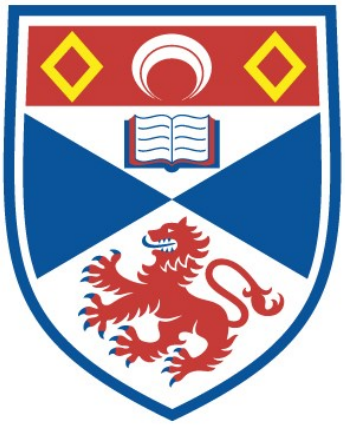
STUDIES ON B1,4-GALACTOSYLTRANSFERASE INHIBITORS AND ON THE IODINE MEDIATED ACTIVATION OF THIOGLYCOSIDES

Peter Cura

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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Studies on β-1,4-galactosyltransferase inhibitors and on the iodine mediated activation of thioglycosides

A Thesis Submitted for the Degree of Doctor of Philosophy

University of St. Andrews

By

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University of St. Andrews

St. Andrews

December 1997

CONTENTS

DECLARATION

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ABBREVIATIONS

Ac	Acetyl
AIDS	Auto Immune Deficiency Syndrome
Asn	Asparagine
Bn	Benzyl
b.p.	Boiling point
Bs	4-Bromobenzene sulfonyl
^{t}Bu	*tert*-Butyl
Bz	Benzoyl
CAN	Ceric ammonium nitrate
CAS	Ceric ammonium sulfate
CDA	Cyclohexane-1,2-diacetal
Cys	Cysteine
DCE	1,2-Dichloroethane
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
2,2-DMP	2,2-Dimethoxypropane
DMSO	Dimethylsulfoxide
DMTST	Dimethyl(methylthio)sulfonium trifluoromethane sulfonate
DNA	Deoxyribonucleic acid
cDNA	Complimentary deoxyribonucleic acid
Et	Ethyl
Ether	Diethyl ether
EtOAc	Ethyl acetate
FT	Fourier transform
Fuc	Fucose
Gal	Galactose

GalT	β-1,4-Galactosyltransferase
Glc	Glucose
GlcNAc	*N*-Acetylglucosamine
GPI	Glycosylphosphotidylinositol
HMPA	Hexamethylphosphoramide
IDCP	Iodonium di-sym-collidine perchlorate
IR	Infrared
IUPAC	International Union of Pure and Applied Chemists
Man	Mannose
*m*CPBA	*meta*-Chloroperbenzoic acid
Me	Methyl
m.p.	Melting point
Ms	Methanesulfonyl
M.W.	Molecular weight
NBS	*N*-Bromosuccinimide
NCS	*N*-Chlorosuccinimide
Neu5Ac	*N*-Acetylneuraminic acid
NIS	*N*-Iodosuccinimide
NMO	*N*-Methylmorpholine *N*-oxide
NMR	Nuclear magnetic resonance
PDC	Pyridinium dichromate
PCC	Pyridinium chlorochromate
Ph	Phenyl
Phe	Phenylalanine
Piv	Pivaloyl
Pro	Proline
TMS	Tetramethylsilane
*p*TsOH	*para*-Toluenesulfonic acid
SLe^a	Sialyl Lewis^a
SLe^x	Sialyl Lewis^x

T. brucei	*Trypanosoma brucei*
TBAHS	Tetrabutylammoniumhydrogen sulphate
T. cruzi	*Trypanosoma cruzi*
TFA	Trifluoroacetic acid
Tf	Trifluoromethanesulfonyl-
THF	Tetrahydrofuran
t.l.c.	Thin layer chromatography
TPAP	Tetrapropylammoniumperruthenate
Ts	*para*-Toluenesulfonyl-
Trp	Tryptophan
Tyr	Tyrosine
UDP	Uridine diphosphate
U.V.	Ultra violet

ABSTRACT

This abstract describes both parts of a two part thesis.

Part 1

Potential acceptor substrate analogue inhibitors of β-1,4-galactosyltransferase were proposed and the following compounds were thus synthesised to assist in understanding the mechanism of this enzyme. Octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (**13**) was synthesised and the 4-hydroxyl group was oxidised to give the corresponding ketone, octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-*xylo*-hexopyranosid-4-ulose (**15**). The ketone (**15**) was reacted with methoxylamine hydrochloride to give the *O*-methyloxime, octyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-methoxyimino-β-D-*xylo*-hexopyranosid-4-ulose (**16**), which was reduced to give both the *gluco*- and *galacto*-configured *O*-methylhydroxylamines, octyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-methoxyimino-β-D-glucopyranoside (**17**) and octyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-methoxyamino-β-D-galactopyranoside (**18**). Both methoxyamino compounds are, however, unstable and hence studies towards the target compounds via an amine at the 4-position were pursued. Octyl 2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (**23**) was prepared by displacement of the mesyl group of octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methanesulfonyl-β-D-glucopyranoside (**22**) with cesium acetate. Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methanesulfonyl-β-D-galactopyranoside (**25**) was prepared from this and the mesyl group was displaced with sodium azide to give octyl 2-acetamido-4-azido-3,6-di-*O*-benzyl-2,4-dideoxy-β-D-glucopyranoside (**26**). Octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (**13**) was reacted with methanesulfonyl chloride to give octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-methanesulfonyl-β-D-glucopyranoside (**28**). The mesyl group was displaced with sodium azide to give the *galacto*-configured azide, octyl 2-acetamido-4-azido-3,6-di-*O*-benzoyl-2,4-dideoxy-β-D-galactopyranoside (**29**). After reduction of the azide functionalities to the corresponding amines access to various amine derivatives will be possible.

Part 2

To enable a better understanding of thioglycoside activation by iodine, the following β-glycosides of 2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside were synthesised: thiomethyl (**39**), thiobenzyl (**48**), thiophenyl (**36**), *p*-methoxythiophenyl (**44**), *p*-nitrothiophenyl (**47**) and *p*-acetamidothiophenyl (**51**). All of these compounds were treated with iodine in the presence of methanol and rates of reaction were compared. Reactivity was found to depend on the nature of the leaving group. Further studies showed that DDQ in combination with iodine gave rise to substantially enhanced rates of reaction. Similar experiments showed that the nature of the solvent also affected the rate of reaction with reaction in acetonitrile substantially faster than in dichloromethane. Further studies, replacing iodine with either IBr or ICl demonstrated that both of these reagents could activate thioglycosides more rapidly than iodine. All compounds tested can be activated in approximately 1 minute depending on the activator(s) and solvent used. The anomeric ratios of all reactions favoured formation of the

methyl α-glycoside. The mechanism of these reactions was studied by analysing the reaction of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) with iodine in the absence of methanol. This demonstrated that the thiomethyl glycoside anomerised rapidly; real time N.M.R. studies revealed that the course of the reaction could be followed by N.M.R.. An analogous study in the presence of methanol demonstrated that anomerisation also takes place simultaneously with the formation of *O*-methyl glycosides and can similarly be followed by N.M.R.. Activation and glycosylation of thioglycosides with iodine vapour was demonstrated on the surface of silica t.l.c. plates. It may therefore be possible to optimise reaction conditions on t.l.c. plates prior to use of significant quantities of reagents.

BACKGROUND

CARBOHYDRATES IN NATURE

There are very many instances in Nature in which carbohydrate molecules are required for life[1,2] and probably many more that have not yet been discovered or recognised. Carbohydrates are frequently thought of as constituents of food and as such have an important role in maintaining a healthy diet. However, cellulose (poly β-1,4-linked glucose) is the most common carbohydrate on earth as it is the main structural element of the plant cell wall. In mammalian systems carbohydrates are also in abundance, and some play crucial roles in the life cycle of organisms, occurring on the cell surface of egg,[3] sperm[3] and embryo.[4] Carbohydrates play a role in disease[5] and infection,[6] for example, with carcinoma development-associated blood group precursor antigens T and Tn [7] as well as other cancer related antigens.[8,9] The extent of T and Tn expression often correlate to carcinoma and they may be involved in specific cell-cell adhesion events required for invasion by cancer cells.[5] During infection with *Trypanosoma cruzi* [10,11] the parasite transfers sialic acid to the parasite surface from the host.[12] This process may enable the parasite to successfully invade host cells.[12] Countering some of these serious ailments are complex natural products[13] such as the calicheamicins, which are potent anti-tumour compounds that contain a carbohydrate component.[4,14,15] Carbohydrate-recognising proteins and their interactions with both naturally occurring and chemically modified ligands are of great interest because of the involvement of these proteins in a number of human disease states, for example diabetes and microbe induced diseases such as influenza and cholera.[16] Hence it can be seen that in all aspects of the life cycle, of plants, animals and microorganisms, carbohydrates have an important role.

There are, as a result, a multitude of reagents and strategies available for glycoside synthesis and activation, some vastly superior to others. However there is as yet no universally applicable method that can be said to be suitable for the majority of applications. Though there are several popular methods, there is still a quest for new, improved versions.

Since carbohydrates play such a wide role in nature, there is often a need for both compounds to test and improved methods of synthesising them. To this end, this thesis is split into two parts, one focusing on the synthesis of potential inhibitors of β-1,4-galactosyltransferase and the other dedicated to new synthetic methods for preparation of oligosaccharides.

CHAPTER 1 - β-1,4-GALACTOSYLTRANSFERASE INHIBITORS

1.1.1 CELL-CELL RECOGNITION

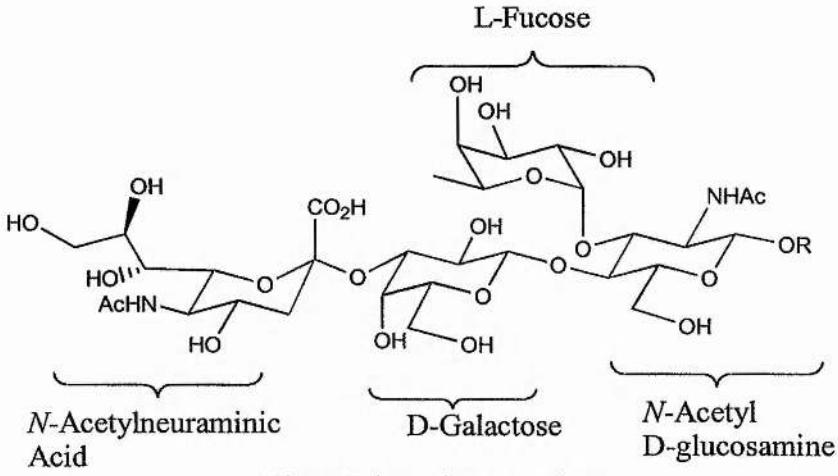
Cell-cell recognition and adhesion are vital properties of cells in all forms of life. Without these functions no plant or animal could maintain structural integrity; diseases that affect this function can cause breakdown of fleshy tissue. Cell-cell recognition in the immune response is attacked by diseases such as AIDS which affect the ability of the bodies defence mechanisms to recognise invading cells. White blood cells (leukocytes) are the major means of defence during infection.[6] Migration of leukocytes into an infected area is the result of a multistep process that takes place during both chronic and acute inflammation, and occurs via a regulated and specific cell-cell recognition process between leukocytes and endothelial cells.[17] Cell-cell recognition occurs by the interaction of a number of membrane bound adhesion receptors with membrane bound ligands.[6] The following three types of receptors are known to be involved: a) selectins, b) integrins and c) members of the immunoglobulin superfamily.[17] While cell-cell recognition is obviously important in maintaining good health, a rare leukocyte adhesion deficiency occurs in some people,[6] due in part to a fault in the biosynthesis of the sialyl Lewis^x (SLe^x) antigen. As a result neutrophils are not able to adhere to the stimulated endothelium and thus they cannot be transported to the inflamed region.[17] (Neutrophils give relatively nonspecific defence mechanisms, after which a more long lived antigen specific response is determined by macrophage monocytes and B and T lymphocytes.)[6] This deficiency is the result of a genetic disorder and leads to an unusually large number of serious bacterial infections, which can prove fatal.[6] In some cases, though the system is highly regulated, too many leukocytes can migrate to an infected region. This causes attack on the host organism by its own defence mechanism. Damage from this event can lead to psoriasis, asthma, arthritis and even multiple organ failure.[17] In addition to the above ailments, a link between tumour formation and SLe^x overproduction is thought to exist since SLe^x expression is upregulated on the surface of tumour and embryonic cells.[4,17]

Given the involvement of SLe^x in cell-cell recognition, a large amount of research has been carried out investigating the various binding requirements of

SLe^x.[17-19] Combined with this, analogue design and synthesis[20] have been intensively investigated with a view to developing medicinal understanding and therefore potential drugs to combat some of the above conditions.

1.1.2 SELECTINS

The sialyl Lewis^x (SLe^x) (Figure 1) antigen is a cell surface ligand for selectins L, E and P. These are all glycoproteins which mediate the initial stage of leukocyte adhesion during the inflammatory response.[17]



Where R is a glycoprotein.

Figure 1. The structure of the sialyl Lewis^x antigen.

The cell surface glycoprotein endothelial leukocyte adhesion molecule-1 (ELAM-1 or E-Selectin) is an endothelial cell adhesion molecule that allows myeloid cells to attach to the walls of blood vessels adjacent to sites of inflammation.[9,18,21,22] The amino-terminal domains of all three selectins have a lectin motif and all three recognise carbohydrate ligands. Destruction of SLe^x in cell cultures causes loss of E-selectin adhesion and thus demonstrates that E-selectin-mediated adhesion of cells is through recognition of cell surface carbohydrate groups that contain SLe^x, confirming that E-selectin recognises carbohydrates (Figure 2).[21] SLe^x containing glycolipids can inhibit E-selectin mediated cellular adhesion and it has been shown that the inhibition is through direct competition with the ligand binding site of E-selectin and not an allosteric effect.[21] Hence it is thought that SLe^x is essential for cell-cell recognition.[4]

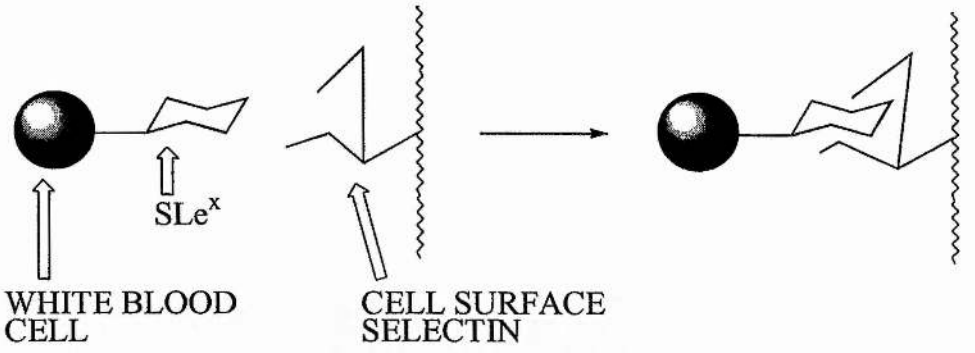


Figure 2. The envisaged cell-cell interaction of surface selectins and SLe^x.

1.1.3 BINDING INTERACTIONS

Conformational studies on SLe^x have shown that unusually close stacking interactions between the Gal and Fuc rings provides a very rigid structure for the Le^x part of the molecule and leads to the formation of a well defined hydrophilic surface along the NeuAc-Gal-Fuc residues and a hydrophobic surface underneath the NeuAc-Gal-GlcNAc residues.[4,18,23] The conformation of the Lewis^x moiety appears to be unaffected by the addition of the sialic acid residue, and calculations on SLe^x predict essentially a single energy minimum for the Lewis^x fragment with some restriction on the flexibility of the sialic acid moiety.[24] Since conformational analysis on SLe^x, SLe^a, Le^x-3'*O*-sulfate and SLe^x glycal (all of which have similar E-selectin inhibition activities) demonstrate that they possess a common topostructure in the space around the Neu5Ac, Gal and Fuc residues, it can be implied that this may be a common E-selectin binding domain (Figure 3).[18,23] The domain of E-selectin which interacts with SLe^x contains positively charged amino acid side chains. Since SLe^x is a negatively charged molecule it is likely that electrostatic interactions also contribute to binding.[6] Bivalent SLe^x (in which both units have roughly the same conformations as that of monovalent SLe^x) is approximately 5 times better at inhibiting E-selectin binding than SLe^x itself in whole cell assays, and thus the binding domain may have a multivalent ligand-receptor interaction.[18]

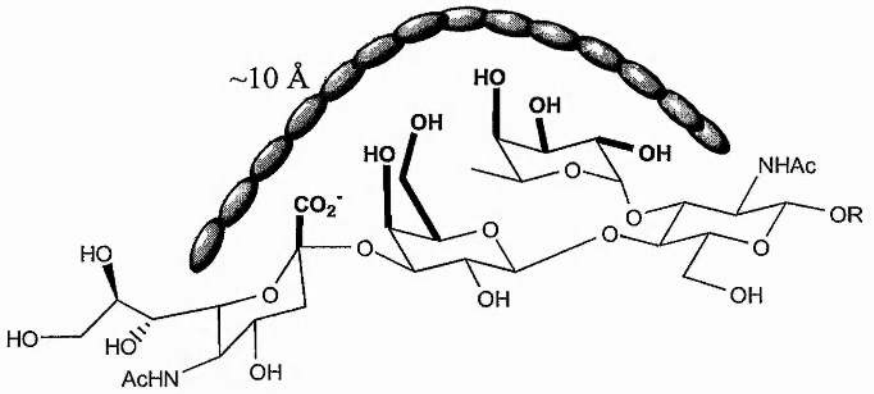


Figure 3. The proposed binding groups of the sialyl Lewis^x antigen and a model of the surface to which it is though to interact at a 10Å distance.

Some oligosaccharides which contain sialylated, fucosylated carbohydrates are recognised by the selectins as similar to SLe^x and indeed two oligosaccharides (SLe^a and sulfated Le^x, Figure 4) exhibit E-selectin binding properties.[18] Carbohydrates analogous of SLe^x with the *N*-acetylglucosamine substituted by a glucose unit have a similar binding affinity to that of SLe^x.[25] This supports the theory that the binding domain of SLe^x in the interaction with E-selectin comes from the carboxylate of the *N*-acetylneuraminic acid residue and the galactose and fucose residues, while the *N*-acetylglucosamine residue does not seem to be important for binding.[26]

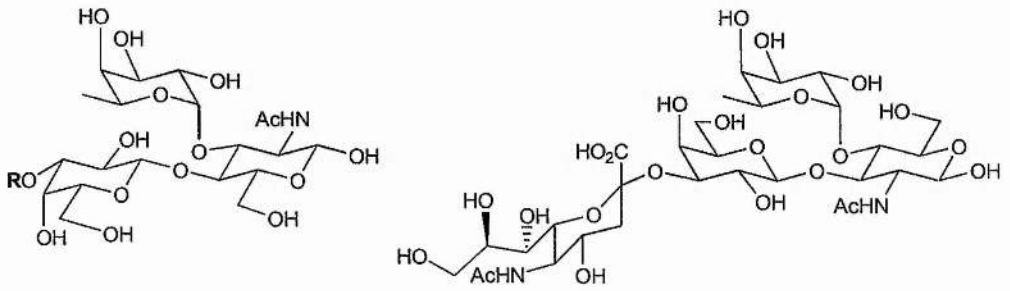


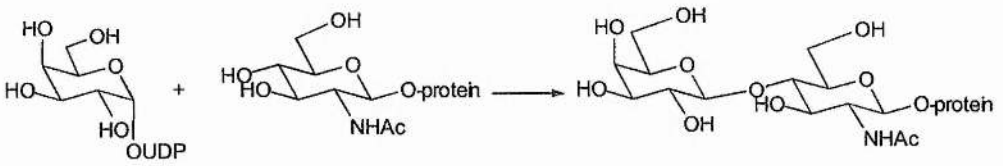
Figure 4. The structures of Lewis^x (R=H), sulfated Lewis^x (R=SO_3H) and Sialyl Lewis^a.

1.1.4 INHIBITION OF SIALYL LEWIS^x BIOSYNTHESIS

Inhibition of cell-cell adhesion may have beneficial effects in combating the illnesses noted in earlier sections. The ability to control ligand receptor interactions in general would potentially allow many advances in medicine, reduction of organ rejection in transplant operations and reduced tissue damage as a result of heart attack or stroke among them.[17] There are several possible methods of inhibiting cell-cell interaction which can be divided into three strategies: use an inhibitor to prevent SLe^x binding, destruction of selectins or SLe^x *in situ*, or prevention of SLe^x being formed at all. Synthesis of inhibitors of SLe^x binding[26] is an ongoing topic of research and, with many groups already studying this aspect,[18-20,27] it was felt that the alternative strategy of inhibiting of SLe^x biosynthesis would present an interesting challenge. Sialyl Lewis^x is biosynthesised by sialylation of the D-galactose - D-GlcNAc disaccharide which is then fucosylated.[18,28] The objective of this study is the prevention of SLe^x formation by inhibition of β-1,4-galactosyltransferase, which is the first step in SLe^x biosynthesis.

1.1.5 MODEL STUDIES USING BOVINE β-1,4-GALACTOSYLTRANSFERASE

Of the glycosyltransferases that function in the biosynthesis of glycoproteins and glycolipids, bovine galactosyltransferase is the most extensively studied.[5,29-34] Gal-T is principally located in the *trans* golgi network of a wide range of animal cell types, where it catalyses the transfer of galactose into a β-1,4 linkage with the nonreducing terminal GlcNAc residues in the glycans of glycoproteins or glycolipids (Figure 5).[35]



UDP-Galactose D-GlcNAc

Figure 5. The coupling reaction catalysed by Gal-T using the natural substrates.

