng, Y.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881-6882.

(257) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1998**, *120*, 435-436.

(258) Wipf, P.; Reeves, J. T. *J. Org. Chem* **2001**, *66*, 7910-7914.

(259) Kim, S.-H.; Augeri, D.; Yang, D.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 1766-1775.

(260) Yan, L.; Taylor, C. M.; Goodnow, R., Jr.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 6953-6954.

(261) Sliedregt, L. A. J. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1994**, *35*, 4015-4018.

(262) Kahne, D.; Raghavan, S. *J. Am. Chem. Soc.* **1993**, *115*, 1580-1581.

(263) Alonso, I.; Khiar, N.; Martín-Lomas, M. *Tetrahedron Lett.* **1996**, *37*, 1477-1480.

(264) Agoston, K.; Kerékgyartó, J.; Hajko, J.; Batta, G.; Lefeber, D. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Chem. Eur. J.* **2002**, *8* , 151-161.

(265) Zhang, H.; Wang, Y. L.; Thurmer, R.; Meisenbach, M.; Voelter, W. *Liebigs Ann. - Rec.* **1997**, 1871-1876.

(266) Gigg, R. Studies on the Synthesis of Serologically Active Glycolipids; American Chemical Society: 1977; pp 253-278.

(267) Iversen, T.; Bundle, D. R. *J. Chem. Soc. ,Chem. Commun.* **1981**, 1240.

(268) Cunningham, J.; Gigg, R.; Warren, C. D. *Tetrahedron Lett.* **1964**, 1191-1194.

(269) Heuer, M.; Hohgardt, K.; Heinemann, F.; Kuhne, H.; Dietrich, W.; Grzelak, D.; Muller, D.; Welzel, P.; Markus, A.; van Heijenoort, Y.; van Heijenoort, J. *Tetrahedron* **1994**, *50*, 2029-2046.

(270) Boss, R.; Schefford, R. *Angew. Chem. Int. Ed. Eng.* **1976**, *15*, 558-559.

(271) Honda, M.; Morita, H.; Nagakura, I. *J. Org. Chem.* **1997**, *62*, 8932-8936.

(272) Hansson, J.; Garegg, P. J.; Oscarson, S. *J. Org. Chem.* **2001**, *66*, 6234-6243.

(273) Hinklin, R. J.; Kiessling, L. L. *J. Am. Chem. Soc.* **2001**, *123*, 3379-3380.

(274) Aly, M. R. E.; Ibrahim, E. S. I.; El Ashry, E. S. H.; Schmidt, R. R. *Carbohydr. Res.* **1999**, *316*, 121-132.

(275) Ratcliffe, A. J.; Fraser-Reid, B. *J. Chem. Soc. , Perkin Trans. 1* **1990**, 747-750.

(276) Lefeber, D. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Chem. Eur. J.* **2001**, *7*, 4411-4421.

(277) Chernyak, A.; Oscarson, S.; Turek, D. *Carbohydr. Res.* **2000**, *329*, 309-316.

(278) Vermeer, H. J.; Kamerling, J. P.; Vliegenthart, J. F. G. *Tetrahedron: Asymmetry* **2000**, *11*, 539-547.

(279) Mash, E. A.; Kantor, L. T.; Waller, S. C. *Syn. Commun.* **1997**, *27*, 507-514.

(280) Iijima, H.; Ogawa, T. *Carbohydr. Res.* **1989**, *186*, 107-118.

(281) Di Deo, M.; Marcantoni, E.; Torregiani, E.; Bartoli, G.; Bellucci, M. C.; Bosco, M.; Sambri, L. *J. Org. Chem.* **2000**, *65*, 2830-2833.

(282) Chong, J. M.; Heuft, M. A.; Rabbat, P. *J. Org. Chem.* **2000**, *65*, 5837-5838.

(283) Lemieux, R. U.; Abbas, S. Z.; Chung, B. Y. *Can. J. Chem.* **1982**, *60*, 58-62.

(284) Durette, P. L.; Meitzner, E. P.; Shen, T. Y. *Tetrahedron Lett.* **1979**, *42*, 4013-4016.

(285) Spijker, N. M.; Westerduin, P.; van Boeckel, C. A. A. *Tetrahedron* **1992**, *48*, 6297-6316.

(286) Ponipom, M. M.; Bugianesi, R. L.; Shen, T. Y. *Tetrahedron Lett.* **1978**, 1717-1720.

(287) Banoub, J.; Boullanger, P.; Lafont, D. *Chem. Rev.* **1992**, *92*, 1167-1195.

(288) Olah, G. A.; Gupta, B. G. B.; Narang, S. C.; Malhotra, R. *J. Org. Chem.* **1979**, *44*, 4272-4275.

(289) Deng, S.; Yu, B.; Guo, Z.; Hui, Y. *J. Carbohydr. Chem.* **1998**, *17*, 439-452.

(290) Garegg, P. J.; Hultberg, H.; Lindberg, L. *Carbohydr. Res.* **1980**, *83*, 157-162.