In the lactating mammary gland, the specificity of Gal-T is modulated by interaction with the lactose synthase regulatory protein α-lactalbumin (Figure 6).[35] α-Lactalbumin interacts with the Gal-T catalytic domain to form the lactose synthase enzyme system. As a result of this interaction Gal-T binds glucose efficiently at physiological glucose levels and catalyses the formation of Gal-β-1,4-Glc linkages (i.e. lactose synthesis).[35] Glucose and other monosaccharide substrate binding is promoted through the highly synergistic binding of α-lactalbumin and monosaccharides to Gal-T. This may indicate proximity of the α-lactalbumin binding site on Gal-T to the acceptor binding site so that extended acceptor substrates inhibit α-lactalbumin binding and *vice versa*.[35]

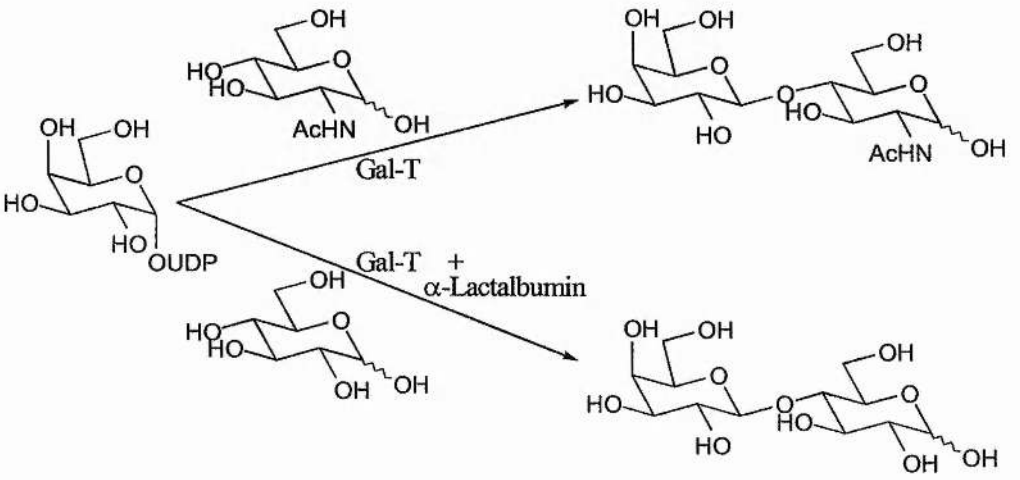


Figure 6. Gal-T and Gal-T/α-lactalbumin mediated β-1→4 glycosylation

cDNA sequences have been reported for galactosyltransferases from bovine, murine, and human sources.[35] These encode polypeptides of about 400 amino acid residues consisting of a short cytoplasmic domain of 23-24 residues, a hydrophobic *trans*-membrane region, a stem region, and a catalytic domain that projects into the lumen of the golgi.[35] Studies have shown that UDP-galactose binding specifically involves the carboxyl terminal residues. Gal-T association with α-lactalbumin affects the amino-terminal region of the catalytic domain and possibly part of the proline rich "stem region" implicating these regions in the binding of substrates.[35] It appears that the soluble form of Gal-T is composed of 2 domains, the amino-terminal 150 residues containing the Cys-134-Cys-247

disulphide bond, which functions in acceptor binding, and the carbonyl-terminal region, which is involved in UDP-galactose binding.[36] Other studies of a hydrophobic region of a Gal-T concluded that amino acids Phe-305, Pro-306, Asn-307 and Asn-308 may be involved in Gal-T catalysis or are located close to the UDP-galactose binding region but are not involved in manganese binding. Kinetic studies of mutated Gal-T have shown that Tyr-284, Tyr-309, and Trp-310 are involved in GlcNAc binding, and Tyr-309 is also involved in UDP-galactose binding.[36]

β-Galactosyltransferases require divalent cations for their enzymic activity and there is a high affinity metal site for Mn^{2+} which must be occupied prior to the binding of any substrate.[37] This site is involved in maintaining the structural integrity of the enzyme. Binding of a second metal ion (either Mn^{2+} or Ca^{2+}) to another lower affinity site appears to occur near the sugar-nucleotide binding site and exhibits a specific synergistic effect upon the binding of UDP-galactose.[38]

Galactosyltransferase is of central importance in lactose biosynthesis and glycoprotein assembly.[39] Gal-T forms β-1,4 linkages to glucose in a stereo- and regio-selective manner,[40] and investigations of its use in chemoenzymatic synthesis have been carried out (see section 1.1.6) and it is the most easily available of all the galactosyltransferases. UDP-galactose is used as a galactose source for Gal-T. Analogues of UDP-galactose and transition state analogues of UDP-galactose have been found to be competitive inhibitors.[41] *In vitro* investigations using galactal containing analogues of nucleoside diphosphate sugars[40] support an S_N1 type transition state in the enzyme catalysed reaction where metal ions or protons function as promoters for the expulsion of the nucleoside diphosphate leaving group. A conformational change in the sugar towards a glycal or half-chair type structure in the transition state might be expected.[41]

1.1.6 SUBSTRATE SPECIFICITY OF BOVINE GALACTOSYLTRANSFERASE

The flexible substrate specificity of Gal-T allows the transfer of 2-deoxygalactose, glucose or glucosamine from the corresponding UDP-hexose

analogues (Figure 6).[39] Although the transfer by bovine milk Gal-T is known to link regiospecifically and stereospecifically to the OH-4 position of glucose analogues[42] it may also form Gal β-1→1 linked disaccharides.[40] Variations of the substituents round the *N*-acetyl glucosamine (Figure 7) sugar ring have shown a range of effects upon the ability of Gal-T to accept substrates.[8,23,43-45] Using UDP-galactose as the galactose donor, the reaction rates for some substrate analogues relative to the natural substrates have been determined. In relation to the natural *N*-acetyl glucosamine substrate the following analogues have the rates for the reaction shown in Figure 7.[43]

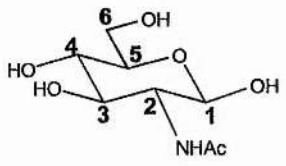
Substrate	**Substituent**	**Rate**
	none	100
Position modified		
3	$CH_3(CH_2)_2O$-	0.5
3	epimeric hydroxyl	0.04
1	CH_3O-	75
1	CH_2=$CHCH_2O$-	25
6	Acetyl	4

Figure 7. Rates of galactosylation of substrate analogues relative to the natural substrate, *N*-acetyl glucosamine.[43]

Gal-T can accommodate large changes in the substituents at the β-anomeric position of GlcNAc, tolerating the substrates shown in Figure 8 to give the Gal-β-1,4-GlcNAc linked products in 30-60% yield.[39] This demonstrates that where these bulky groups are accommodated in the active site of the enzyme there must be a large space without any steric or electronic constraints.

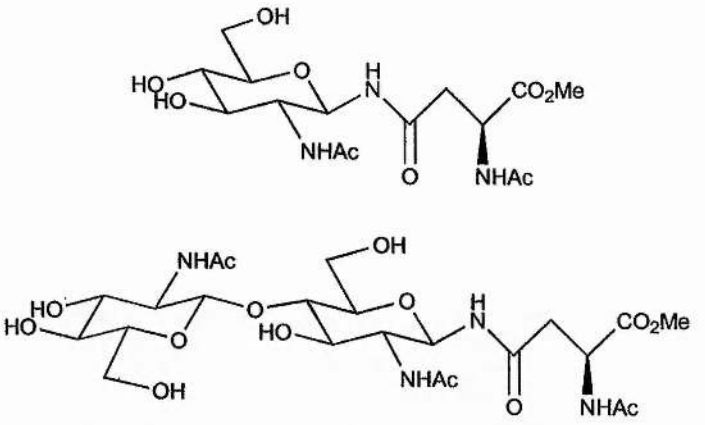
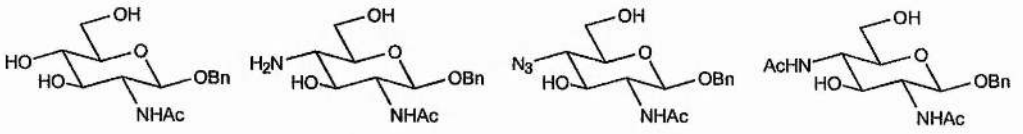


Figure 8. Variations in the groups attached to the β-1-position of substrate analogues.[39]

The 4-position of the glucosamine acceptor substrate has been shown to be vital for enzyme recognition, since removal of a hydrogen bonding moiety at this position adversely affects substrate binding (Figure 9).



Rate	100%	2%	not detectable	not detectable

Figure 9. Variations at the 4-position of substrate analogues of β-benzyl GlcNAc and their rate of galactosylation relative to β-benzyl GlcNAc(100%).[30]

It has therefore been proposed that there is a crucial hydrogen bond between the 4-position of the substrate to be glycosylated and a basic residue at the active site of the enzyme (Figure 10).[30]

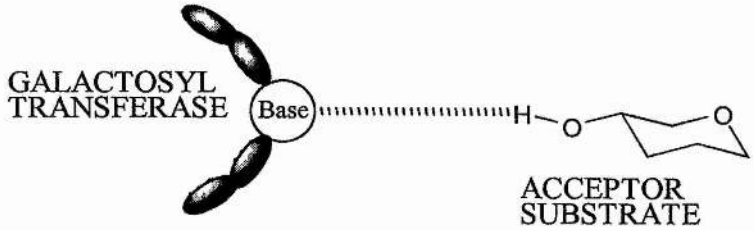


Figure 10. The proposed hydrogen bond between Gal-T and the substrate.

Using the data from different substrate analogues as a guide to the orientation of the molecules and the interactions of the substituents round the sugar ring with residues within the active site of the enzyme, the following model of the active site has been proposed by Thiem (Figure 11).[40] The model explains the observed ease with which the enzyme will tolerate modifications at the 1 and 6 positions of the sugar, with space around these positions in addition to proposed hydroxyl binding sites and a hydrophobic pocket.

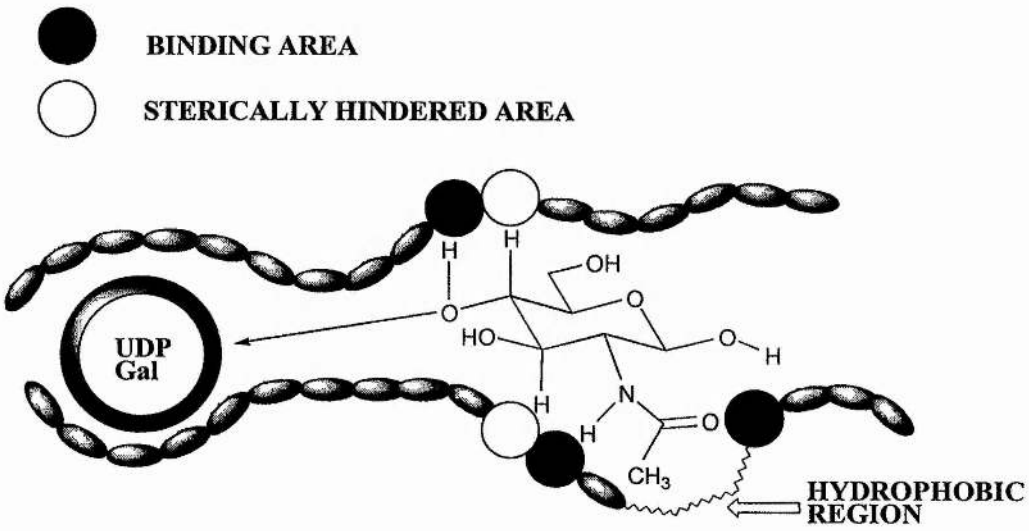


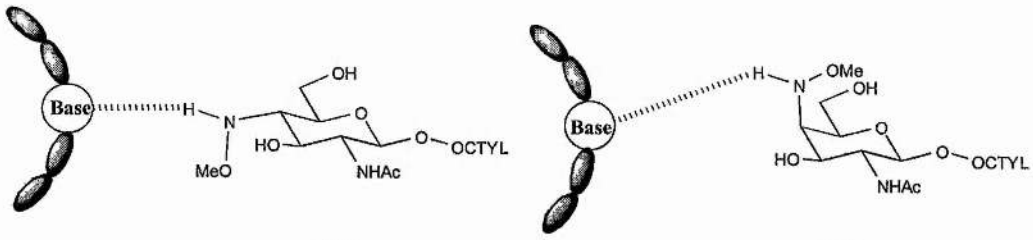
Figure 11. The proposed structural map of the bovine galactosyltransferase active site.[40]

1.2.1 AIMS AND OBJECTIVES

The aim of this project was to investigate the inhibition of D-glucose β-1,4-D-galactosyltransferase (Gal-T), an enzyme which forms β-1,4-linkages to glucose or *N*-acetylglucosamine. The aim was to be achieved by synthesising structural analogues of the *N*-acetylglucosamine unit as preferential acceptor analogue inhibitors of β1,4-Gal-T.

1.2.2 TARGET MOLECULES: METHOYXAMINO COMPOUNDS

As a result of the structural map proposed in Figure 11 and the observed need for a hydrogen bonding moiety at the 4-position of the substrate, several synthetic targets were designed to investigate binding to and acceptance by the enzyme. The analogues chosen (Figure 12) were primarily designed to mimic the hydrogen bonding thought to be present at the C-4 hydroxyl on the GlcNAc unit. Specific targets were *gluco-* and *galacto*-configured-*O*-methyl hydroxylamino sugars (**1**) and (**2**) (Figure 12).



(1) **(2)**

Figure 12. Putative substrate analogue inhibitors of β-1,4-Gal-T and their proposed interaction with the enzyme.

O-Methylhydroxylamine targets were chosen in preference to free hydroxylamines as free carbohydrate hydroxylamines are known to oxidise in air to give unstable radicals (see section 1.2.5).[46,47] The *gluco*-configured target was chosen because it has the same configuration as the natural substrate.[48] This should position the NH hydrogen atom in a manner suitable for hydrogen bonding with the putative basic residue at the active site of the Gal-T enzyme. The *galacto*-configured *O*-methylhydroxylamino sugar was chosen because, in spite of the fact that the configuration is different than that of the natural acceptor, the conformation adopted may still allow hydrogen bonding between the NH hydrogen atom and the basic residue at the active site of the Gal-T enzyme (Figure 12). These targets were also chosen since the pK_a of the methoxylamino compound is lower than that of the corresponding amine (Figure 13), thus allowing investigation of the nature of the putative hydrogen bond to the basic residue at the active site of galactosyltransferase. By lowering the pK_a of the amino compound, the character of the basic residue-NH hydrogen bond

interaction will become less of a charge-charge interaction, since a compound with pK_a 4.75[49] will not be charged at pH7. Thus any effects demonstrated on substrate acceptance will give an indication of the nature of the interaction with the natural substrate.

	pK_a
Guanidinium ion	13.59
$(CH_3NH_3)^+$	11.49
Aziridinium ion	8.01
$(MeNH_2NH_2)^+$	7.87
$(CH_3NH_2OH)^+$	5.96
$(CH_3NH_2OCH_3)^+$	4.75

Figure 13. pK_a values for representative nitrogen containing bases.[49]

Aziridines and hydrazines might also be used to vary the pK_a of the nitrogen functionality of the 4-position.

1.2.3 AMINO ANALOGUES

Further investigation of the effects of hydrogen bonding and pK_a upon the 4-position of Gal-T substrates were planned to compliment the above study. Synthesis of both the *gluco*- and *galacto*-configured amines would allow derivatisation to a variety of N-R compounds, where R = Me, Ms, amidine (Figure 14). These compounds could also be used to investigate the nature of the substrate H-bonding with Gal-T.

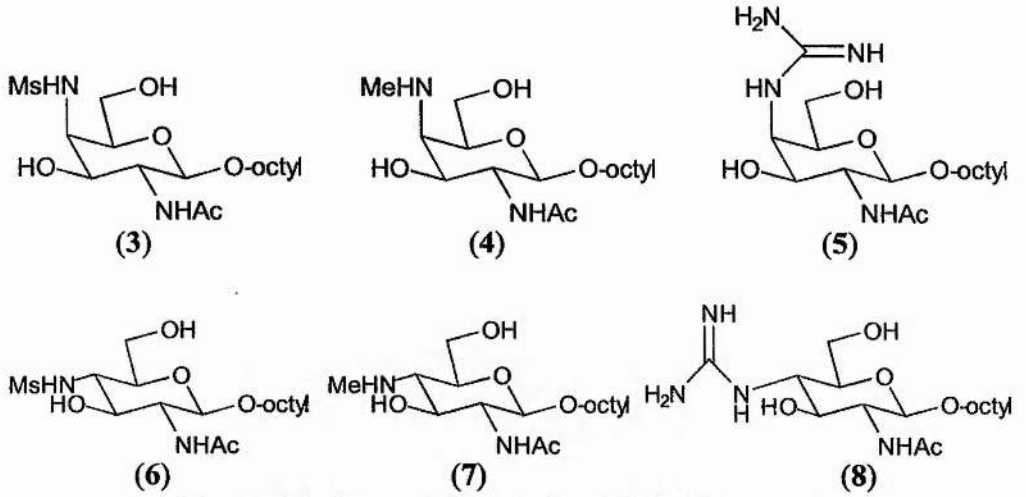


Figure 14. Some amine derived GlcNAc analogues

1.2.4 THE OCTYL CHAIN

In order to protect the anomeric centre from adverse reactions, a functional group was required that would not interfere with substrate binding. An octyl chain was considered suitable, as it is known that Gal-T can tolerate large groups at the anomeric centre.[31] In addition, a hydrophobic group such as an octyl chain can be used to aid purification by reverse phase chromatography.[50,51]

1.2.5 SYNTHETIC CONSIDERATIONS: PREPARATION OF N-O HYDROXYLAMINE LINKAGES

There are many methods of synthesising hydroxylamines and several relevant examples arise from the various recent syntheses of calicheamicin γ_1^I which contains a hydroxylamino glycosidic linkage (Figure 15).

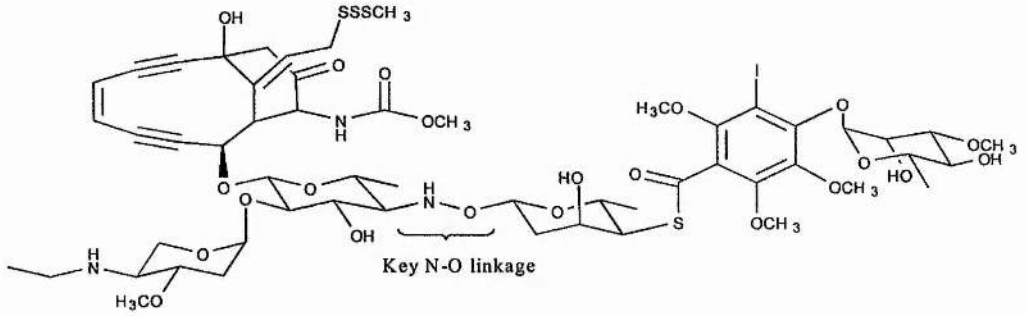
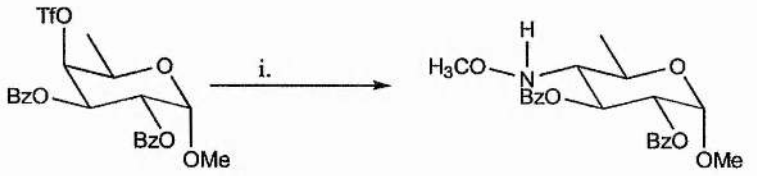


Figure 15. The structure of Calicheamicin γ_1^I, highlighting the key N-O linkage.

Calicheamicin is a member of the ene-diyne-containing class of potent antibiotics and anti-tumour agents which include esperamicin A_{1a}, the kedaricidin chromophore and C-1027 chromophore.[52] It is probable that the cytotoxicity of these molecules is due to their ability to cleave double stranded DNA. The ene-diyne portion cleaves the DNA while the carbohydrate containing section is responsible for targeting the molecule to the correct portion of DNA.[53]

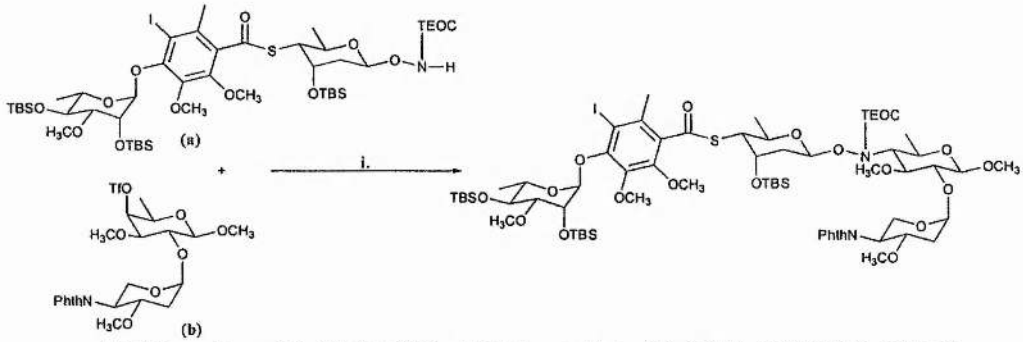
Several methods have been developed for the synthesis of the calicheamicin hydroxylamino glycosidic linkage and some of these are outlined below.[14,15,48,52,54-58] Kahne and Danishefsky have both used S_N2 displacement of a triflate in their respective syntheses of calicheamicin. Kahne used a triflate displacement by a *O*-methylhydroxylamine to form the hydroxyamine (Figure 16)[48] as a model for disaccharide formation in the approach to his synthesis of calicheamicin.



i. $MeONH_2$:DMF (2:1), 25°C, 3 h (89%).

Figure 16. Kahnes' model hydroxyamine synthesis via S_N2 displacement.

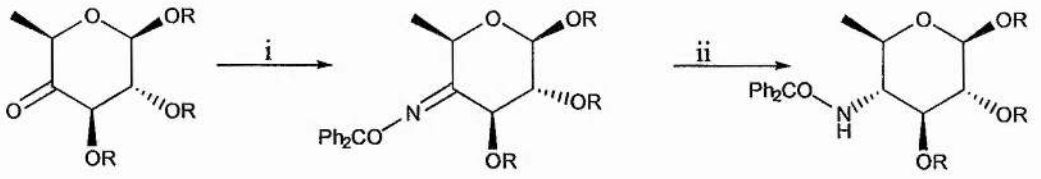
Danishefskys' similar method displaced a triflate with a urethane group to form the hydroxyamine linked disaccharide (Figure 17).[14]



i. NaH, urethane (**a**), DMF, 0°C → RT then triflate (**b**), DMF, 0°C (80%); TEOC = $Me_3SiCH_2CH_2OC(O)$-

Figure 17. Danishefskys' hydroxyamine synthesis from a urethane via an S_N2 displacement.

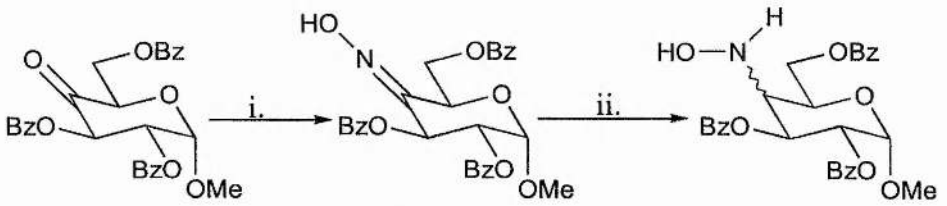
Another approach is to form an oxime and then reduce it to the hydroxylamino compound. Nicoloau synthesised calicheamicin using this methodology resulting in a stereospecific reaction to give the required linkage (Figure 18).[54] The stereo specificity is presumably due to steric hinderance from the large ene-diyne-containing fragment on the opposite face of the molecule, although an identical result was obtained when this moiety was replaced with a benzyl group.[54]



i. 1.1 equiv $BnONH_2$, PPTS cat., benzene, 25°C, 30 mins. (90%); ii. 30 equiv of $NaCNBH_3$, 13 equiv of $BF_3.OEt_2$, DCM, -60 → -40°C, 1.5 h (86%) (6:1 mixture of isomers)

Figure 18. Synthesis of an oxime and its subsequent reduction to the hydroxyamine.

In the preparation of hydroxylamines Tronchet[46,47] used a route similar to that of Nicoloau, making first an oxime from a ketone followed by reduction to the methoxyamine (Figure 19). Using commercially available methoxylamine hydrochloride salt to form the oxime (rather than the noxious free base used in Kahnes studies[48]) followed by cyanoborohydride reduction yields both the axial (preferentially) and the equatorial isomers which could be separated by column chromatography.[46] Use of the methoxylamine is preferential to the free hydroxylamine as free hyroxylamines tend to oxidise to radical species as demonstrated by Tronchet in spin labelling studies.[46,47]



i. $HONH_2.HCl$, EtOH, pyridine (80%); ii. $NaCNBH_3$, MeOH, HCl/MeOH pH3 (*gluco*- 7%, *galacto*- 47%)

Figure 19. Synthesis of an oxime and its reduction to the hydroxyamine.

1.3 RESULTS AND DISCUSSION

1.3.1 SYNTHETIC STRATEGY

For the synthesis of the required methylhydroxylamine targets the route of Tronchet[46,47] was chosen, forming an *O*-methyloxime from a ketone. This oxime would then be reduced to give the *gluco*- and *galacto*-*O*-methylhydroxylamines which should be separable by column chromatography.[46] This method was chosen in preference to the S_N2 displacements that Kahne[56] and Danishefsky[52] had used since reduction of the oxime would give access to both the *gluco*- and *galacto*-configured products from one synthetic route. In contrast, S_N2 displacements would require synthesis of GlcNAc and GalNAc derivatives to obtain the required hydroxylamines. The ketone could be formed from the free 4-hydroxyl of 3,6-protected octyl GlcNAc which could be formed by conventional means and this then manipulated to give the required target molecules (Figure 20).

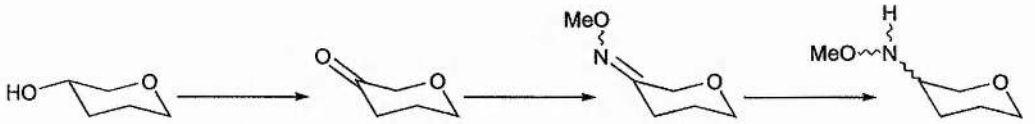
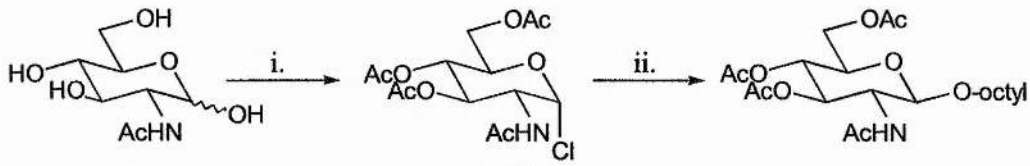


Figure 20. General scheme of hydroxylamine synthesis.

1.3.2 OCTYL GlcNAc PREPARATION

2-Acetamido-2-deoxy-D-glucopyranoside (**9**) was the starting point for the synthesis of the required 2-acetamido-2-deoxy-β-D-glucopyranoside derivatives. *N*-Acetyl glucosamine was dissolved in HCl saturated acetyl chloride and reacted overnight; this method affords higher yields than without presaturation as in the conventional procedure.[60] The resultant chloride (**10**) was glycosylated with octanol in the presence of a mercury cyanide promoter to yield octyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**11**) in 76% yield (Figure 21).



(9) **(10)** **(11)**

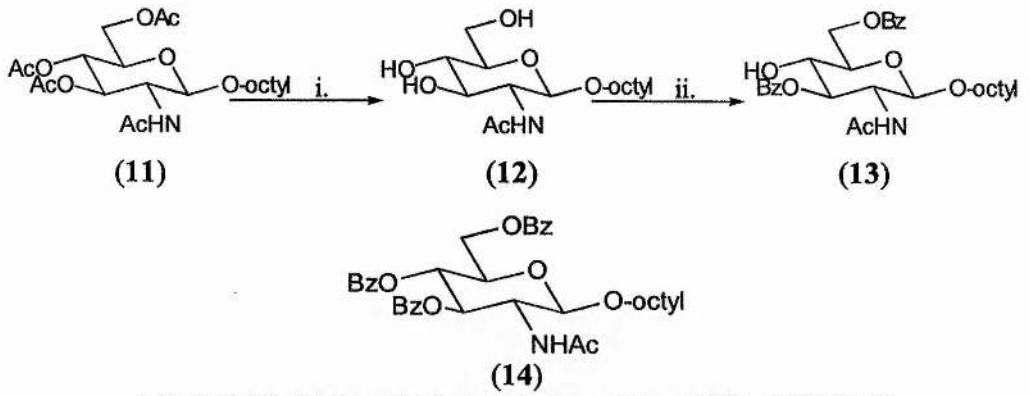
i. acetyl chloride (75%); ii. octanol, $HgCN_2$, $CaSO_4$, toluene (76%)

Figure 21. Preparation of the octyl glycoside (11).

1.3.3 SELECTIVE PROTECTION OF OCTYL GlcNAc

To obtain the free hydroxyl group at the 4-position of octyl GlcNAc necessary for functionalisation, protecting group manipulations were required. There were two main ways to achieve this; protect all functionalities and selectively deprotect the 4-position or selective protection of all functionalities except the 4-position.[61-65] With careful control of temperature selective benzoylation of a glycoside can be achieved to give the 4-position free.[65] Similarly, the more reactive primary hydroxyl can be protected first with a silicon reagent, and then with careful control of temperature the 3-position can be protected to leave the 4-hydroxyl free. The second strategy is frequently carried out by 4,6-*O*-benzylidene acetal protection followed by protection of the remaining hydroxyl groups. The fully protected sugar then undergoes selective benzylidene acetal cleavage[61-64] to give either the 4- or the 6-hydroxyl free. Other methods utilising total cleavage of the acetal followed by selective reprotection of the required hydroxyl are more involved.[64]

Deacetylation of compound (**11**) with sodium methoxide, generated *in situ*, lead to a quantitative yield of deprotected octyl GlcNAc (**12**). Selective protection[65] of (**12**) with benzoyl chloride in pyridine at -10°C gave a moderate yield (55%) of the 3,6-dibenzoate (**13**)(Figure 22). Formation of the dibenzoyl compound was monitored for formation of the unwanted tribenzoate (**14**), which was also prepared as an authentic standard.



i. NaOMe, MeOH (quantitative); ii. BzCl, pyridine, DCM, -10°C (55%)

Figure 22. Preparation of the 4-OH compound (13).

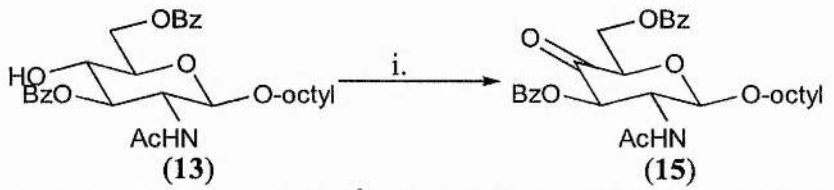
1.3.4 KETONE PREPARATION

In order to prepare oximes suitable for reduction to the corresponding hydroxylamines a ketone functionality was required at the 4-position of the GlcNAc saccharide. There are numerous methods available for oxidation of free alcohols, some of the more popular of which are detailed below. Over the years reagents have been developed to allow higher selectivity and greater functional group tolerance, allowing preparation of increasingly complex molecules. Despite these advances, the transformation of an alcohol to a carbonyl function can still cause problems. While pyridinium chlorochromate,[66,67] pyridinium dichromate[68,69] and other such reagents[70-72] are known to be good oxidants, improved methods have also been claimed using the likes of 3-carboxypyridinium dichromate[73,74] (also known as nicotinium dichromate). Improvements may be made to chromium (VI) oxidising reagents by addition of molecular sieves[75,76] (activated and added immediately prior to reaction) and addition of anhydrous acetic anhydride (used as co-solvent).[69]

Considering the above reagents, oxidants of alcohols have changed from the aggressive (often refluxing conditions)[66,67] and strongly acidic or basic chromium systems to milder and more selective procedures. An improvement over reagents such as ruthenium tetroxide,[77,78] is Leys' reagent tetrapropylammonium perruthenate (TPAP), which is a mild selective reagent with a wide range of useful applications.[79] The Swern procedure using oxalyl chloride, DMSO, and triethylamine,[80] is another useful oxidant and as with Leys' procedure this has

demonstrated its versatility in many reactions.[81-83] The oxidation procedure of Dess-Martin,[84] using periodinanes, is also popular[83,85-87] despite the fact that intermediates in the synthesis of these reagents carry the risk of explosion.[84] There are other oxidation procedures using hypervalent iodine species, some of which are similar to that of the Dess-Martin reagent[88] however, none have as yet become as common place. The methods of Ley,[79] Swern[80] and Dess-Martin[84] are all very useful but as yet there is no single reagent or system that can be guaranteed to succeed in all cases. As a result, several different methods may be required during the course of a single complex synthesis.[85] Considering this, it is probable that the oxidation of a compound may take several attempts before the best procedure is found.

Oxidation of octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside **(13)** to octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-*xylo*-hexopyranosid-4-ulose **(15)** was attempted by several methods. Leys' reagent tetrapropylammonium perruthenate (TPAP)[79] failed to oxidise even with addition of acetonitrile as suggested by Ley;[79] acetonitrile would appear to dissolve some of the TPAP[79] which, making the reagent homogeneous, is in some cases more efficient. The Swern procedure using oxalyl chloride, DMSO, and triethylamine,[80] did not yield the required product either. Nicotinium dichromate oxidation as reported by Roldan[73] was then attempted in refluxing toluene and pyridine. This experiment did not yield the required product. Following improvements made to other chromium(VI) oxidising reagents by Antonakis,[75,76] the nicotinium dichromate procedure was modified to a room temperature reaction in which 4Å molecular sieves were activated and added immediately prior to addition of pyridine and acetic anhydride (Figure 23).[69]



(13) **(15)**

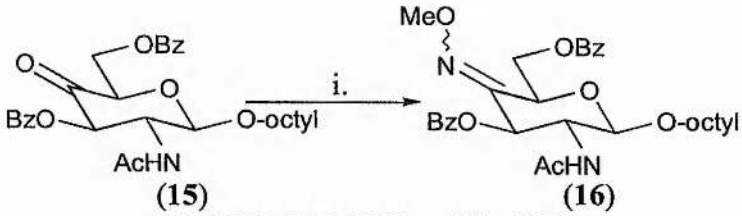
i. nicotinium dichromate, DCM, 4Å molecular sieves, pyridine, Ac_2O, RT (37%)

Figure 23. Oxidation of the 4-OH compound (13).

This reaction proved successful and fast for small (50 mg) quantities of alcohol (less than 10 minutes). When either pyridine or acetic anhydride were omitted the reaction proceeded extremely slowly. Upon addition of the omitted reagent the reaction went to completion almost immediately. The one remaining problem was successful and efficient removal of the chromium residue during workup. The chromium residue was difficult to separate and probably accounted for the lower than expected yield (yields should be good since there were no by-products visible on t.l.c.s of the completed reaction). Antonakis speculated that the sieves assisted orientation of the chromium-oxygen bonds acting as a site of reaction or that the sieves stabilise the ionic intermediates.[75,76] Hence it is possible that product (**15**) was still bound to the molecular sieves. As yet optimisation of the low yield (37%) has not been possible.

1.3.5 OXIME FORMATION

Formation of the *O*-methyloxime (**16**) from methoxylamine hydrochloride and ketone (**15**) was preferential to use of methoxylamine free base, which is unstable. Formation of the free methoxylamine is achieved from reaction of the hydrochloride salt with KOH, subsequent distillation of the product is required,[89] whereas the salt is an easy to handle solid which can be used directly. Thus reaction of the ketone (**15**) with methoxylamine hydrochloride in the presence of pyridine[46,47,90] gave the *O*-methyl oxime in reasonable yield (**16**)(89%)(Figure 24).



(15) **(16)**

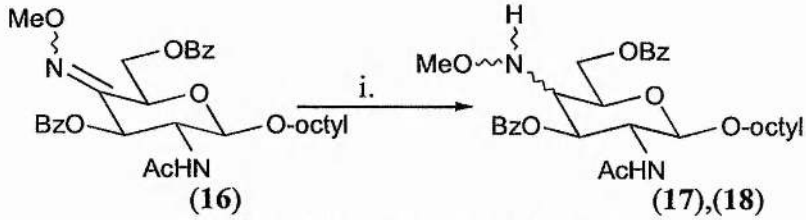
i. $MeONH_2$, HCl, EtOH, pyridine (73%).

Figure 24. Preparation of the oxime (16).

1.3.6 OXIME REDUCTION[91]

Though there are many reagents available for the reduction of oximes to their hydroxylamine counterpart, including allyl zinc,[92] diborane,[96] $NaBH_4/H^+$,[97] BH_3-

THF complex,[98] Et_3SiH/CF_3CO_2H[99] and SmI_2[100], the $NaBH_3CN$[46,47,93,94,99,101] method that Tronchet had used was obviously suited to carbohydrate synthesis. Hence reduction of the oxime **(16)** was carried out with sodium cyanoborohydride (Figure 25).[46,47] The two isomeric products formed **(17)** and **(18)** were separated by column chromatography[46] but proved to be unstable. T.l.c. analysis suggested that isomerisation/interconversion of these compounds took place even when refrigerated. Identification of the *gluco*-**(17)** and *glacto*-**(18)** configured compounds was based on 200 MHz 1H N.M.R. (which gave complex spectra that were difficult to assign) and comparison with the results of Tronchet (in which the *galacto*-configured compound was the more mobile of the two isomeric hydroxylamines).[46] Since a further deprotection step would be required to reach the target molecules, it is likely that the yield of this step would be unfeasibly low and hence synthesis of the *O*-methylhydroxylamino compounds was not continued.

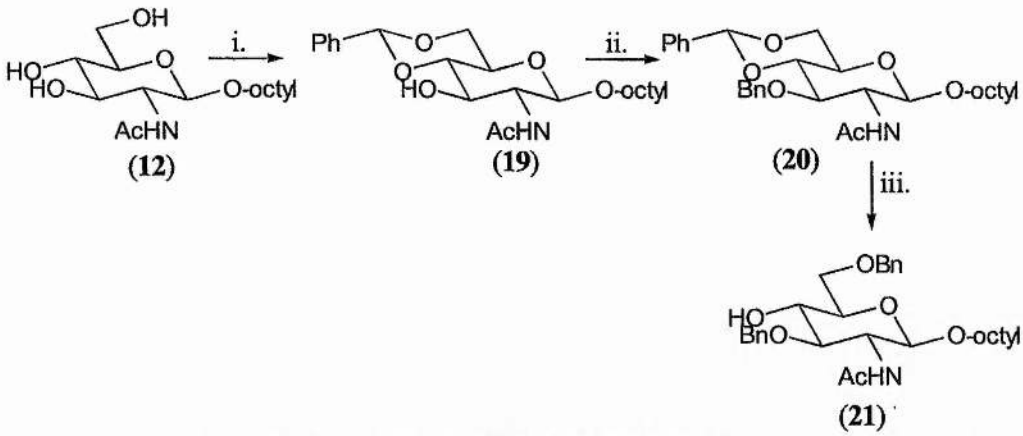


i. AcOH, $NaCNBH_3$ (yield undetermined).

Figure 25. Reduction of the oxime.

1.3.7 ALTERNATIVE PROTECTING GROUPS

An alternative synthesis for formation of a 4-hydroxyl octyl GlcNAc derivative, using benzyl groups in place of the benzoyl protecting groups was then considered (Figure 26).



i. Benzaldehyde dimethyl acetal, MeCN, *p*-TSA (98%); ii. BnBr, DMF, NaH (85%); iii. $NaCNBH_3$, 4 Å molecular sieves, THF, HCl/diethyl ether (57%)

Figure 26. Preparation of the 4-OH compound (21).

Formation of octyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**19**) from the previously prepared octyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**12**) was achieved with benzaldehyde dimethylacetal and catalytic *p*-toluenesulfonic acid in near quantitative yield. The resulting acetal was then benzylated at the 3-position to give octyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**20**) in 85% yield. Regioselective reduction of the benzylidene acetal protected compound (**20**) with sodium cyanoborohydride/HCl[62,63] to octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**21**) was achieved in 57% yield (Figure 26). Alternative methods of forming this compound were also attempted. Selective triethylsilane/TFA reduction[61] gave poorer yields while total cleavage of the benzylidene acetal to give the 4- and 6-positions free followed by selective benzyl protection of the 6-position with bis(tributyltin)oxide and benzyl bromide[64] was unsuccessful in the latter stage. Oxidation of octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**21**) by some of the methods previously attempted was even more problematic than for the analogous 3,6-di-*O*-benzoyl compound (**13**). With the prospect of an even poorer yield of hydroxylamine products, this route was terminated.

1.3.8 AMINE ANALOGUES

Since both the *gluco*- and *galacto*-hydroxylamine targets proved too unstable to be useful, synthesis of GlcNAc derivatives containing an *N*-methyl amine at

the 4-position were considered. These would allow derivatisation to a variety of N-R compounds, where R may be Me, Ms or amidine for example. These analogues would hopefully prove more stable than the hydroxylamines but still be amenable to binding studies with Gal-T. A brief outline of the strategy for synthesis of these analogues is given below (Figure 27).

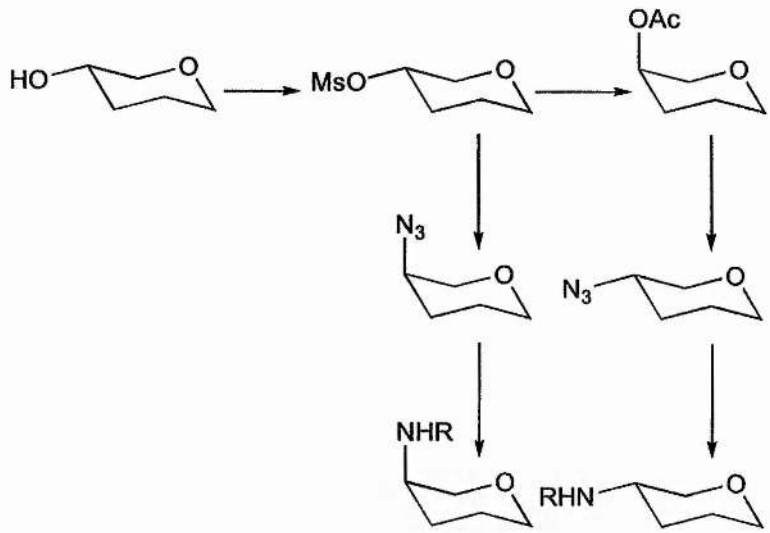


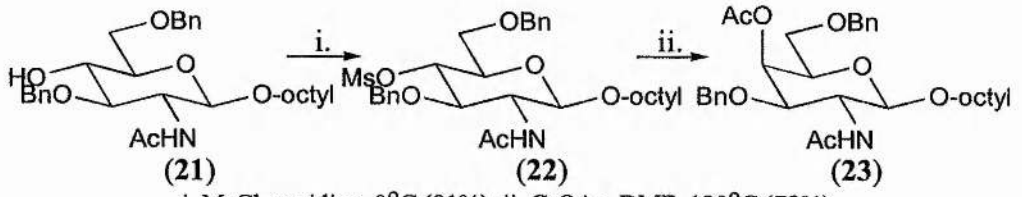
Figure 27. Strategy for preparation of amine derived targets.

1.3.9 INVERSION CHEMISTRY

In order to obtain the *gluco-* and *galacto*-configured amine derivatives, methods of inverting the stereochemistry and of introducing nitrogen functionality were required. There are many available methods for inverting the stereochemistry of hydroxyl groups, however not all were compatible with the protecting groups and functionality required. Most methods involve conversion of hydroxyl functions to a leaving group such as mesylate,[102,103-105] triflate,[85,106-109] tosylate[103,110] or brosylate[111-113] and displacement with various metal acetate[56,114-116] or azide salts.[103] Other reactions such as modified Mitsunobu[105,117] reactions, and variations upon these, have also been reported These yield inverted stereochemistry with either hydroxyl (or derivative) or amine (or derivative) functionalities. Considering the above choices, a suitable procedure for inversion of configuration to an oxygen containing functionality was considered to be the common mesylate displacement with a metal acetate. Similarly, mesylation

followed by subsequent sodium azide displacement was deemed to be the most obvious choice of procedure to introduce the nitrogen functionality.

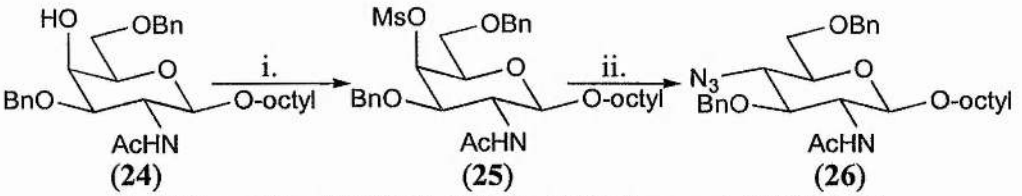
Hence octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**21**) was mesylated with mesyl chloride in pyridine to give octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methanesulfonyl-β-D-glucopyranoside (**22**) in 91% yield. Cesium ions are large and polarisable allowing the counter ions to be more nucleophilic than smaller, harder metal ions.[114,115,118] As a result cesium acetate was the preferred reagent for acetate displacement. Subsequent displacement of the mesyl group of compound (**21**) with cesium acetate[114,115,118] in refluxing DMF for 2 days gave octyl 2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (**23**) in 78% yield (Figure 28).



i. MsCl, pyridine, 0°C (91%); ii. CsOAc, DMF, 120°C (78%).

Figure 28. Inversion of stereochemistry with cesium acetate.

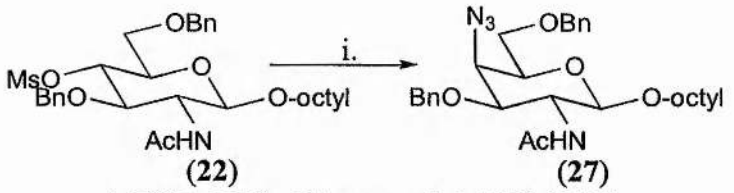
Deacetylation of compound (**23**) with sodium methoxide gave a 94% of the 4-hydroxyl compound (**24**). Subsequent mesylation as above, gave octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methanesulfonyl-β-D-galactopyranoside (**25**) in 82%. The *galacto* configured mesylate (**25**) was then reacted with sodium azide in refluxing DMF to give octyl 2-acetamido-4-azido-3,6-di-*O*-benzyl-2,4-dideoxy-β-D-glucopyranoside (**26**) in 93% yield (Figure 29).



i. MsCl, pyridine, 0°C (82%); ii. NaN_3, DMF,15-crown-5, 120°C (93%).

Figure 29. Preparation of the azide (26).

Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methanesulfonyl-β-D-glucopyranoside (**22**) was also treated with sodium azide in refluxing DMF to give 2-acetamido-4-azido-3,6-di-*O*-benzyl-2,4-dideoxy-β-D-galactopyranoside (**27**) in 61% yield (Figure 30).

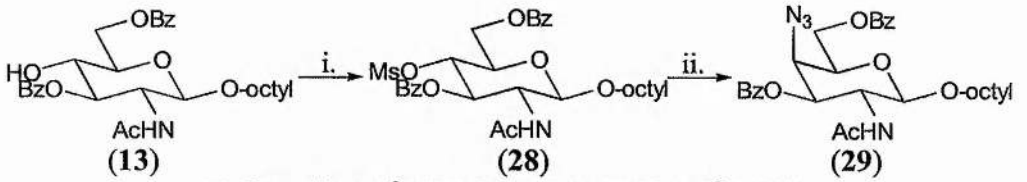


(**22**) (**27**)

i. THF, NaN_3, 15-crown-5, 140°C (61%)

Figure 30. Preparation of the azide (27).

The analogous 3,6-di-*O*-benzoate was also prepared. Octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (**13**) was mesylated as before to give octyl 2-acetamido-4-*O*-methanesulfonyl-3,6-di-*O*-benzoyl-2-deoxy-β-D-gluco-pyranoside (**28**) in 87% yield. The mesyl group was displaced with sodium azide in refluxing DMF after 4 days to give 2-acetamido-4-azido-3,6-di-*O*-benzoyl-2,4-dideoxy-β-D-galactopyranoside in 50% yield (**29**)(Figure 31).



(**13**) (**28**) (**29**)

MsCl, pyridine, 0°C (87%); ii. NaN_3, DMF, 110°C (50%)

Figure 31. Preparation of the azide (29).

1.3.10 AZIDE REDUCTION

Once the *gluco*- and *galacto*-azides (**26**), (**27**) and (**29**) had been obtained, reduction to the free amine was required. As with oxidations there are many methods available, the choice of which depends on the circumstances. In this case, if the 4-position is obtained via benzylidene acetal cleavage, conversion of the product of this reaction to the corresponding azide will result in a benzyl protected sugar. The most convenient method to approach reduction here, would be palladium/charcoal hydrogenation[64] which should reduce the azide and

deprotect the sugar in one reaction.[64] However, since the amine thus formed will still require functionalisation, free hydroxyl groups may give side reactions. If on the other hand the protecting groups were benzoate esters, then other reduction methods could be considered. These methods include hydrogen sulfide,[119] triethyl amine/1,3-propane dithiol,[120,121] tri-*n*-butyl tin hydride[122] or triphenylphosphine.[123,124] A more direct method would be to use the modified Staudinger reaction reported by Boullanger,[125] in which an azide is condensed with an acid chloride in the presence of triphenylphosphine. However as yet this reaction has only been reported for anomeric azides; the procedure may not be suitable.