(291) Hashimoto, S.; Honda, T.; Ikegami, S. *J. Chem. Soc. ,Chem. Commun.* **1989**, 685-687.

(292) Fried, J.; Walz, D. E. *J. Am. Chem. Soc.* **1949**, *71*, 140-143.

(293) Lam, S. N.; Gervay-Hague, J. *Org. Lett.* **2002**, *4*, 2039-2042.

(294) Miquel, N.; Doisneau, G.; Beau, J. M. *Phys. Chem. Chem. Phys.* **2000**, *2*, 2347-2348.

(295) Caputo, R.; Kunz, H.; Mastroianni, D.; Palumbo, G.; Pedatella, S.; Solla, F. *Eur. J. Org. Chem.* **1999**, 3147-3150.

(296) Hadd, M. J.; Gervay, J. *Carbohydr. Res.* **1999**, *320*, 61-69.

(297) Gervay, J.; Hadd, M. J. *J. Org. Chem.* **1997**, *62*, 6961-6967.

(298) Jung, M. E.; Yang, E. C.; Kiankarimi, M.; Spyrou, E.; Kaunitz, J. *J. Med. Chem.* **1999**, *42*, 3899-3909.

(299) Mitchell, S. A.; Pratt, M. R.; Hruby, V. J.; Polt, R. *J. Org. Chem.* **2001**, *66*, 2327-2342.

(300) Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2001**, *123*, 6819-6825.

(301) Lay, L.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1998**, *310*, 157-171.

(302) Xia, J.; Alderfer, J. L.; Piskorz, C. F.; Matta, K. L. *Chem. Eur. J.* **2000**, *6*, 3442-3451.

(303) Zhang, Y. M.; Brodzky, A.; Sinaÿ, P.; Saint-Marcoux, G.; Perly, B. *Tetrahedron: Asymmetry* **1995**, *6*, 1195-1216.

(304) Numomura, S.; Iida, M.; Numata, M.; Sugimoto, M.; Ogawa, T. *Carbohydr. Res.* **1994**, *263*, C1-C6.

(305) Gan, Z. H.; Cao, S.; Wu, Q.; Roy, R. *J. Carbohydr. Chem.* **1999**, *18*, 755-773.

(306) Deschaux, E.; Savy, P.; Bouyain, S.; Monneret, C.; Florent, J.-C. *J. Carbohydr. Chem.* **2000**, *19*, 485-501.

(307) Fletcher, H. G. Tetra-O-benzoyl-a-D-glucopyranosyl bromide; In *Methods in Carbohydrate Chemistry*; Whistler, R. L., Wolfrom, M. L., BeMiller, J. N., eds. Academic Press: New York, 1963; pp 226-228.

(308) Roelen, H. C. P. F.; Bijsterbosch, M. K.; Bakkeren, H. F.; Van Berkel, T. J. C.; Kempen, H. J. M.; Buytenhek, M.; van der Marel, G. A.; van Boom, J. H. *J. Med. Chem.* **1991**, *34*, 1036-1042.

(309) Xia, J.; Abbas, S. A.; Locke, R. D.; Piskorz, C. F.; Alderfer, J. L.; Matta, K. L. *Tetrahedron Lett.* **2000**, *41*, 169-173.

(310) Wright, J. A.; Yu, J. Q.; Spencer, J. B. *Tetrahedron Lett.* **2001**, *42*, 4033-4036.

(311) Liao, W. S.; Locke, R. D.; Matta, K. L. *Chem. Commun.* **2000**, 369-370.

(312) Lefeber, D. J.; Arevalo, E. A.; Kamerling, J. P.; Vliegenthart, J. F. G. *Can. J. Chem.* **2002**, *80*, 76-81.

(313) Moller, H.; Serttas, N.; Paulsen, H.; Burchell, J. M.; Taylor-Papadimitriou, J.; Meyer, B. *Eur. J. Biochem.* **2002**, *269*, 1444-1455.

(314) Meinecke, R.; Meyer, B. *J. Med. Chem.* **2001**, *44*, 3059-3065.

(315) Mayer, M.; Meyer, B. *J. Am. Chem. Soc.* **2001**, *123*, 6108-6117.

(316) Kooistra, O.; Herfurth, L.; Luneberg, E.; Frosch, M.; Peters, T.; Zahringer, U. *Eur. J. Biochem.* **2002**, *269*, 573-582.

(317) Biet, T.; Peters, T. *Angew. Chem. Int. Ed.* **2001**, *40*, 4189-4192.

(318) Maaheimo, H.; Kosma, P.; Brade, L.; Brade, H.; Peters, T. *Biochemistry* **2000**, *39*, 12778-12788.

(319) Cappa, A.; Marcantoni, E.; Torregiani, E.; Bartioli, G.; Bellucci, M. C.; Bosco, M.; Sambri, L. *J. Org. Chem.* **2000**, *65*, 4782.