Reduction of octyl 2-acetamido-4-azido-3,6-di-*O*-benzyl-2,4-dideoxy-β-D-glucopyranoside (**26**) (Figure 32) proved troublesome and a number of different methods were explored.

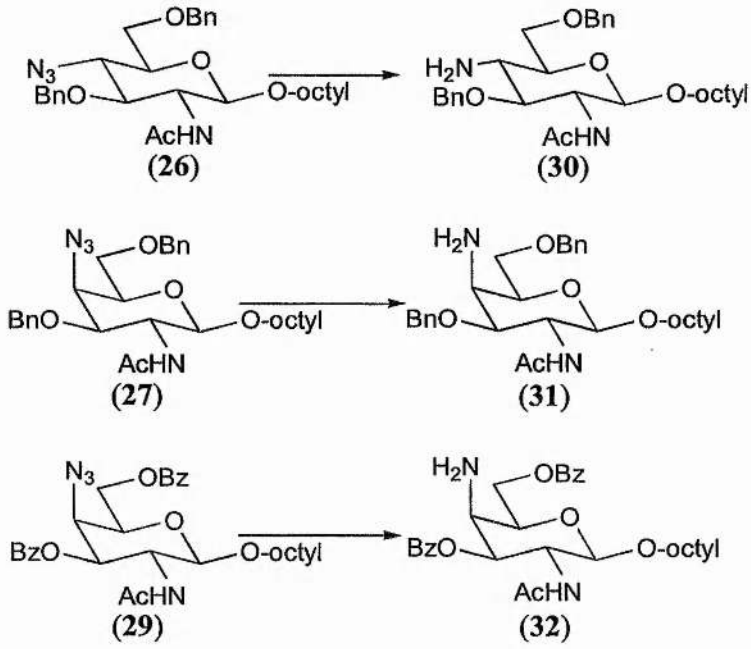


Figure 32. Reduction of the azides (26), (27) and (29) to their corresponding amines (30), (31) and (32).

Total reduction of both the azide and the benzyl functions with hydrogen and catalytic palladium/charcoal[64,121] surprisingly gave a complex mixture of products and incomplete reaction. This is possibly because the amine may poison the

palladium/charcoal catalyst and prevent further reaction. Reduction with triphenylphosphine[123,124] in THF and a small amount of water did not provide a clean reaction; addition of ammonium hydroxide[126] did not improve matters. Similar reduction with polymer-supported triphenylphosphine/ammonia in pyridine appeared to work by t.l.c., but very low yields were obtained upon workup probably due to the product sticking to the solid support. 1,3-Propane dithiol[120] in the presence of sodium borohydride,[121] an alternative reducing agent, gave much cleaner reaction but purification with acidic ion exchange resin gave very low yields, possibly due to the product sticking to the resin beads. Similar attempts at reduction of octyl 2-acetamido-4-azido-3,6-di-*O*-benzoyl-2,4-dideoxy-β-D-galactopyranoside (**29**) (Figure 32) with triphenylphosphine gave several products, though distinctly cleaner than for compound (**26**) above. When 1,3-propane dithiol/sodium borohydride was used the reaction was much cleaner but isolation of the product again proved difficult. Although neither the *gluco*- or *galacto*-configured azide has successfully been reduced to the corresponding amine and isolated, the propanedithiol/sodium borohydride method appears to be the most promising. Once optimisation of the workup and purification procedures has been achieved, the required products should be obtainable without further complication. Unfortunately time constraints did not permit this part of the project to be completed.

1.4 CONCLUSION

The synthesis directed towards nitrogen containing 4-OH analogues of GlcNAc has illustrated some of the unexpected difficulties that may arise during a synthesis. Although a target may be designed and synthesised there is no sure way to know in advance (other than perhaps molecular modelling) whether it will be stable or biologically active. To obtain the required target many synthetic methods may need to be investigated for the successful completion of each step. Although many literature methods exist for most reactions they may not be suitable to the problem presented. While at least one of the available methods is often usable with some modification, optimisation of procedure, workup and purification may still be necessary. This was the case for several steps in this project.

1.5 FUTURE WORK

While synthesis of the target molecules for binding studies with Gal-T has not been completed there remains only reduction of the azide, functionalisation of the amine and deprotection to be achieved. After these steps enzyme assays with β-1,4-Gal-T will be necessary and the results of these studies will determine the nature of any subsequent targets. In addition the information obtained may lead to further understanding of the nature of the 4-OH-enzyme interaction. Hence inhibitors of the Gal-T enzyme and thus SLe^x biosynthesis may lead to development of therapeutic targets.

CHAPTER 2 - IODINE MEDIATED ACTIVATION OF THIOGLYCOSIDES

2.1.1 SYNTHESIS OF OLIGOSACCHARIDES

In the construction of carbohydrate molecules one of the most challenging aspects is construction of the glycosidic linkage. To form these linkages it is usual to employ a saccharide with at least one free (unprotected) hydroxyl group - the acceptor, which will react with a saccharide with a leaving group at the anomeric position - the donor.[127-131] Most of the chemistry of the anomeric centre arises because of its electrophilic character, the reactive intermediate being the oxocarbenium ion formed by departure of the appropriate leaving group. The oxocarbenium ion may then be attacked by a nucleophile, usually the free hydroxyl group of an acceptor saccharide (Figure 33).[131]

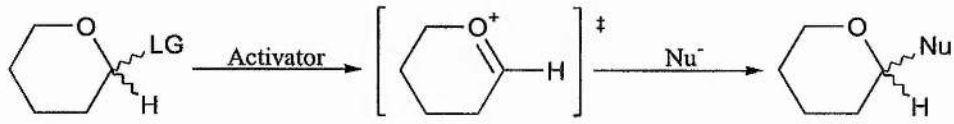


Figure 33 Typical glycosylation mechanism.

2.1.2 THE ANOMERIC EFFECT

The first major advance in understanding the factors influencing the stereochemical outcome of glycosylation reactions was made by Lemieux.[132] The term "anomeric effect" was introduced by Lemieux to describe a stereoelectronic influence on chemical bonding that had been noticed while studying the anomerisation of sugars with known conformation. The anomeric effect tends to make electronegative substituents at the anomeric position favour the axial orientation. In addition repulsion between the negative end of the C1-β-substituent dipole and the unshared electrons of the ring oxygen atom also acts against the formation of equatorial isomers.[133-136] Another vital contribution is made by the *exo*- and *endo*-anomeric effects which are generally taken to arise from anti-periplanar n-σ^* interaction of the axial oxygen lone pair orbital with

the antibonding orbital of the adjacent C-O bond, which hinders rotation about the *exo*-cyclic C-O bonds at the anomeric centre (Figure 34).[132]

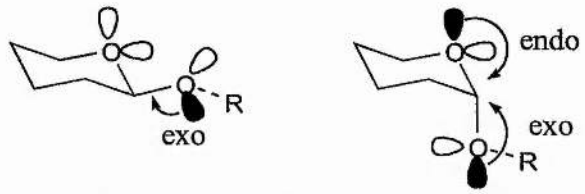


Figure 34 Exo and Endo anomeric effects.

In addition, groups attached to the anomeric centre which reverse the dipole of the bond attaching them to the anomeric carbon, show a reverse anomeric effect resulting in a preference for β-configured saccharides, as in the case of pyridinium glycopyranosides.[136,137] These stereoelectronic effects are influenced by, among other things, polar molecules such as solvents[132,138] and the size of the anomeric substituent[139]. The anomeric effect is responsible for stereocontrol in many reactions (such as anomeric halides being formed as the α-isomer exclusively in most conventional syntheses) and therefore it is important to take it into account whenever synthesis of a glycoside is planned. Not only does the anomeric effect affect the stereocontrol of conventional reactions but also those involving formation of radicals[140] at the anomeric centre.

2.1.3 1,2-*trans*-GLYCOSYLATION

From a historical perspective the most significant advance in the formation of glycosidic linkages was the Koenigs-Knorr reaction.[131,141] As a result, the classical glycosyl donors in glycosylation chemistry are glycosyl halides. In the Koenigs-Knorr[142] reaction acetylated glycosyl chlorides or bromides are reacted with an acceptor, under promotion by heavy metal salts, into glycosides. The stereoselectivity depends on the position of the substituent at C2, in the *gluco*- and *galacto*- series the β-glycosidically linked oligosaccharides are usually obtained and the *manno*- series gives α-linked glycosides.[143] This is known as

neighbouring group participation, in which the *O*-acetyl group at C2 forms a cyclic intermediate cation after the halide departs. The cyclic intermediate reacts stereoselectively to give the 1,2-*trans* product (β-D-*gluco* or α-D-*manno*). This principle of directing the stereoselectivity at the anomeric centre is effective in most methods, even those that use quite different leaving groups at the anomeric centre (Figure 35).

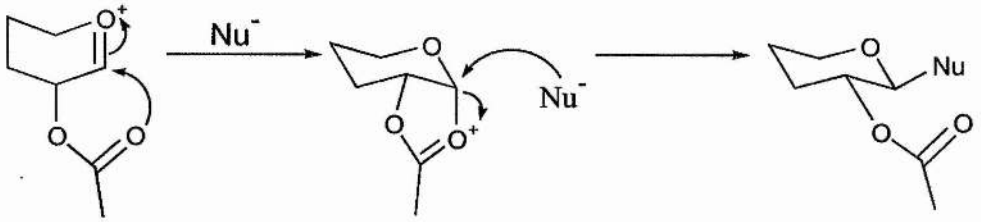


Figure 35 Neighbouring group participation.

2.1.4 SOLVENT EFFECTS ON STEREOSELECTIVITY

The influence of temperature[144] and solvents[132] on the stereochemical outcome of glycosylations is well known, for example in many cases acetonitrile leads to the formation of an 1,2-*trans*-glycosidic linkage even in the absence of a participating C-2 sunstituent (Figure 36).[131,145,146]

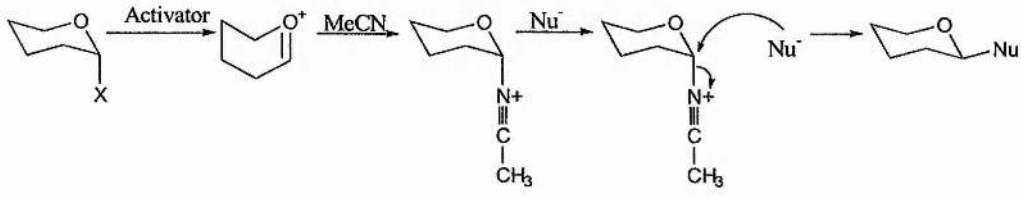


Figure 36 The stereochemical effect of acetonitrile as a solvent.

Use of diethyl ether as a solvent generally leads to the formation of the axial glycoside as the main product. The α-directional effect of diethyl ether may be due to participation of the oxygen atom of diethyl ether with a sugar oxonium ion

intermediate.[138] Additionally, the α-anomer is more thermodynamically stable and the anomeric effect is stronger in solvents of low polarity (such as diethyl ether). Recent studies by Boons and co-workers have shown that the α-selectivity of glycosylation reactions with thioglycosides can be drastically improved by using toluene/dioxane as a solvent.[138] This effect was ascribed to the oxygen donating effect (as with diethyl ether) and the relatively low polarity of the solvent. Furthermore toluene slowed down the reaction. It was also shown that the iodonium ion source and its counter ion, the concentration and the presence of molecular sieves also have an effect on the stereochemical result of a glycosylation.[138] Even the composition of the molecular sieves may influence the outcome of some reactions.[147]

2.1.5 "ARMED" AND "DISARMED" EFFECTS IN GLYCOSYLATION REACTIONS

As the synthesis of oligosaccharides has been studied, an increasing number of donors and methods of activating them has emerged. However forming linkages in the correct order is still a troublesome task. Ideally a synthesis could be completed with all the reagents present in one pot reacting of their own accord (once activated) to form the correct oligosaccharide. To this end, methods of fine tuning the reactivity of donors have been intensively investigated.[148-153]

The effect of some common protecting groups on glycoside reactivity was probed with *n*-pentenyl glycosides by Fraser-Reid,[148] and the "armed"/"disarmed" strategy for oligosaccharide synthesis came directly from these investigations. Glycosyl donors can be "armed" or "disarmed" by a C2 ether or ester group respectively, as the glycosyl oxygen is a poorer nucleophile when C2 is OAc than when C2 is OBn because of the electron withdrawing effect of the ester.[148] Reaction of a glycosyl donor with an appropriate electrophile gives a positively charged intermediate which is less favourable when there is an adjacent electron withdrawing group (e.g.: OCOR, as in a "disarmed" donor) than when there is an adjacent alkoxy group (as in the "armed" counterpart). The latter therefore reacts faster, and if there is a "disarmed" species carrying a free hydroxyl group present,

this leads to cross coupling with (virtually) none of the self coupled byproduct (Figure 37).

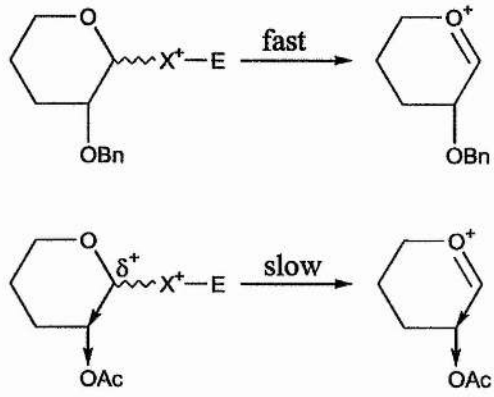


Figure 37 "Armed" and "disarmed" effects of protecting groups.

The "armed"/"disarmed" protocol for oligosaccharide synthesis has general applicability and it is now possible to couple a donor having a C2 ether ("armed") with an acceptor having a C2 ester ("disarmed") (Figure 38).[148]

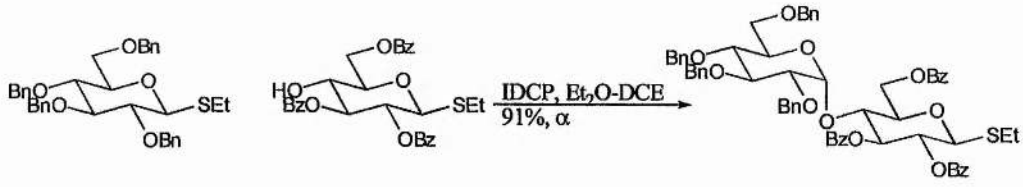


Figure 38 Coupling of an "armed" donor with a "disarmed" acceptor.[153]

The anomeric centre of the resulting disaccharide may be activated with a more powerful activator, and reaction with a suitable acceptor will yield a trisaccharide. It has also been demonstrated that a saccharide may be regarded as "disarmed" when a cyclic acetal is attached to the pyranosyl ring.[127,151] Recently Ley *et al* reported thioglycosides bearing a dispiroketal (dispoke) or cyclohexane-1,2-diacetal (CDA) protecting group. The reactivity of a C2 benzylated CDA thioglycoside is an order of magnitude different to that of ethyl

thioglycosides having a fully "armed" ether and "disarmed" protecting group on C2. This disarming effect is due to the rigidity of the bicyclic (*trans*-decalin-type structure) protected sugar as this opposes flattening of the carbohydrate ring during oxocarbenium ion formation (Figure 39).

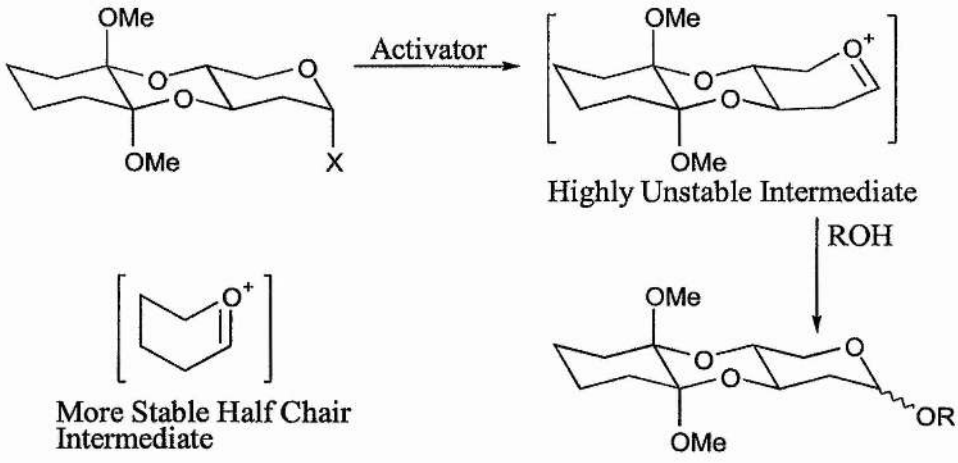


Figure 39 Torsional disarming effects of CDA protecting groups.

These thioglycosides may therefore be regarded as "semi-disarmed" substrates, with reactivities between that of "armed" and "disarmed". In addition, Boons[152] has reported that the bulk of the anomeric leaving group affects the leaving group lability (Figure 40).

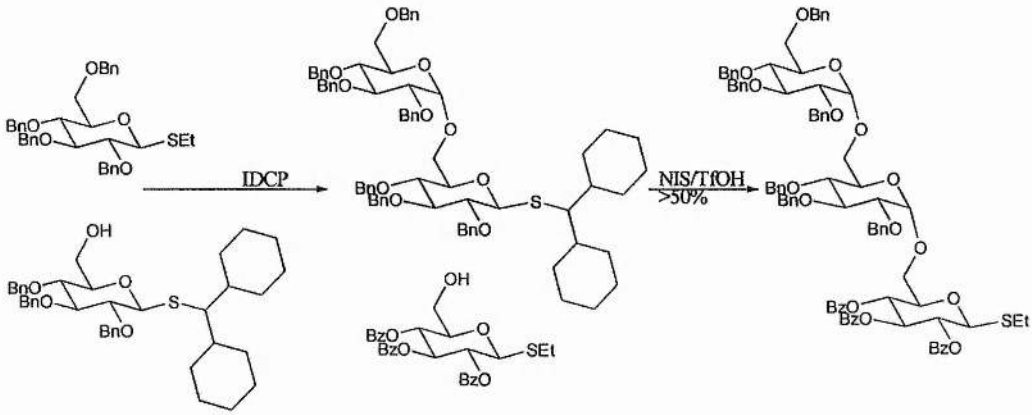


Figure 40 Steric bulk as a "disarming" method.

Thus esters "disarm" electronically, while benzylidene and isopropylidene rings "disarm" by torsional strain.[151] Thus several different levels of anomeric reactivity for thioglycosides are now available allowing chemoselective coupling of benzylated glycoside donors with benzylated glycoside acceptors.

2.1.6 COMMON GLYCOSYL DONORS

There are at present numerous chemical methods for linking saccharide units. Most current use of glycosyl halides is based on the classic Koenigs-Knorr[142] reaction. In its modern form, this method is useful for certain types of reactions, although there are also a range of applications for glycosyl fluorides.[127,151,152] A second important method is the thioglycoside method.[129] Thioglycosides may either be activated by conversion into a glycosyl halide and then reacted, or directly with an electrophilic reagent.[127,129] A range of other methods exist such as the trichloroacetimidate method which is very flexible, requires low temperatures, and often needs surprisingly short reaction times.[145] The following subsections give a brief summary of topical classes of glycosyl donor.

2.1.6.a BROMIDE AND CHLORIDE DONORS

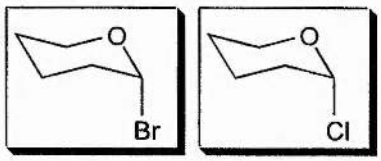


Figure 41 Glycosyl bromide and chloride donors.

The Koenigs-Knorr[142] reaction has been continuously improved: from silver salts[156] (silver carbonate)[142] to mercury salts[156-158] (e.g.: mercury(II)cyanide) and back to silver salts, of which silver triflate[158-161] is one of the most effective promoters. The heavy metal salts act as halophilic reagents. By careful selection of reaction conditions and type of protection, both α- and β-glycosidic linkages can be prepared with high stereoselectivity.[162] Tetramethylurea and various

hindered pyridine derivatives are used as acid scavengers[141] and molecular sieves can also be used to absorb any water produced during reaction.[141] However, difficulties may arise in the conversion of many oligosaccharide derivatives (e.g.: *O*-glycosides or glycosyl esters) into glycosyl halides resulting in unsatisfactory yields.[129] In addition glycosyl halides are often unstable making handling and purification potentially troublesome. They require relatively harsh preparation conditions, silver salts are expensive and their use, especially in large scale reactions, is often dangerous as silver perchlorate is explosive.[145] The other common type of halide activators – mercury salts, are highly toxic.[145] The problems with the use of halides described above may make use of them unfeasible in some procedures. Hence many alternative glycosyl donors have been developed, some of which equal or indeed supersede glycosyl halides as donors.

2.1.6.b GLYCOSYL FLUORIDE DONORS

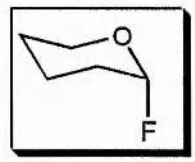


Figure 42 Glycosyl fluoride donors.[141,151,153,162-165]

A related alternative to the chloride or bromide strategies is the use of fluoride donors. Fluorides have several advantages over chloride and bromide donors; they are formed by mild methods and more importantly they are more stable. Glycosyl fluorides when used as glycosyl donors may be activated in the presence of silver perchlorate/tin dichloride,[129] lithium perchlorate[163] or BF_3:OEt_2[166] to couple with an acceptor. The mechanism of activation of fluorides is thought to be similar to that of chloride and bromide activation. The stereoselectivity of the reactions are as for Koenigs-Knorr reactions and neighbouring group participation leads to β-anomers while without this effect the α-anomer is formed as expected.

2.1.6.c IODIDE DONORS

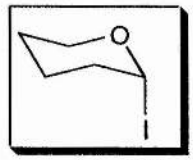


Figure 43 Glycosyl iodide donors.[163,166-173]

Although rarely used because of their instability glycosyl iodide donors may be generated *in situ* and used directly.[163,168] The majority of iodides formed are the α-anomers as with chlorides, bromides and fluorides.[169,170] Glycosyl iodides may be formed from either glycosyl chlorides or bromides by reaction with sodium iodide[159], hydrogen iodide/glacial acetic acid,[149] reaction of a variety of glycosides with TMS-iodide[168,170] or reaction of a hemiacetal with 1-iodo-*N*,*N*,2-trimethylpropenylamine.[169] α-Iodides of acetyl, benzoyl and isopropylidene protected sugars have been made and characterised[167,169,170,173] but the corresponding benzyl protected iodides are usually regarded as far too unstable to isolate.[167] However Waldmann successfully isolated and identified a benzyl protected α-iodide as a reaction intermediate in the activation of glycosyl phosphates with LiI in $LiClO_4$ solutions.[163] Upon reaction with the acceptor this intermediate gives predominantly α-glycosides. More recently Gervay successfully synthesised, isolated and characterised both α and β benzyl protected glucosyl iodides as glycosyl donors.[170]

2.1.6.d TRICHLOROACETIMIDATE DONORS

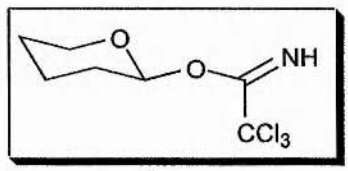


Figure 44 Trichloroacetimidate glycosyl donors.

The trichloroacetimidate group is an excellent leaving group which may be used in several ways as a glycosyl donor.[52,131,145,] The reaction is fast (≈10 minutes[141] catalysed by boron trifluoride etherate[145] or trimethylsilyltriflate[174]) and

a reaction influenced by neighbouring group participation will always yield the β-glycosides in the *gluco-* and *galacto-* series and α-glycosides in the *manno-* series, regardless of whether α or β-trichloroacetimidate donors are used. β-Trichloroacetimidates of benzylated saccharides without participating neighbouring groups can be transformed into α-glycosides in the *gluco-* and *galacto-* series. Using optimised conditions it is also possible to transform α-trichloroacetimidates into β-glycosides without participation of neighbouring groups. Glycosyl trichloroacetimidates are usually stable enough for storage, and require no metal salts for their activation.

2.1.6.e *n*-PENTENYL GLYCOSYL DONORS

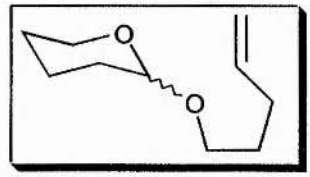


Figure 45 *n*-Pentenyl glycosyl donors.[148,149,175]

A novel method for glycosylation with wide scope for application in oligosaccharide synthesis has been developed by Fraser-Reid. He uses 4-pentenyl glycosides as glycosyl donors. These can be activated by bis(2,4,6-trimethyl pyridine)iodonium perchlorate, NIS-TfOH or NIS-TMSOTf. Addition of a halogen to the pentenyl double bond produces a halonium ion intermediate. This intermediate then rearranges to a second intermediate containing the 2-halomethyl tetrahydrofuran leaving group. Elimination of this results in a glycosyl cation which reacts to form the glycoside (Figure 46).

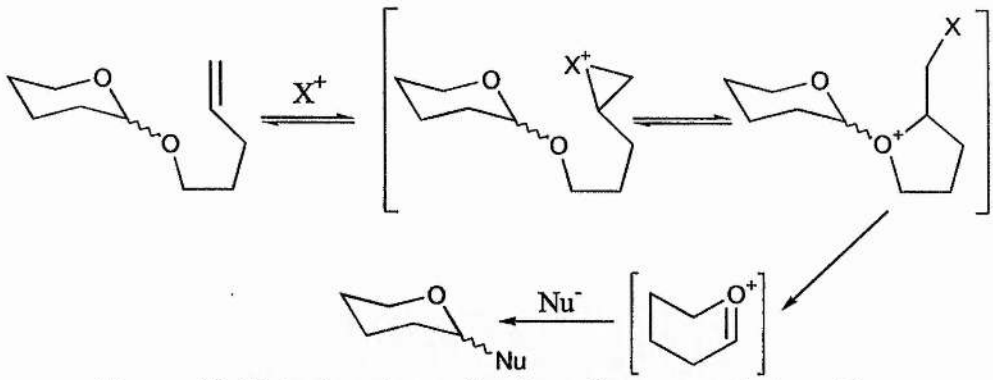


Figure 46 Halonium ion activation of *n*-pentenyl glycosides.

This method usually produces 1,2-*trans* glycosides and is best suited to synthesis of larger oligosaccharide units. *n*-Pentenyl glycosides are prepared by standard glycoside forming procedures and although they are stable to a wide range of reagents, they are easily activated by treatment with halonium ions.

2.1.6.f GLYCAL DONORS

Figure 47 Glycal donors.[131]

Other than the methods of glycosylation discussed above there are several other methods that may be used to synthesise glycosides. Glycals are convenient to use in some cases as the free hydroxyl in the acceptor glycal only requires that two (rather than four) other alcohols must be protected. This method has the advantage that manipulations at the anomeric centres are unnecessary, since coupling is initiated by attack of an oxidant at the donor glycal. The subsequent coupling in the sequence is straight forward since the disaccharide formed is itself a glycal ready for reaction.[176-178] Alcohols with a high nucleophilicity can lead to 1,2-*trans*-glycosides, while alcohols with low nucleophilicity may also yield small amounts of 1,2-*cis*-glycosides. A drawback of the preparation of 2-deoxy oligosaccharides by the methods described above is the absence of a substituent at C2, i.e.; there can be no neighbouring group participation to control

the stereoselectivity of the glycosidic linkage formed. It is therefore sometimes more practical to prepare 2-deoxy-2-iodo compounds first, which are obtained from addition reactions to glycals (Figure 48).

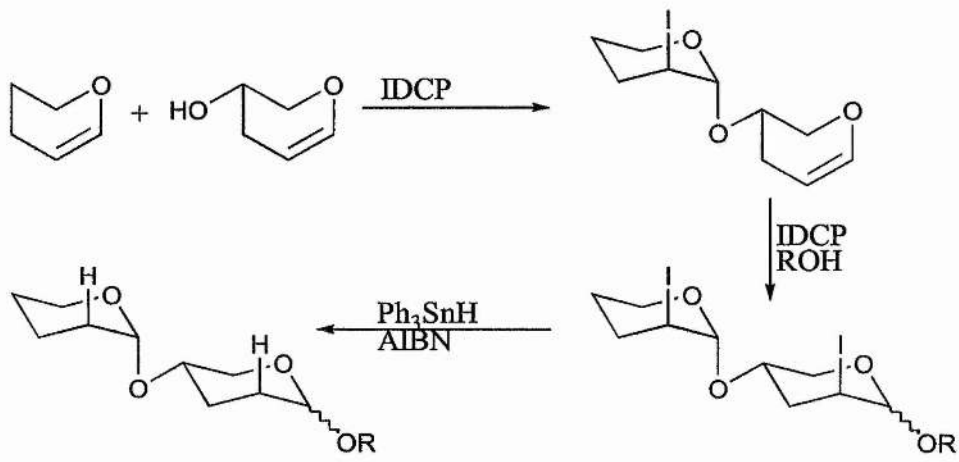


Figure 48 IDCP activation of glycal donors.

Addition of saccharide acceptors to glycals in the presence of bis(2,4,6-trimethyl pyridine)iodonium perchlorate yields 2-deoxy-2-iodo glycosides, from which the iodine can be removed by reduction.[177,179] Glycals may also react with saccharide acceptors in the presence of NIS to commonly give 2-iodo-α-*trans*-glycosides.[180] After cleavage of the iodide by reduction, α-linked 2-deoxy-glycosides are produced.[179] In addition to these methods iodine alone has been shown to efficiently activate glycals for glycosylation via Ferrier reaction in high yield under mild conditions.[181] The reaction results in generation of a glycosidic bond, shift of the double bond from the 1-2 to the 2-3 position and without formation of an iodide product.

2.1.6.g EPOXIDE DONORS

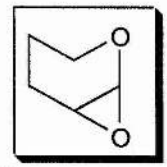


Figure 49 Glycosyl epoxide donors.

Glycals can be made more reactive by oxidation to 1,2-epoxides. The use of 1,2-epoxides as donors was introduced by Lemieux[182] in the work on his sucrose synthesis in the 1950s. More recently Danishefsky[178] investigated this method again with the advent of 2,2-dimethyl dioxirane, a reagent for the epoxidation of enol ethers. This reagent makes it possible to convert glycals into 1,2-epoxides much more easily. These couple with saccharide acceptors yielding 1,2-*trans*-glycosides at low temperatures with anhydrous zinc chloride as the catalyst.[178]

2.1.6.h PHOSPHOROUS BASED DONORS

Figure 50 Glycosyl phosphite and phosphate donors.

Recently it has been found that dialkyl phosphite groups are suitable leaving groups for oligosaccharide synthesis.[183,184] In the presence of Lewis acids (TMSOTf, or TfOH generated *in situ*[183]) dialkylphosphites can be coupled with saccharide acceptors. This method has been employed successfully for the synthesis of *N*-acetyl neuraminic acid glycosides giving good yields of α-glycoside. In addition activation of glycosyl phosphates has also been investigated.[163,185] These may be activated by *in situ* conversion to glycosyl iodides which then react to give glycosylation in moderate yield.

2.1.6.i THIOGLYCOSIDE DONORS

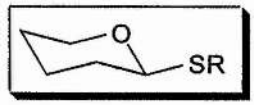


Figure 51 Thioglycoside donors.

Although known for many years,[186,187] until relatively recently thioglycosides were not widely used in oligosaccharide synthesis. Thioglycosides are amenable to the reactions usually found in glycoside synthesis and are adaptable, allowing flexible strategies for the synthesis of oligosaccharides.[129] The versatility of thioglycosides in carbohydrate chemistry arises because the sulfur atom in a thioglycoside is a soft nucleophile, and is therefore able to react with soft electrophiles, such as heavy metal cations, halogens or alkylating reagents.[131,188] The anomeric sulfur can then be selectively activated with a soft electrophile, to give a reactive glycosylating species that may form a new glycosidic bond. The first conversion of a thioglycoside into a glycosylating agent was reported in 1948 by Bonner,[186] who used bromine in acetic acid to convert *O*-acetylated monosaccharide thioglycosides into glycosyl 1-acetates, *via* an initially formed glycosyl bromide (Figure 52). The activation of thioglycosides is discussed in more detail in section 2.2.

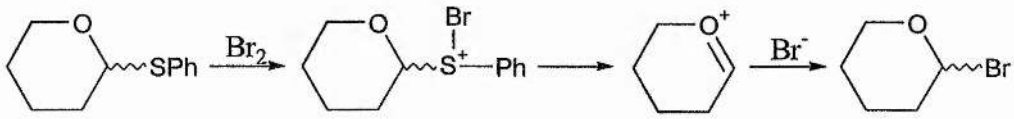


Figure 52. Thioglycoside activation with bromine.

2.1.6.j SULFOXIDE DONORS

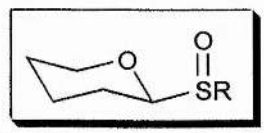


Figure 53 Glycosyl sulfoxide donors.

As shown by Kahne,[189-192] glycosyl sulfoxides (prepared from the corresponding thioglycosides) can be converted to reactive glycosylating agents, using triflic anhydride as promoter. Several oligosaccharides have been synthesised by this method,[193] which has also been used in intramolecular glycosylations, and more recently, in solid phase synthesis of oligosaccharides.[192] They may also be used as precursors to glycals.[52]

2.1.6.k SULFONE DONORS

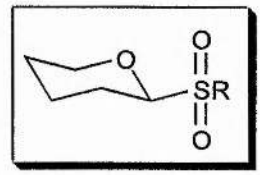


Figure 54 Glycosyl sulfone donors.

Preliminary experiments with the less reactive glycosyl sulfones have also been performed, using magnesium bromide etherate as a promoter.[194] Reactions of cyclic ether phenyl sulfones (as analogues of more complex saccharides) with magnesium bromide etherate gave high yields. Most notable from a carbohydrate point of view was the mildness of the procedure, allowing (at room temperature) the presence of double and triple bonds, acetals and carbonyl functionalities[194] without their reaction.

2.1.6.l XANTHATE DONORS

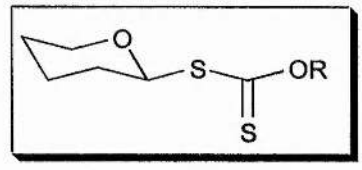


Figure 55 Glycosyl xanthate donors.[184,195-198]

S-Xanthates may be used as donors being activated by DMTST[195-198] methylsulfenyl triflate,[144] or copper(II)triflate,[195-198] to couple with an acceptor to

give disaccharides in good yield.[144,195-198] *S*-Xanthate donors have been used successfully in syntheses with unprotected acceptors giving selective glycosylation[195] thus allowing more efficient synthesis with fewer steps. In specific cases[196] *S*-xanthates have been activated for glycosylation with the single electron transfer reagent tris(4-bromophenyl)ammoniumyl hexachloroantimonate to give coupling in good yield.

2.1.6.m SELENIDE DONORS

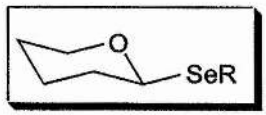


Figure 56 Glycosyl selenide donors.

The use of phenyl selenoglycosides as glycosyl donors in glycosylation reactions was first described by Mehta and Pinto.[199] They have since been used in a variety of ways.[194,200] Phenyl selenoglycoside donors are crystalline, odourless and stable to storage. They may be activated with silver triflate and the activation may be suppressed with proton acceptors like collidine and 1,1,3,3-tetramethyl urea while anhydrous bases such as potassium or silver carbonate do not prevent reaction from occurring. Photochemical synthesis of a disaccharide has also been achieved by U.V. activation of a methoxy protected phenyl selenoglycoside,[200] illustrating a novel variation on conventional glycoside synthesis.

2.1.6.n TELLURIDE DONORS

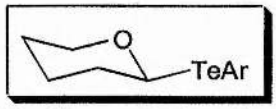
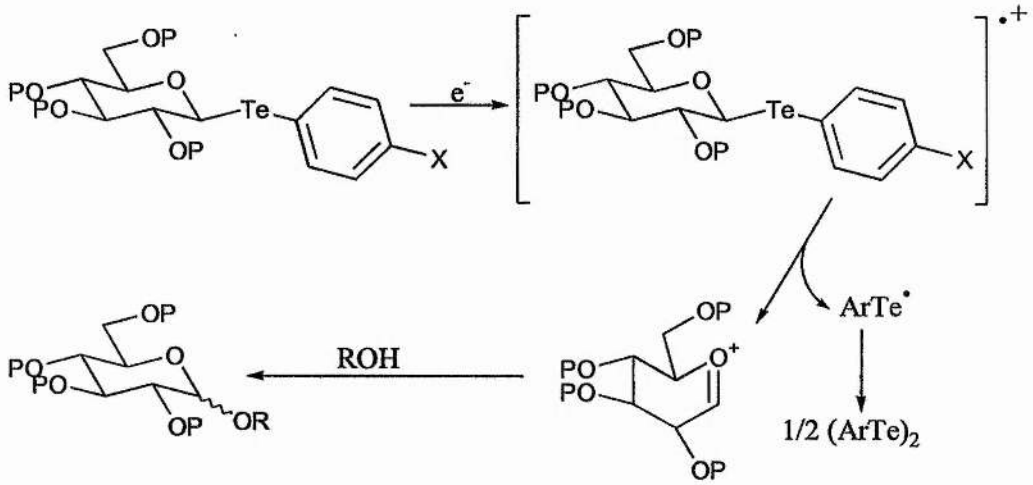


Figure 57 Glycosyl telluride donors.

Tellurium glycosides are prepared from sodium aryl telluride and the relevant glycosyl bromide.[201] The oxidative activation (*via* a short lived radical cation) of

these has been achieved, in good yield, using electrolysis in the presence of an acceptor alcohol in a 0.1M solution of lithium perchlorate (the electrolyte) in acetonitrile (Figure 58).[201]



P = Bz or Bn; X = H, Me, OMe or NMe_2

Figure 58 Oxidative activation of tellurium glycosides.

The efficiency and stereocontrol of these reactions is heavily influenced by the *p*-substituent on the aryl telluride and their reactivity (due to the difference in oxidation potential) is the opposite of that normally observed – i.e.: "armed" benzyl protected telluroglycosides are less reactive than "disarmed" benzoyl protected telluroglycosides.[201,202] This raises interesting possibilities for oligosaccharide synthesis, using conventionally "disarmed" saccharides as "armed" donors.

2.1.7 ORTHOGONAL ACTIVATION

Given the "armed"/"disarmed" approach, synthesis of oligosaccharides is made somewhat easier. However, in the stepwise approach to the synthesis of oligosaccharides, in which one monosaccharide unit is added at a time to a growing molecule, it inevitably becomes necessary to perform protecting group manipulations on large oligosaccharides. Block condensations have an

advantage over stepwise additions as manipulations are kept to a minimum, instead being performed on smaller fragments in which blocks of di- or trisaccharides are coupled. However block condensations raise the problem of activating donor oligosaccharide building blocks selectively in the presence of others. Here the anomeric groups may be used as protecting groups until they are required as donors. Hence activation of the anomeric centre should not require many steps or better, should be direct. A good anomeric blocking group must be stable under the reaction conditions used in the synthesis leading up to the point requiring its' selective removal or transformation. This is important where deblocking is performed late in a long synthetic sequence, a bad choice of anomeric substituent may mean that modifications cannot be made. Given the concerns described above, there are therefore cases where using more than one type of glycosyl donor during a synthesis is advantageous. These typically arise when two or more potential donors are present in the same glycosylation reaction – obviously activation (orthogonal activation) of one without any affect on the other is desirable.

The concept of orthogonal activation led to investigation into the versatility of phenyl selenoglycoside donors. Selective activation of both "disarmed" and "armed" phenyl selenoglycosides over "armed" ethyl thioglycoside acceptors was demonstrated.[199] Additionally selective activation of glycosyl bromide donors over phenyl selenoglycosides with silver triflate in the sequential synthesis of a trisaccharide[199] confirmed of the value of this strategy. Selectivity was also noted in the activation of α-glycosyl trichloroacetimidate donors in the presence of selenoglycoside acceptors with triethylsilyl triflate.[199] Phenyl selenoglycoside donors were also used by Ley[151] in the presence of anomeric fluorides – the fluoride being activated with silver triflate and Cp_2HfCl_2, leaving the phenylseleno function untouched. This product was then in turn activated with NIS/TfOH to couple with an acceptor bearing a thioethyl group which remained intact (Figure 59).

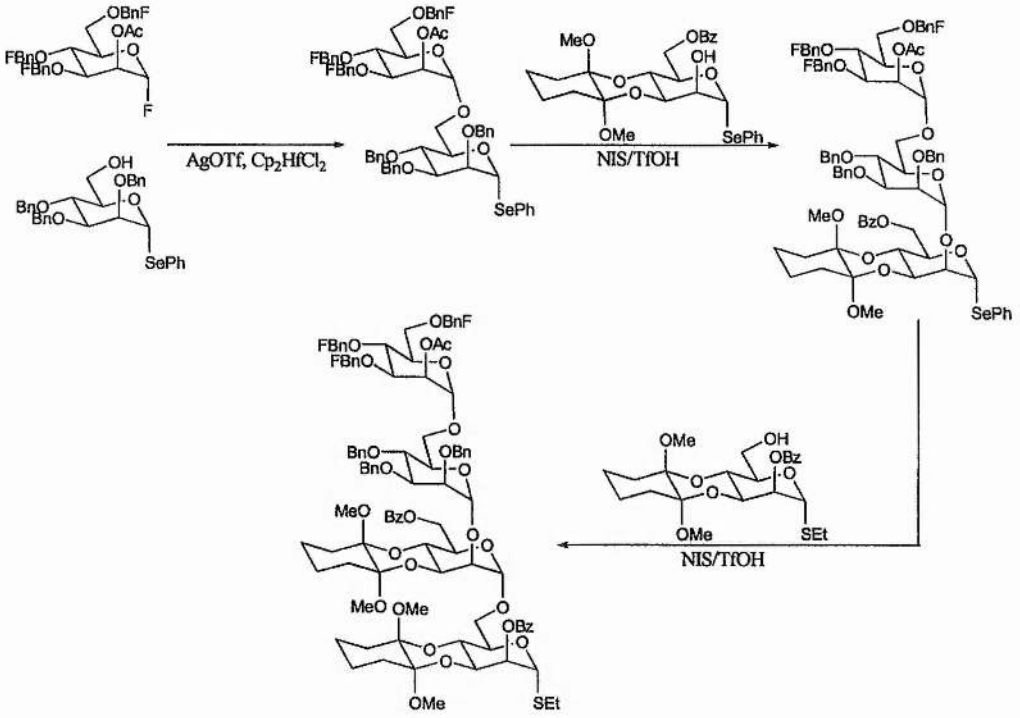


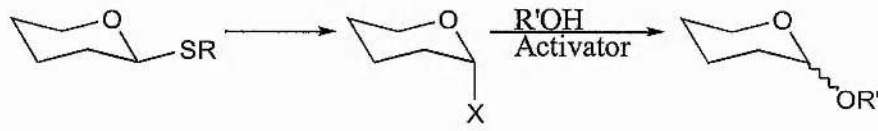
Figure 59 An example of orthogonal oligosaccharide synthesis.

Although the thioglycoside functionality is introduced in the final step in the synthesis above, thioglycoside donors may also be used in conjunction with fluoride donors in orthogonal synthesis.[203] As with selenoglycosides, thioglycosides are stable to the mild conditions that activate fluoride donors.[203]

2.2 DEVELOPMENT OF THIOGLYCOSIDE CHEMISTRY

2.2.1 TWO-STEP HALIDE ACTIVATION OF THIOGLYCOSIDES

The oldest method of thioglycoside activation is by conversion to a glycosyl halide (chloride, bromide and fluoride). The halide is then activated either with or without isolation, to allow glycosylation in the presence of a halophilic reagent and an acceptor.[186,204,205] Suitable halophiles are heavy metal salts (Figure 60) used in conditions mild enough to leave sensitive protecting groups such as benzylidene acetals unaffected. However, with some oligosaccharide thioglycosides difficulties can be encountered in preparing such halides in good yields without side-reactions.



X = Br, Cl, F

Figure 60 Two-step activation of thioglycosides.[205]

The advantage of the two-step procedure is that thioglycoside acceptors as well as donors can be used. Such acceptors cannot be used if "one step" activation (i.e., with methyl triflate[144,207] or DMTST[208,209]) is used, since the acceptor thioglycosides are also affected by the activating reagent.

2.2.2 SINGLE-STEP HALIDE ACTIVATION OF THIOGLYCOSIDES

Bromides can also be used in single-step activation of thioglycosides being generated *in situ* from bromine and reacted immediately in the presence of a suitable promoter (eg: AgOTf or $HgCN_2$).[210] However most direct activation of thioglycosides is mediated by a highly reactive promoter, common examples of which are given in Figure 61.

Activator (in order if reactivity)	**Disadvantages**
PhSeOTf[165]	Toxic
DMTST[209,210,212]	Prepared with MeOTf
MeSTf[188]	
MeSBr[210]	Reaction may be slowed by formation of anomeric bromide
MeOTf[144,207]	Highly toxic, may cause *O*-methylation
NIS/TfOH[213]	Corrosive
NIS[213]	Toxic
NBS/TfOH[214]	Corrosive
IDCP[215]	Potentially explosive, low stereocontrol
Br_2[186,210]	Requires heavy metal salts
NBS[216-218]	Not reactive enough for most applications
Heavy metal salts[155]	Toxic, not reactive enough for most applications

Figure 61 Single-step activation of thioglycosides.

Modern single-step activation of 1-thioglycosides[129,209] usually involves the formation of a reactive sulfonium intermediate through a 2-electron process, taking advantage of the well know affinity of the sulfide group for soft electrophiles (heavy metal salts). Ferrier[155] attempted to activate thioglycosides with mercury salts with limited success in order to utilise them for glycoside synthesis to form a disaccharide derivative. Methyl trifluoromethanesulfonate (methyl triflate) was the first efficient non-metal ion electrophile to be used for thioglycoside glycosylations.[144,207] The thioglycoside sulfur is methylated to form an intermediate sulfonium ion even in the presence of a glycosyl acceptor hydroxyl group. The glycosyl sulfonium ions (or ions derived from them) are good glycosylating reagents. The yields are very good and the stereochemical outcome follows the same general rules as with other donor/promoter combinations.

A safer and faster alternative to methyl triflate is dimethyl(methylthio)sulfonium trifluoromethane sulfonate (DMTST).[209] The

presence of tetrabutyl ammonium bromide in these reaction mixtures may give an *in situ* conversion of the thioglycoside into the corresponding bromide which gives slower but highly stereoselective halide-ion promoted formation of a *cis*-1,2-glycoside.[209] Presently DMTST, with its less nucleophilic triflate counter ion, is the most extensively used sulfenium ion like reagent (other examples being methyl sulfenyl bromide and methyl sulfenyl triflate) a similar more reactive reagent is phenyl selenyl triflate.[161,165,188,208-211] Sulfenium ion like reagents presumably alkylsulfenylate the thioglycoside to form a reactive intermediate (Figure 62).

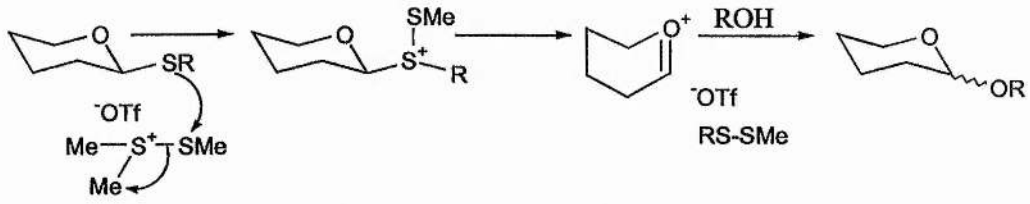


Figure 62 Thioglycoside activation by DMTST.

An alternative to the above methods is halonium ion activation of thioglycosides. Examples of halonium type electrophiles are the halogens[210] themselves, *N*-iodo succinimide/triflic acid (NIS/TfOH)[213] and (iodonium di-sym-collidine perchlorate) IDCP.[215] While IDCP contains an iodonium species, the NIS/TfOH reagent generates iodonium ions *in situ* by protonation of the NIS imide carbonyl which allows release of the iodonium ion (Figure 63).[148]

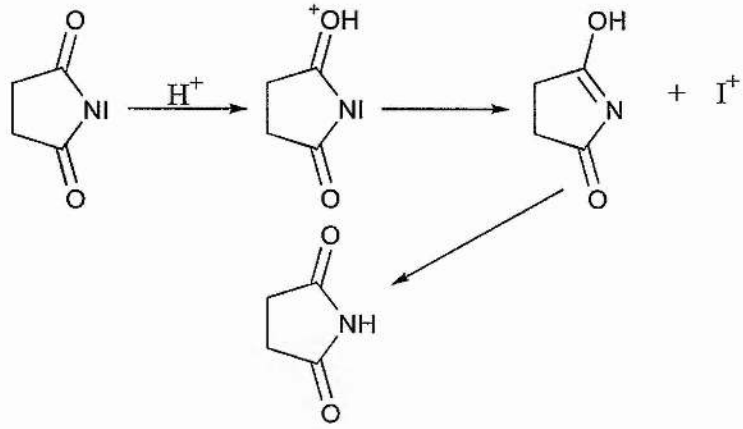


Figure 63 Iodonium ion generation from NIS/TfOH.

Because few side reactions have been reported, NIS/TfOH has become the most popular halonium type glycosylation reagent in recent years.[165] The use of NBS[214] or NIS[213] together with triflic acid is effective for activation of "armed" and "disarmed" thioglycosides. These reagents are useful when thioglycoside acceptors are to be glycosylated with thioglycoside donors representing an alternative to two-step glycosylations.

2.2.3 OTHER PROMOTERS FOR THIOGLYCOSIDE ACTIVATION

The reactivity of methyl triflate implied that other alkylating agents could also act as good glycosylation promoters. Indeed, trimethyloxonium tetrafluoroborate can be used to activate thioglycosides, but has few advantages over methyl triflate.[188] Methyl iodide in combination with 2-pyridyl thioglycosides also promotes glycosylations.[219] Production of 2-*N*-methyl thiopyridone and its salt indicates that activation of the 2-pyridyl thioglycoside by MeI is initiated by *N*-methylation.[219]

NBS itself can activate thioglycosides[211-213] but has not been used in practical synthesis since the rate of glycosidation reaction with this reagent alone is not sufficiently high even for reactive, so-called "armed" thioglycosides.[220] The use or release of triflic acid in NBS reactions in many instances renders reaction media strongly acidic, which causes problems in reactions with acid labile substrates. However a similar activation effect can be obtained by using neutral

salts of strong acids such as triflates or perchlorates.[220] Stereoselectivity of the glycosylation of thioglycosides using such salts under mild and neutral conditions has been extensively studied by Fukase.[220] Results from some of these studies using NBS or iodosobenzene with a catalytic amount of various strong acid salts are illustrated in Figure 64 and Figure 65. In all cases the donor is methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside and the acceptor is methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside.[220]

Additive[a,d]	Solvent	Time	%	α:β
Ph_2IOTf	CH_3CN	<1 min	90	1:12
Bu_4NOTf	CH_3CN	<1 min	98	1:13
Bu_4NClO_4	CH_3CN	<1 min	97	1:12
None	ether	45 h	60	1:0.11
None[b]	ether	50 min	44	1:0.02
$LiNO_3$[b]	ether	1 h	60	1:0

a) 1.5 x NBS, 0.5 x additive, donor/acceptor;1:0.8; b) 200 mg silica; c) 0.05 x additive; d) other salts tried: KOTf, $KClO_4$, $NaClO_4$, $Mg(ClO_4)_2$, $LiBF_4$, LiOTf, $LiClO_4$

Figure 64 Studies on activators for thioglycosides.[220]

NBS/salt promoted reactions are very fast in many cases but lack stereocontrol. In the studies with iodosobenzene (Figure 65) the effect of solvent on anomeric stereocontrol is distinct and illustrates clearly the role solvents play in determining the anomeric configuration of glycosylation products. For both types of system most reactions were fast and their yields were good.[220]

Additive[a]	Solvent	Time (Average)	α:β
TMSOTf, Tf_2O, TfOH, $Sn(OTf)_2$,	CH_3CN	5-20 mins.	6.4:94
$Sc(OTf)_3$, $Yn(OTf)_3$, $Lu(OTf)_3$	CH_3CN	5-20 mins.	6.4:94
Tf_2O, TfOH, $Sn(OTf)_2$, $Sc(OTf)_3$, $Yn(OTf)_3$	$(CH_2Cl)_2$	5mins-24 hrs	0:100

a 1.3 x PhIO + 0.6 x additives

Figure 65 Investigations on the effect of solvent on reactivity and stereocontrol.

A variety of reagents other than those mentioned above have been used for the activation of thioglycosides. These include nitrosyl tetrafluoroborate, triflic anhydride and radical cations.[188] Activation of thioglycosides can also be effected by anodic oxidation of arylthioglycosides (electrochemical glycosylation).[221,220] The mechanism involves the formation of a radical cation that cleaves into a glycosyl cation and an arylthiyl radical (which dimerises to form a diaryl to sulfide).[220] The glycosyl cation, in the presence of an alcohol, reacts to form a glycoside or, under certain conditions, the glycosyl fluoride (presumably by reaction between the glycosyl cation and the tetrafluoroborate counter ion from the $Bu^n_4NBF_4$ electrolyte).[188] *O*-Acylated, *O*-benzylated and unprotected thioglycosides can be used in this type of electrochemical glycosylation.

2.2.4 STEREOCONTROL OF THIOGLYCOSIDE REACTIONS

While available thioglycoside activating reagents have increased in number over the last decade, the reaction mechanisms that govern stereocontrol with use of these reagents are not well understood. Anomerisation reactions are known for halides,[223,224] thioglycosides,[224] various *O*-glycosides[224] and glycosylation of fully unprotected sugars in the Fischer-Helferich procedure.[131] Despite this knowledge until recently the only in depth study on anomerisation processes that had been done was with halides.[113,201] More recently Boons[225] reported that treatment of thioglycosides with a catalytic amount of iodonium ions may result in anomerisation and that the nature and size of the *S*-substituent affects the anomerisation process. Methyl and ethyl thioglucosides gave anomerisation with equilibrium favouring the α-anomer. A similar anomeric ratio was obtained when the corresponding α-anomers were treated with IDCP, demonstrating that a thermodynamic equilibrium had been reached.[225] Prolonged treatment of α or β benzylic tetrahydronaphthyl thioglycosides and a bulky *t*-butyl thioglycoside with IDCP gave no significant anomerisation, though some decomposition was observed. Thus increase in the steric bulk of the leaving group results in prevention of anomerisation or at most incomplete reaction. Additionally it was

shown by ^{13}C labelling studies that anomerisation proceeds by intermolecular exchange of alkylthio groups (Figure 66).[225]

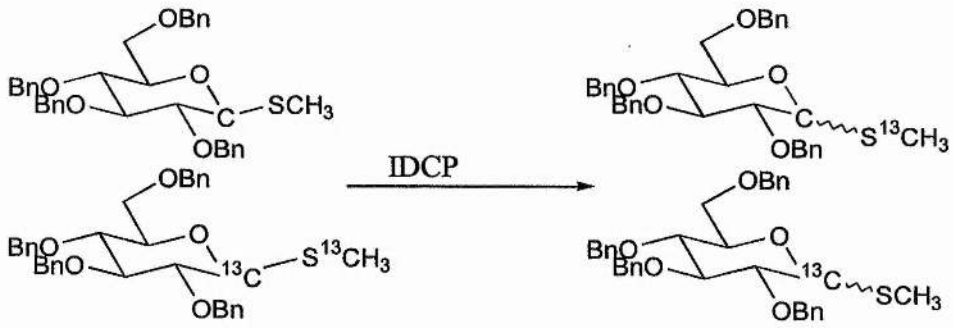


Figure 66 ^{13}C Labelling studies on anomerisation catalysed of iodonium ion thioglycosides.

These results indicated that anomerisation was important for the stereochemical outcome of glycosylation. Activation of an anomeric thio-group by iodonium ions may result in the formation of a solvated carboxonium ion, the reactive intermediate during glycosylation. On the other hand during glycosylation alkyl sulfenyl iodide or a derivative may be associated with the activated species. If this type of intermediate can adopt α and β configurations which are in rapid equilibrium then the stereochemical course of a glycosylation may be controlled by such a process. In the latter case the *S*-substituent of the thio-group should affect the stereochemical outcome of a glycosylation although anomerisation by other processes should not be ruled out. In the case of a bulky thio group, activation with iodonium ions will probably result in the formation of a solvated carboxonium ion which undergoes glycosylation.[225]

2.3 PROJECT BACKGROUND

2.3.1 IODINE AS A THIOGLYCOSIDE ACTIVATOR

Looking at the majority of common glycoside donors and methods of activating them, one can easily see that there is no single, ideal system. Most of the reagents are either corrosive, carcinogenic, explosive, produce an unbearable stench, or are light sensitive. They are all sensitive to moisture and are expensive. Alternative cheap and easy-to-handle reagents would therefore be welcome. In light of the methods employed to activate thioglycosides previously described, consideration of the promoters IDCP and NIS gave rise to interest in their mechanism of activation. They release iodonium ions *in situ*[148] which when attacked by a sulfur-based nucleophile form the respective sulfonium ion that rapidly fragments to give an oxocarbonium ion and sulphenyl iodides. Considering the work of Kihlberg[209] and the seminal observations by Bonner[186] on the cleavage of a thiophenyl glycoside with bromine, it seemed reasonable, therefore, to consider the potentially analogous reactions with iodine. Even in the absence of iodonium ions, in an "armed" thiomethyl glycoside the nucleophilicity of the sulfur atom should be sufficient to result in it attacking a molecule of iodine directly, resulting in release of iodide and oxocarbonium ion formation (Figure 67). In the presence of an alcohol this could lead to the formation of a new glycosidic bond.

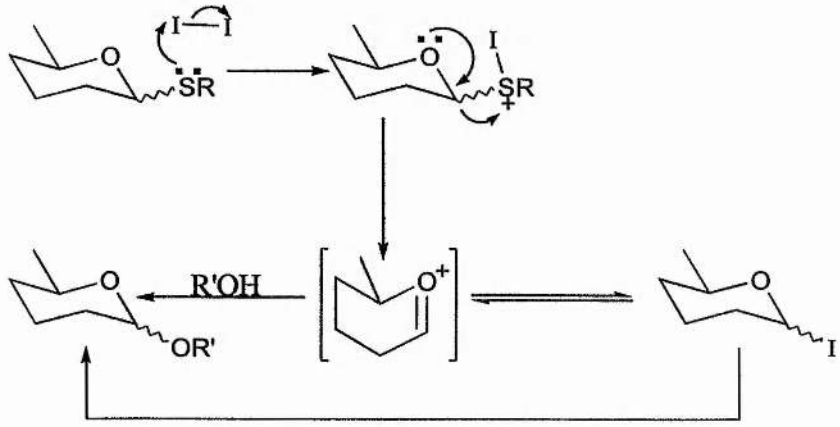
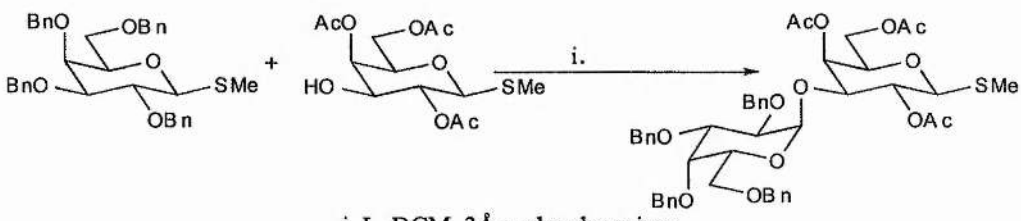


Figure 67 Proposed mechanism of iodine activation of a thioglycoside.

There are a range of reagents other than $I_{2(s)}$ which are available for many applications, as discussed earlier there are many iodonium ion sources (e.g.: NIS, IDCP as used in thioglycoside activation), there are also several hypervalent iodine reagents (e.g.: [bis(trifluoroacetoxy)iodo] benzene)[226] used, for example in the formation of sulfur heterocycles. Were iodine to activate well however, it would clearly be far superior to these reagents in terms of cost, safety and environmental friendliness. To this end, recent work by the Field group has demonstrated that a number of different reactions can be facilitated by the use of iodine.[227-229] Iodine was shown to activate both thiomethyl glycosides and glycosyl halides efficiently for glycosylation.[227, 229] The mechanism of iodine activation of thioglycosides is unknown, however 0.5 x I_2 still gives complete reaction (albeit slower than for 1 equivalent or more).[227] It is thus possible that the MeSI generated *in situ* also activates the thioglycoside donor.

2.3.2 IODINE MEDIATED ACTIVATION OF "ARMED" THIOGLYCOSIDES

As was shown by the Field group, benzyl protected methyl 1-thio-β-galactoside is highly reactive towards iodine in the presence of an acceptor; for simple alcohols reactions can take as little as 20 minutes.[227] Importantly, disaccharides were easily obtained in high yield using iodine as the promoter and reaction conditions were amenable to the use of a variety of protecting groups on the acceptors.[227] Additionally it was shown that a "disarmed" thioglycoside acceptor could be glycosylated with an "armed" thioglycoside donor[227](Figure 68).



i. I_2, DCM, 3Å molecular seives

Figure 68 Iodine mediated disaccharide synthesis using an "armed"/"disarmed" approach.

2.3.3 IODINE MEDIATED ACTIVATION OF "DISARMED" HALIDES

The Field group also showed that iodine could activate acetyl protected, "disarmed" glycosyl halides to conveniently give simple glycosides in high yield.[229] As with the "armed" thioglycosides, the "disarmed" halides reacted quickly with simple alcohols under iodine promotion in as little as 5 minutes. The mechanism of glycosyl halide activation by I_2 is not known, however it is possible that iodine acts as a halophile, resulting in iodobromonium ion formation, followed by fragmentation to give IBr or ICl and a carbohydrate derived oxocarbonium ion (Figure 69).

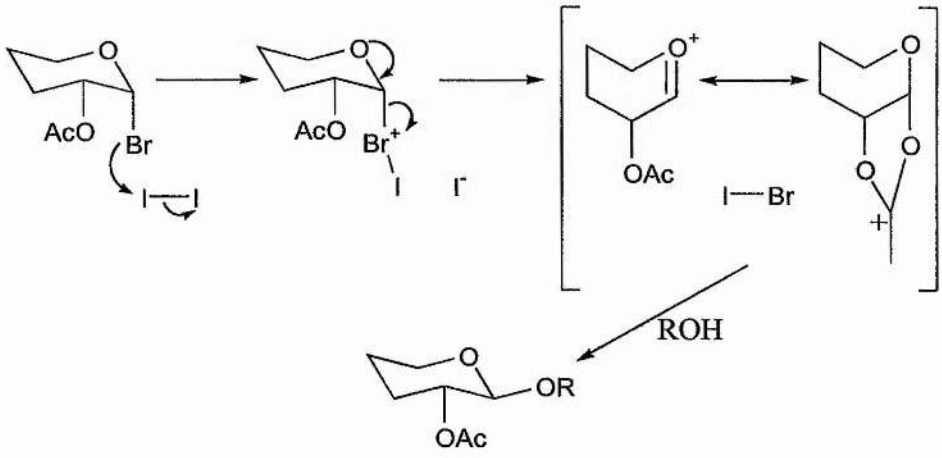


Figure 69 Proposed mechanism for iodine activation of glycosyl halides.

There may, or may not, be reversible trapping of the carbonium ion by the released iodide ion to give a reactive glycosyl iodide, which might be expected to undergo spontaneous alcoholysis. While sodium iodide[230] in acetone is known to convert acetobromoglucose into the corresponding iodide[171] substitution of sodium iodide for iodine in this case does not, however, cause facile methanolysis.[229] This indicates that direct displacement of bromide by iodide is not the key step in this activation process. The potential liberation of I-Br (a more potent I^+ source than iodine) may explain efficient methanolysis with only 0.5 mol equivalents of iodine.[229]

2.3.4 SINGLE ELECTRON TRANSFER REAGENTS

The powerful oxidants[231] DDQ, CAN and CAS are also known for their ability to act as electron transfer reagents.[232,233] Oxidation by DDQ may proceed by either ionic or radical means[234] and DDQ has been used in the selective oxidative cleavage of 4,6-*O*-*p*-methoxybenzylidene acetals, which is thought to proceed by an ionic mechanism.[234,235] DDQ is thought to behave as a Lewis acid in some cases[236] and during the preparation of carbohydrate isopropylidene mixed acetal derivatives (in which catalytic amounts of DDQ are used) it was suggested that an ethereal oxonium type species may be generated.[237] In some cases DDQ reactions are proposed to proceeded via a charge-transfer complex.[238] CAN is one of the strongest oxidants known,[231] it is a single electron oxidant that may sometimes give nitration products. CAS may be used where nitration with CAN is a problem. CAN has a wide variety of uses including the generation of radicals.[239] Interestingly a CAN-I_2 system has been successfully used for the oxidative aromatisation of cyclohexenones; CAS was also shown to carry out this function, though with lower yields.[240] Our group has shown that DDQ improves the rate and efficiency of iodine promoted glycosylations.[229] While DDQ alone does not activate either thioglycosides or glycosyl halides, addition of DDQ or CAN was shown to improve the efficiency of I_2 activated halide and thioglycoside glycosylations.[229] Though the mechanism of iodine ± DDQ activation processes are unknown it is possible that DDQ acts as an electron transfer reagent. In view of the potential for electron transfer behaviour on sulfur[241] by DDQ and CAN[233] it could be expected that they might mediate glycosylation as with tris(4-bromophenyl)ammoniumyl hexachloroantimonate (a stable radical cation single electron transfer reagent which is capable of effecting methanolysis of acetobromogalactose) (Figure 70).[196] However, "disarmed" thiomethylglycosides are not activated either by iodine alone, or in combination with DDQ.

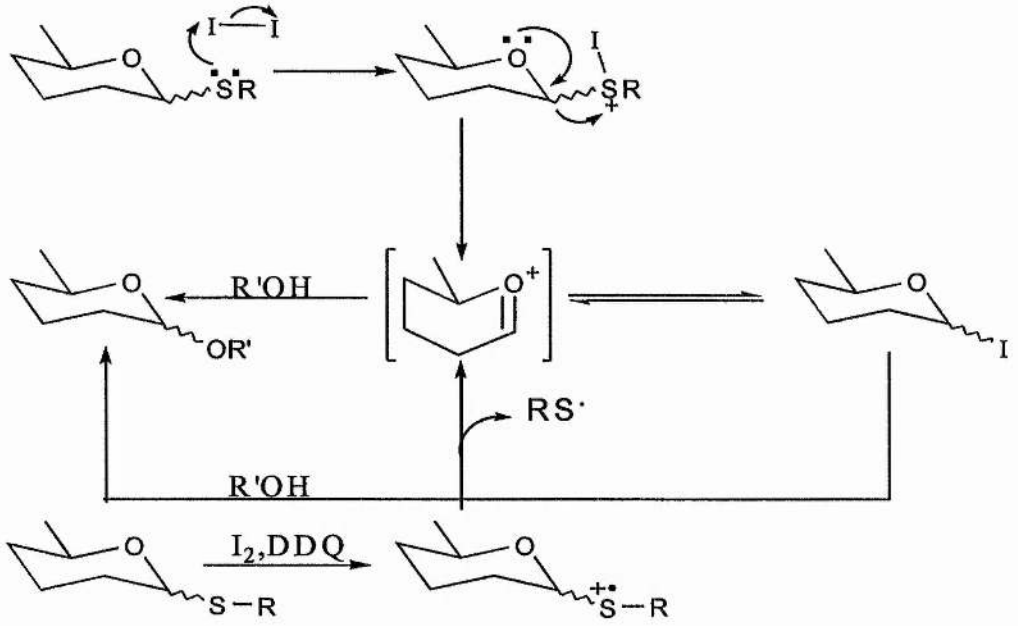


Figure 70 Proposed mechanism of I_2 and I_2/DDQ mediated activation of thioglycosides.

2.3.5 PERIOD 16 ELEMENTS

Before discussing thioglycosides, which are the focus of this section, the elements from Period 16 of the table of the elements require closer discussion, as their properties have some bearing on their use in glycoside synthesis. Importantly from a thioglycoside viewpoint, they may form ionic species with one bond and one negative charge (e.g.: RS^-, HS^-) and species with three bonds and one positive charge (e.g.: R_3S^+). In addition to such divalent species, the elements form compounds in formal oxidation states IV and VI with four, five or six bonds and tellurium may give an eight coordinate ion TeF_8^{2-}. The S-Po elements employ *d*-orbitals together with their *s* and *p*-orbitals to form more than four σ bonds (as in SF_6), and they may also make use of their *dπ* orbitals when forming multiple bonds. Thus sulfur may form hexacoorinate compounds.[244] Although the heteroatomic bonds to sulfur are most important it should be noted that sulfur also has a strong tendency to catenation (i.e.: bonding to itself) to form polymeric structures and disulfides e.g. CH_3SSCH_3.

2.3.6 IODINE CHEMISTRY

The chemistry of iodine in organic synthesis has been explored in a wide range of fields[245-260] often in oxidation (as for Cl_2 and Br_2, I_2 is a two electron oxidant)[250] or iodination[252] reactions. One of the most interesting iodine mediated reactions is solid-phase polystyryl diphenylphosphine-iodine promoted peptide bond synthesis,[254] which couples amino acids in very high yield. Because the reagent is polymer bound the workup is very easy and the conditions are compatible with a wide range of standard protecting groups. More relevant to this project, iodine has been used as a catalyst for the protection[255,256] and deprotection of carbohydrates,[257] deprotection of amines[258] as well as deprotection of hydroxyl functions.[257,259,260]

2.3.7 IODINE-SULFUR CHEMISTRY

Iodine-sulfur chemistry is extensive[261-263] and many reactions and novel compounds may be formed as a result. For example sulfur and iodine may form a three electron intermolecular radical cation species, generated by pulse radiolysis of thiirane, MeI in MeCN during the synthesis of 1-iodo-2-(methylthio)ethane and also during the oxidation of 1-iodo-3-(methylthio)propane[261] (Figure 71).

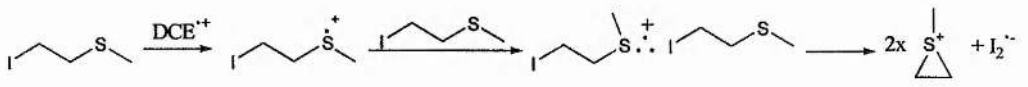


Figure 71 Generation of unusual sulfur-iodine species during radical reactions.

Sulfur-iodine bonds are important in thioglycoside activation. Sulfur-iodine and selenium iodine bonds are unstable probably due to their low ionic resonance stabilisation energies and are even more unstable in the solid state. This instability is demonstrated by compounds such as CF_3SI and CH_3SI which are only stable as gasses at very low temperatures, and it is worth noting with regard

to the mechanisms of thioglycoside activation by iodonium ions, that CH_3SI decomposes to give CH_3SSCH_3 and I_2.

2.4 AIM AND OBJECTIVES

Given the obvious utility of iodine in synthesis the aim of this project was to develop the use of iodine as a cheap, convenient and safe alternative to conventional heavy metal glycosylation promoters in the synthesis of glycosides and potentially, oligosaccharides. Following published work by our group,[227-229] further investigation into the use of iodine as an activator was required. With reference to the previously discussed aspects of thioglycoside glycosylations the aims of this project were thus:

- To determine the effect of the *S*-substituent on the leaving group upon rate of reaction in iodine promoted glycosylations.
- To investigate whether iodine could activate disarmed thioglycosides.
- To investigate the effect of solvents upon the rate of iodine promoted glycosylations.
- To identify activators for unreactive thioglycoside donors.
- To demonstrate the utility of the above methodology for achieving selective glycosylation reactions.

The overall aim of these investigations was to create a series of shelf stable building blocks for oligosaccharide synthesis. Ideally these will be assembled in one pot or on a solid phase support into a linear or branched order which is determined by their reactivity (Figure 72). Reactivity should be controlled by varying the protecting groups, the leaving groups or the promoter.

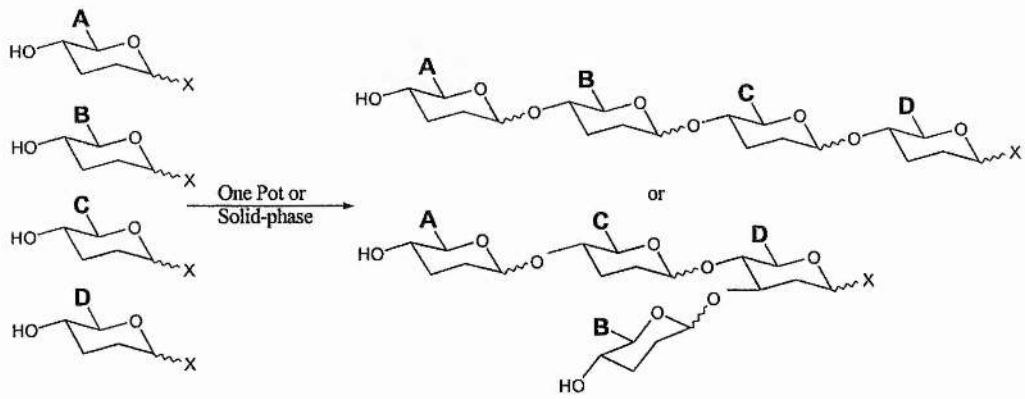


Figure 72 Prospective one pot oligosaccharide synthesis.

2.5 RESULTS AND DISCUSSION

2.5.1 SYNTHESIS OF BUILDING BLOCKS

A range of thioglycoside donors, varying in size and electronic character, were required in order to establish those most suitable for use as building blocks in iodine promoted oligosaccharide synthesis. There are numerous methods for the synthesis of thioglycosides and some of the most common are shown in Figure 73; most of these methods give predominantly 1,2-*trans* derivatives.[188,264] For the synthesis of thiomethyl and thiophenyl glycosides Hanessians' zinc iodide[265] promoted reaction with trimethylsilyl thiol derivatives was chosen as convenient.[211,215,266] Although such TMS reagents are expensive, this method had the fortuitous effect of raising interest in the role of the iodide species in the reaction. Work within this group has subsequently demonstrated that iodine alone may easily and efficiently promote glycosylation to form various thioglycosides from anomeric acetates and various thiols.[267] For the preparation of the *p*-methoxythiophenyl and *p*-nitrothiophenol glycosides, the phase transfer method of Roy[268] was chosen as suitable.[160,162,269]

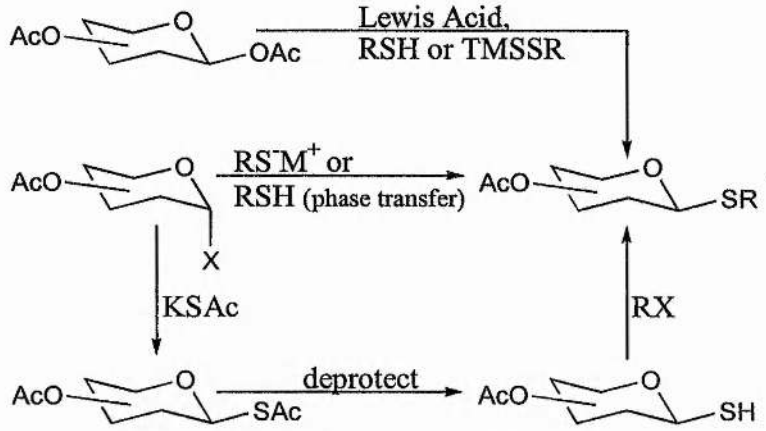
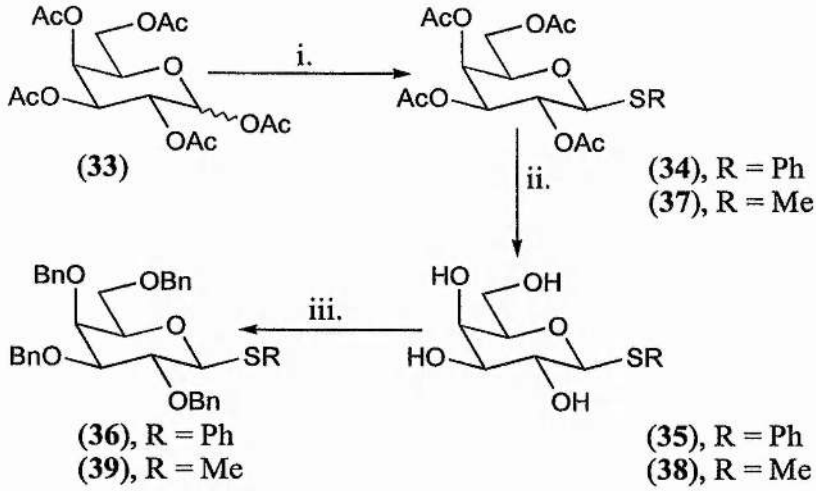


Figure 73 Common methods of preparing thioglycosides.[188,206,209,264-272]

Reaction of penta-*O*-acetyl-D-galactopyranose (**33**) with TMSSPh in the presence of excess ZnI_2 at 50°C gave phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**34**) in 90% yield (Figure 74).[266] Deacetylation of compound (**34**) with sodium methoxide (generated *in situ*) gave phenyl 1-thio-β-D-galactopyranoside (**35**) which was benzylated without further purification to give

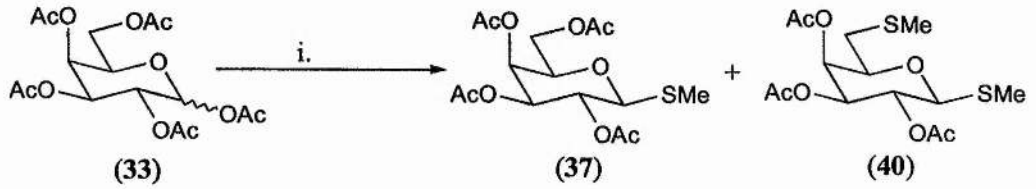
phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**36**) in 98% yield (Figure 74).



R = Ph; i. TMSSPh, DCE, ZnI_2, 50°C (90%); MeOH, NaOMe; iii. THF, KOH, BnBr (98%)
R = Me; i. TMSSMe, DCE, ZnI_2 (90%); ii. MeOH, NaOMe; iii. THF, KOH, BnBr (55%)

Figure 74 Synthesis of (36) and (39) from (33).

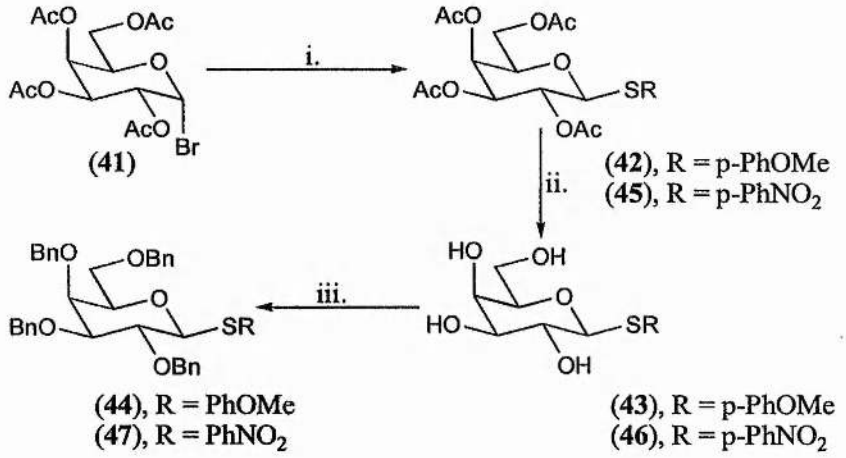
Similar preparation of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) was achieved by reaction of (**33**) with TMSSMe in the presence of ZnI_2 to give methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**37**) in 90% yield. Compound (**37**) was then deacetylated to (**38**) and benzyl protected without further purification as before to give methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) in 55% yield. It is worth noting that in the preparation of thiomethyl galactoside (**37**) the reaction should not be heated and the use of less ZnI_2 and less TMSSMe than the corresponding TMSSPh reaction was required. These modifications were important as otherwise the 6-position of the sugar was also thiomethylated to give methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-methylthio-1-thio-β-D-galacto-pyranoside (**40**) in 44% yield (Figure 75).



i. ZnI_2, DCE, TMSSMe, 50°C [(**40**) 44%], [(**37**) 43%]

Figure 75 Side reaction of (33) during synthesis of (37).

Reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-galactosyl bromide (**41**) (easily obtained by treating D-galactopyranose with 45% HBr/acetic acid)[255] under the phase-transfer conditions of Roy[268] with *p*-methoxythiophenol gave *p*-methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactoside (**42**) in 61% yield. Deacetylation of this compound with sodium methoxide (generated *in situ*) gave *p*-methoxyphenyl-1-thio-β-D-galactoside (**43**) which was used without further purification. This compound was benzyl protected to give *p*-methoxyphenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactoside (**44**) in 94% yield. Analogous preparation of *p*-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactoside (**45**) from the bromide (**46**) gave the required product (**45**) in 63% yield. Deacetylation to (**46**) and subsequent benzyl protection as before, gave *p*-nitrophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactoside (**47**) in 80% yield (Figure 76).



R = *p*-PhOMe: i. DCM, TBAHS, $Na_2CO_{3(aq)}$, *p*-methoxythiophenol (61%); ii. MeOH, NaOMe; iii. BnBr, KOH, 18-crown-6, THF (94%); R = *p*-$PhNO_2$: i. DCM, TBAHS, $Na_2CO_{3(aq)}$, *p*-nitrothiophenol (63%); ii. MeOH, NaOMe; iii. BnBr, KOH, 18-crown-6, THF (80%).

Figure 76 Phase-transfer mediated synthesis of (44) and (47).

2.5.2 RATE OF IODINE MEDIATED ACTIVATION OF "ARMED" THIOGLYCOSIDES

Compounds (**36**), (**39**), (**44**), (**47**) and benzyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**48**) were reacted with methanol under various conditions to assess the utility of iodine as an activator for thioglycosides.[127,129] The reactions were chosen to establish optimum conditions for selective activation of the various "armed"[148-150] benzyl protected thioglycosides. These conditions revealed distinct reactivity differences between the donors (Figure 77), ranging from the thiomethyl glycoside (**39**) (most reactive) to the *p*-nitrothiophenyl glycoside (**47**) (least reactive). While yields are good, stereoselectivity is modest as shown on Figure 77.

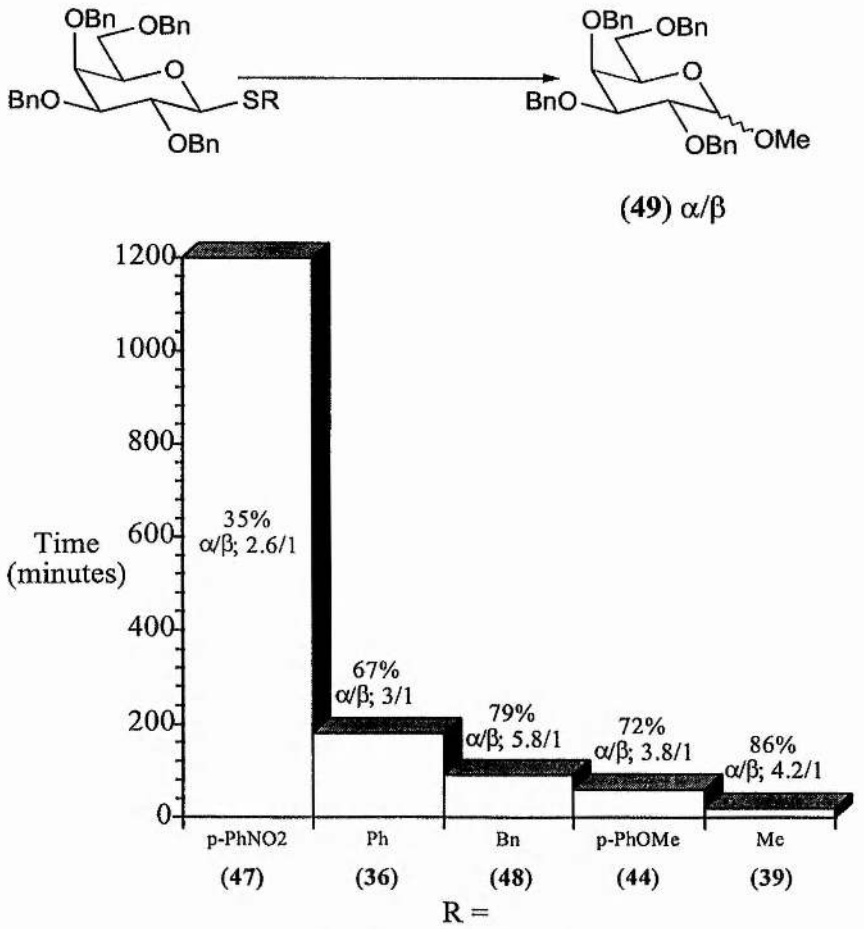
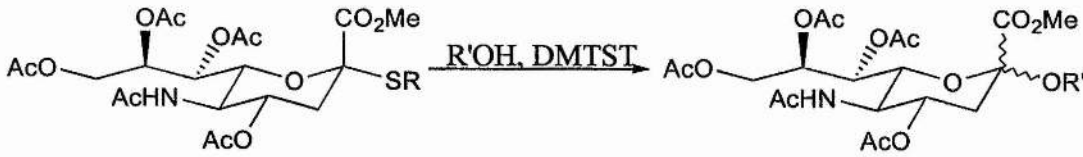


Figure 77 Methanolysis promoted by iodine in DCM.
Conditions: I_2 (2 equivalents), MeOH (100 equivalents), DCM: N.B. (**47**) does not react completely.

Results were in accord with the work of Roy[210,268] on sialosides (Figure 78).



Donor (R')	Solvent	Time	Yield	α/β
SMe	DCM	1 h	82	60:40
SPh	DCM	24 h	81	60:40
S-*p*-MeOPh	MeCN:DCM	8 h	80	55:45
S-*p*-NO_2Ph	MeCN:DCM	no reaction	-	-
S-*p*-NHAcPh	MeCN:DCM	0.5	81	75:25

Figure 78 Difference in reactivity of thioglycoside derivatives of sialic acid.

The nature of the *S*-substituent of a thioglycoside was expected to affect the rate of reaction during glycosylation. For example, 4-nitrothiophenyl substituted sugars are inert towards the thiophilic promoters DMTST and NIS/triflic acid due to the presence of the electron withdrawing nitro substituent on the thioaryl moiety. However, conversion of the nitro group into an electron donating NH-acetyl function by reduction and subsequent acetylation gives an active glycoside which can be condensed under the influence of DMTST, with a suitable acceptor (ROH).[210,274] Corroborating the importance of the *S*-substituents effect on reactivity is Boons report that the size of *S*-substituent effects the reactivity of thioglycosides (see section 2.1.5).[152]

2.5.3 IODINE/DDQ MEDIATED ACTIVATION OF THIOGLYCOSIDES

Since our group has shown that addition of DDQ to iodine mediated reactions can improve their rate and efficiency, comparison of this system with purely iodine mediated reactions was of interest. Thus thioglycosides (**36**), (**39**), (**44**), (**47**) and (**48**) in DCM as solvent were activated with iodine and DDQ. As expected this system activated the thioglycosides significantly faster (Figure 79) than with iodine alone. Importantly iodine/DDQ mediated activation retained the previously observed differences in relative reactivity of the thioglycosides.

Yields were generally good but stereocontrol was still only modest with the exception of nitrophenylthioglycoside (**47**) which gave exclusively the α-anomer.

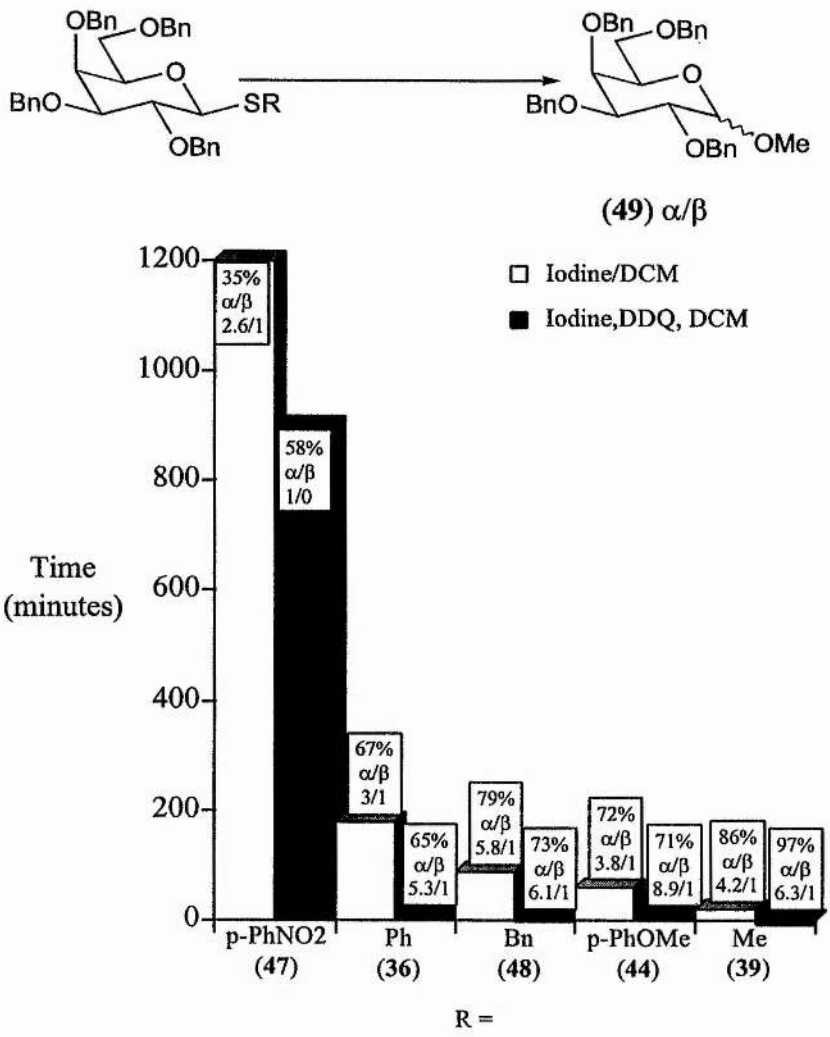


Figure 79 Methanolysis promoted by iodine or iodine and DDQ in DCM.

Conditions: I_2 (2 equivalents), DDQ (1 equivalent), MeOH (100 equivalents), DCM: N.B. (**47**) does not react completely.

As the solvent acetonitrile may effect the stereochemical outcome of glycosylation reactions,[131,145,146] investigation of its effect on iodine/DDQ mediated glycosylations was clearly relevant. Using dry acetonitrile as solvent with iodine and DDQ as activators, the benzylated thioglycosides (**36**), (**39**), (**44**),

(47) and (48) glycosylated considerably faster than in DCM. Increase in rate was most noticeable with the *p*-nitrothiophenyl glycoside (47), reducing reaction time from 15 hours to 1 hour 20 minutes (Figure 80).

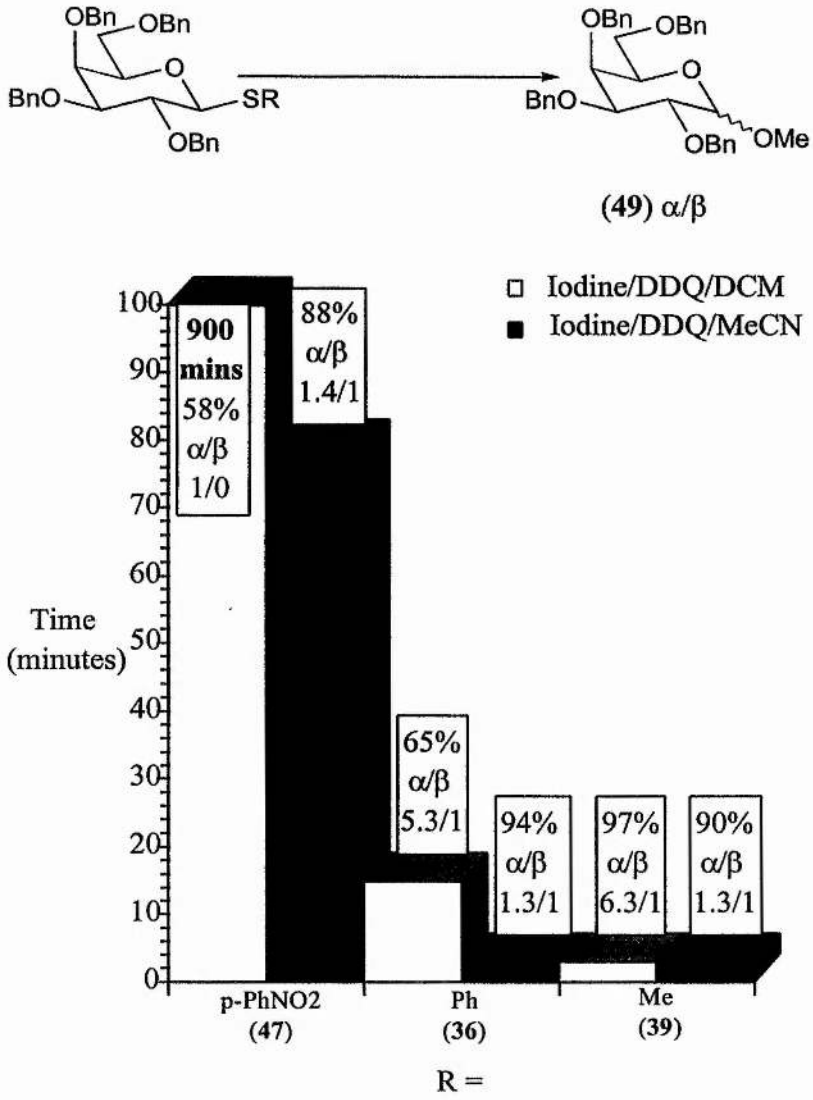


Figure 80 Methanolysis promoted by iodine and DDQ in DCM or MeCN.

Conditions: I_2 (2 equivalents), DDQ (1 equivalent), MeOH (100 equivalents), MeCN

As expected the stereochemical outcome of the reaction was different with MeCN than with DCM, increasing the percentage of β-anomeric products in MeCN.

2.5.4 IODINE MONOHALIDE ACTIVATION OF THIOGLYCOSIDES

As with iodine,[229] IBr has been shown by the Field group to activate thioglycosides for glycosylation.[275] IBr also activates halides for disaccharide synthesis.[275] Since IBr is more potent than either iodine or iodine/DDQ the rate of activation of thioglycosides with IBr was investigated.[275] IBr promoted glycosylation reactions with thioglycosides **(36)**, **(39)**, **(44)**, **(47)** and **(48)**, using DCM as solvent and methanol as acceptor were seen to be considerably faster than all for previous conditions (Figure 81).

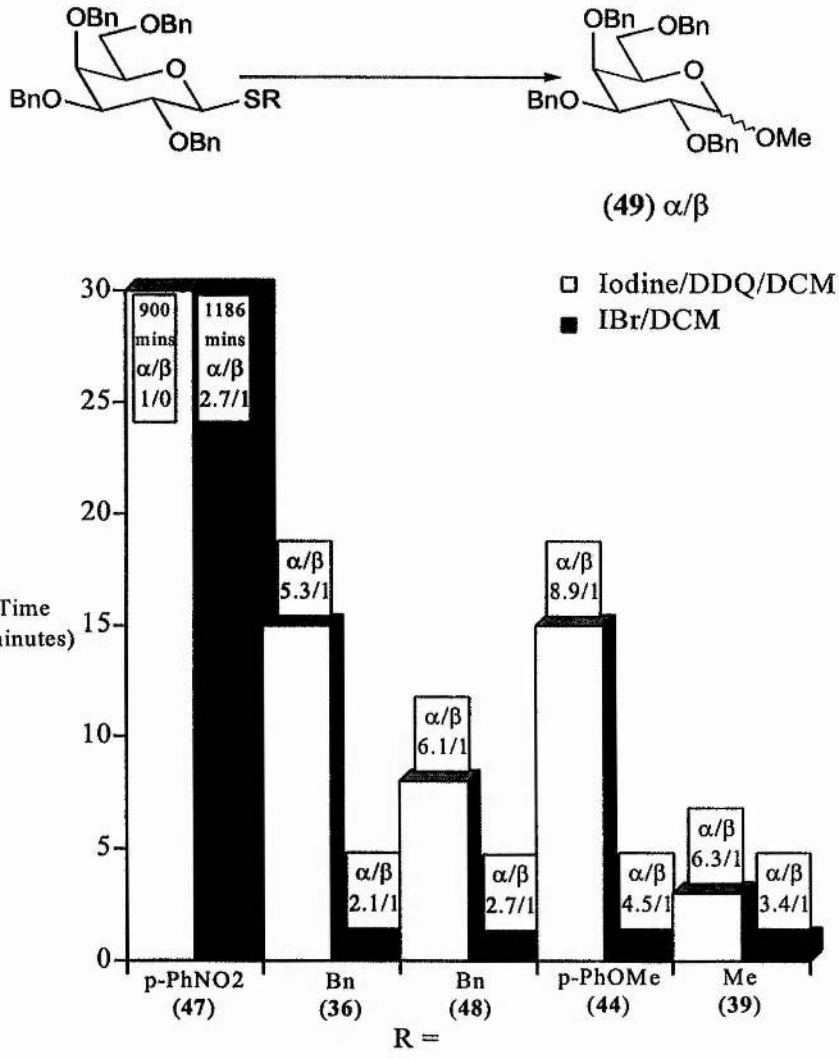
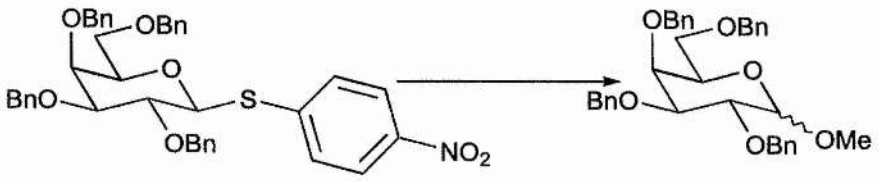


Figure 81 Methanolysis promoted by iodine and DDQ or IBr in DCM.

Conditions: IBr (2 equivalents), MeOH (100 equivalents), DCM

The stereocontrol of the IBr reactions was poorer than for the iodine and iodine/DDQ promoted reactions. Looking at the results from the use of the various activating reagents an apparent correlation between increase in rate and decrease in stereoselctivity can be seen.

However the *p*-nitrothiophenyl glycoside (**47**) was much slower (≈19 hours 20 minutes) than the iodine/DDQ procedure in acetonitrile (1hour 20 minutes). To accelerate reaction of the *p*-nitrothiophenyl glycoside (**47**) further the IBr reaction was carried out using acetonitrile as solvent which reduced reaction time to 13 minutes. Since the other thioglycosides (**36**), (**39**), (**44**) and (**48**) tested could all be activated with the IBr/DCM system in ≈1 minute it was decided that this would be desirable for the *p*-nitrothiophenyl glycoside (**47**) as well. Investigation of the more potent[275] ICl (2 equivalents) with (**47**) in DCM with methanol as acceptor gave a reaction time of ≈25 minutes, when acetonitrile was used as solvent instead the reaction was complete as required in 1 minute. It has thus been demonstrated that even extremely unreactive thioglycosides can be activated rapidly with the correct combination of activator and solvent (Figure 82).



(47) (49) α/β

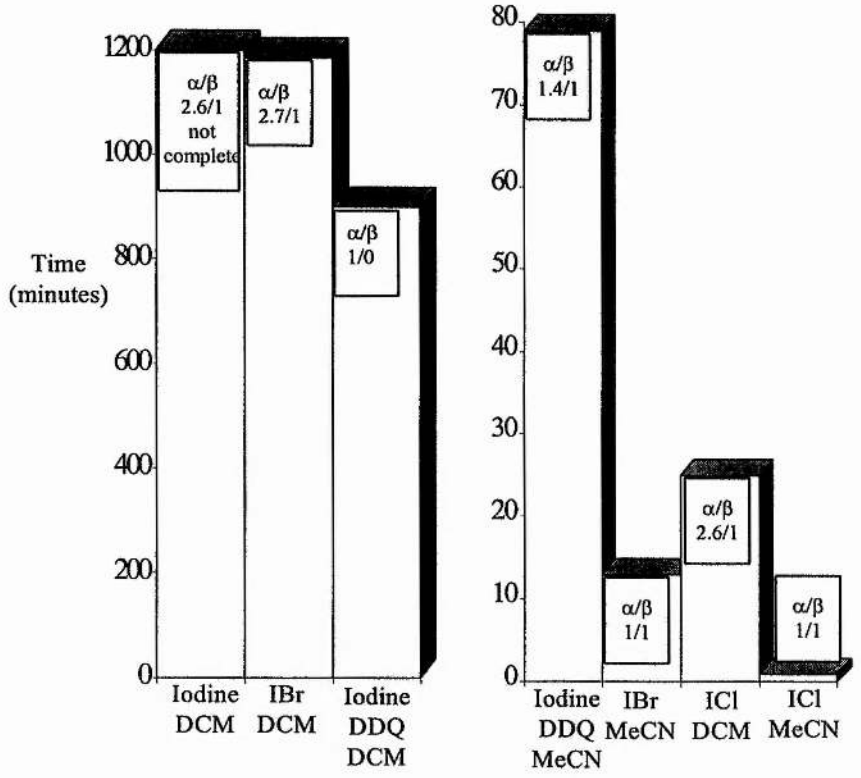
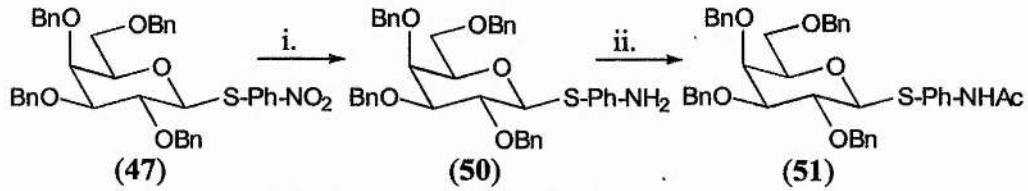


Figure 82 Methanolysis of (47) with various activators.

The activation of *p*-nitrophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactoside (**47**) directly is an alternative to the "latent"/"active" strategy of Roy.[210,268] To compare the convenience of this method *p*-nitrophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactoside (**47**) was reduced with tin (II) chloride[276,277] under reflux to *p*-aminophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactoside (**50**). This compound was then acetylated without further purification to give *p*-acetamidophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactoside (**51**) in poor yield (35%)(Figure 83).[268]



(47) (50) (51)

i. $SnCl_2$, EtOH; ii. Ac_2O, pyridine, [35% from (47)]

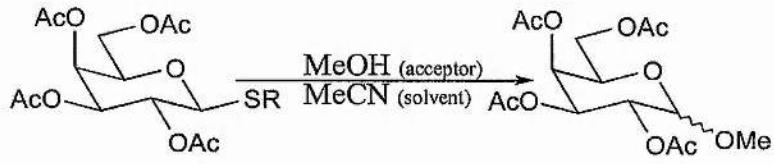
Figure 83 Synthesis of (51) from (47).

p-Acetamidophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactoside (**51**) was then activated with iodine with DCM as solvent and methanol as acceptor. This reaction took 30 minute swith $\alpha/\beta = 3.5/1$, while this was a distinct improvement over the iodine activation of *p*-nitrophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactoside (**47**) (20 hours) the activation with ICl in acetonitrile was direct, required no extra steps and was faster.

2.5.5 ACTIVATION OF "DISARMED" THIOGLYCOSIDES

While "armed" thioglycosides can be activated with iodine, previous investigations had shown that "disarmed" thioglycosides cannot.[227] However it was considered likely that with use of a more potent iodine-based activating system activating system that activation of "disarmed" thioglycosides might also be possible. To this end phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**34**) was reacted with iodine (2 equivalents) and DDQ (2 equivalents) in acetonitrile in the presence of methanol. This reaction took approximately 18 hours and gave 10% yield of glycosylated product (**52**) (Figure 84). Similarly methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galacto-pyranoside (**37**) was reacted in acetonitrile with iodine (1.1 equivalents) and DDQ (1.1 equivalents) in the presence of methanol for approximately 23 hours. 28% of product (**52**) was obtained in addition to some partially deacetylated by-products of (**52**) indicating that much optimisation was required. Use of other potential electron transfer reagents in place of DDQ was then considered. Ceric ammonium nitrate and ceric ammonium sulfate were used in place of DDQ in reaction of phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**34**) with iodine. Combinations of iodine (1.1 equivalents) with either CAN (1.1

equivalents) or CAS (1.1 equivalents) were investigated as activators using acetonitrile as solvent and methanol as the acceptor. The reaction with ceric ammonium nitrate gave a complex mixture of products including partially deacetylated compounds after reaction overnight. With addition of potassium carbonate as an acid scavenger to the ceric ammonium nitrate/iodine mixture, no reaction was observed indicating that any reaction that had taken place was due to formation of an acidic species (presumably HI). The experiment with ceric ammonium sulfate gave no reaction.



R = Ph (**34**), (**52**) α/β

R = Me (**37**)

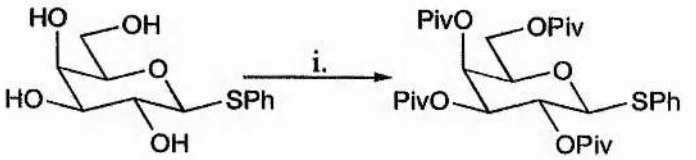
Donor	Activator	Yield(%)	Time(minutes)
(**34**)	I_2/DDQ	10	1080
(**34**)	I_2/CAN	partial deacetylation	1440
(**34**)	I_2/CAS	no reaction	1440
(**37**)	I_2/DDQ	28	1380

Figure 84 Attempted activation of disarmed thioglycosides.

2.5.6 ACTIVATION OF PIVALOYL PROTECTED THIOGLYCOSIDES

Since activation of the acetyl protected thioglycoside donors (**34**) and (**37**) resulted in partial deacetylation, an alternative more stable "disarming" protecting group was required. The bulky pivaloyl protecting group was seen to be suitable for this purpose. Phenyl 2,3,4,6-tetra-*O*-pivaloyl-1-thio-β-D-

galactopyranoside (**53**) was synthesised from phenyl 1-thio-galactopyranoside (**35**) (Figure 85) in poor yield (20%), presumably because pivaloyl chloride is sterically hindered and therefore relatively unreactive even when used in excess.



(**35**) (**53**)

i. pyridine, pivaloyl chloride (20%)

Figure 85 Pivaloyl protection of (35).

Iodine (1.1 equivalents) promoted activation of compound (**53**) in acetonitrile with methanol as an acceptor gave 61% yield of methyl 2,3,4,6-tetra-*O*-pivaloyl-α/β-D-galactopyranoside (**54**) after 1440 minutes. Inclusion of DDQ (1.1 equivalents) with these conditions improved the yield (71%) but did not increase the rate of reaction noticeably. Upon use of CAN, an alternative electron transfer reagent in place of DDQ the reaction was complete in only 10 minutes and in much improved yield (87%). Unfortunately there was no stereoselectivity with any of the three sets of conditions; with all reactions giving 1/1 α/β ratios of products. These results show that pivaloyl protecting groups are less "disarming" than acetyl protecting groups and that I_2/CAN is a more potent thioglycoside activator than either I_2 alone or I_2/DDQ combined. It is worth noting that syntheses using pivaloyl protected acceptors and NIS/TfOH activated thioglycoside donors have been carried out successfully by Russo.[278]

2.5.7 STUDIES ON OLIGOSACCHARIDE SYNTHESIS

The reactions investigated show that there is a clear reactivity difference between the "armed" thioglycosides chosen. Thus it might be expected that these donors could be used in an orthogonal fashion for oligosaccharide synthesis. In the field of thioglycoside donors, activation of one thioglycoside in the presence

of another is also possible using a very unreactive donor in the presence of a much more easily activated species. Such a strategy was used by van Boom,[274] using a *p*-nitrophenylthio glycoside (as introduced by Roy[210,268]) in the presence of a thioethyl glycosyl donor. The *p*-nitrophenylthio group being inert to NIS/TfOH and DMTST allowed a thioethyl function to be activated selectively to give disaccharide products. However, when the *p*-nitrophenylthio function was reduced and acetylated to the corresponding *p*-*N*-acetamidothiophenyl group (which was electron donating), it could be activated with DMTST (Figure 86). This further level of reactivity in anomeric groups was termed "latent/active".[268]

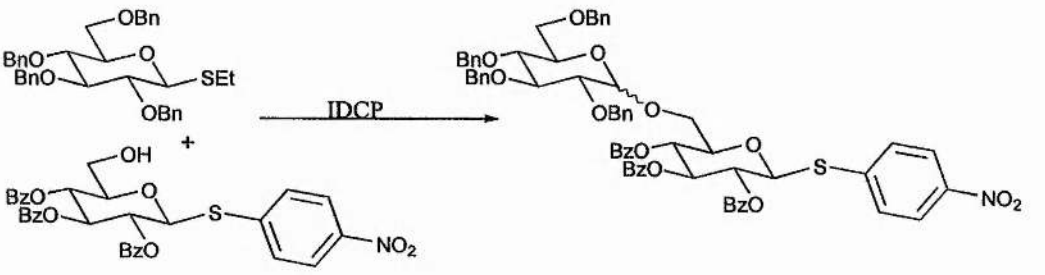


Figure 86 "Latent"/"active" thioglycoside activation.

Combining both the "latent/active" strategy and the principles of orthogonality, Kahne[189] effected a one-pot synthesis of a trisaccharide using thioglycosides and sulfoxides as glycosyl donors and TfOH as the activator. A *p*-methoxyphenyl sulfoxide donor was reacted with the accompanying thiophenyl acceptor in the presence of a phenyl sulfoxide donor. Of the remaining donors the phenyl sulfoxide was more reactive, allowing (after deprotection to acceptor of the newly formed disaccharide) a one pot synthesis of a trisaccharide with selective order of activation. Start to finish (individual monosaccharides to trisaccharide), the procedure took three hours, including purification. In addition to this the remaining thiophenyl group may then be oxidised to the phenyl sulfoxide allowing further range of selective coupling (Figure 87).[189]

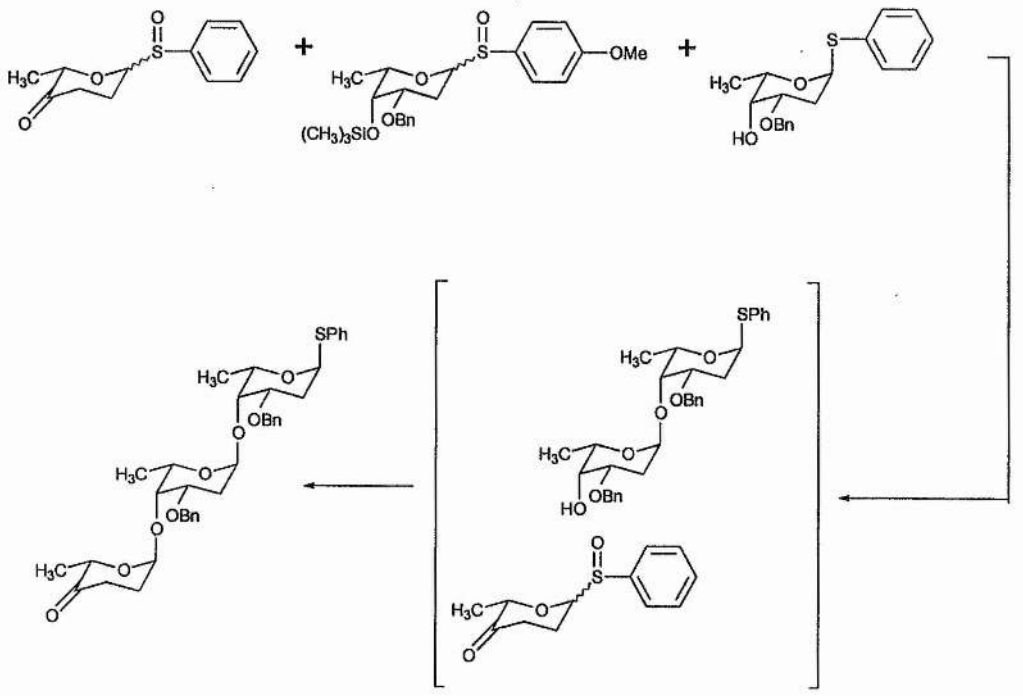


Figure 87 One-pot orthogonal synthesis of oligosaccharides.

Hence glycosylations of reactive thioglycoside donors with unreactive acceptors were investigated. An oligosaccharide target was chosen based on a fragment of the GPI anchor (Figure 88) found on the surface of the parasite *Trypanasoma brucei*.[279-281] The Field group are currently collaborating with Prof. M. A. J. Fergusons' group on the biosynthesis of this type of molecule.[281]

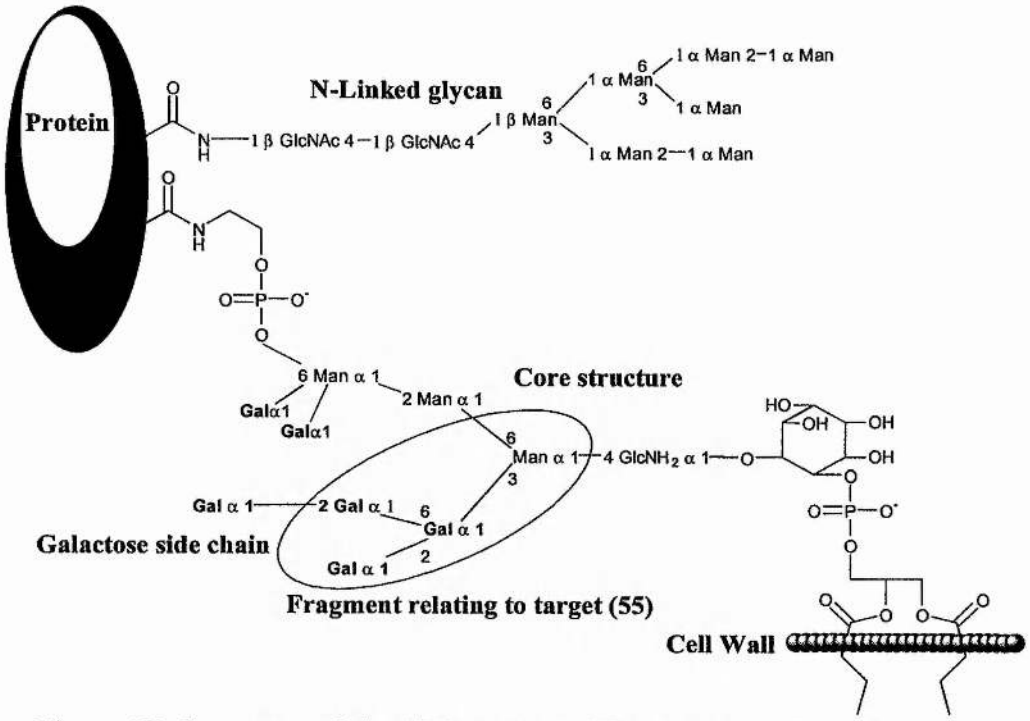


Figure 88 Structure of the GPI anchor of Trypanosoma brucei variant surface glycoprotein.

A retrosynthetic analysis of the fragment of interest, **(55)**, is shown in Figure 89.

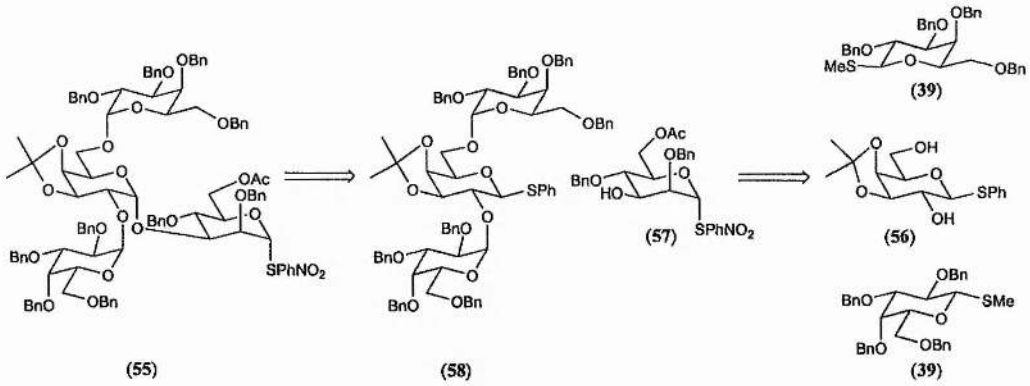
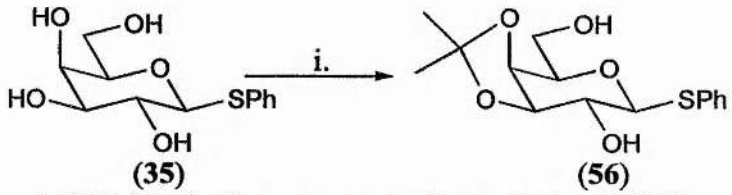


Figure 89 Retrosynthetic strategy for GPI fragment.

The acceptor chosen was 3,4-isopropylidene protected thiophenyl galactoside **(56)** to react with a tetrabenzyl thiomethyl galactose **(39)** donor. This approach

should be ideal as selective activation of the thiomethyl glycoside (**39**) over the less reactive 3,4-isopropylidene protected thiophenyl galactoside (**56**) could be expected to allow a one pot synthesis of the galactose trisaccharide (**58**) (Figure 89). Subsequent activation of the less reactive thiophenyl group (**56**) with a more potent activating system would then give access to tetrasaccharide (**55**). (**55**) could then be extended through the 1- or 6-position to give larger fragments of the GPI anchor.

Initial studies focused on the synthesis of the galactose trisaccharide portion (**58**). Hence phenyl 3,4-isopropylidene-1-thio-β-D-galactopyranoside (**56**) was synthesised from phenyl 1-thio-β-D-galactopyranoside[279] (**35**) in 52% yield (Figure 90).

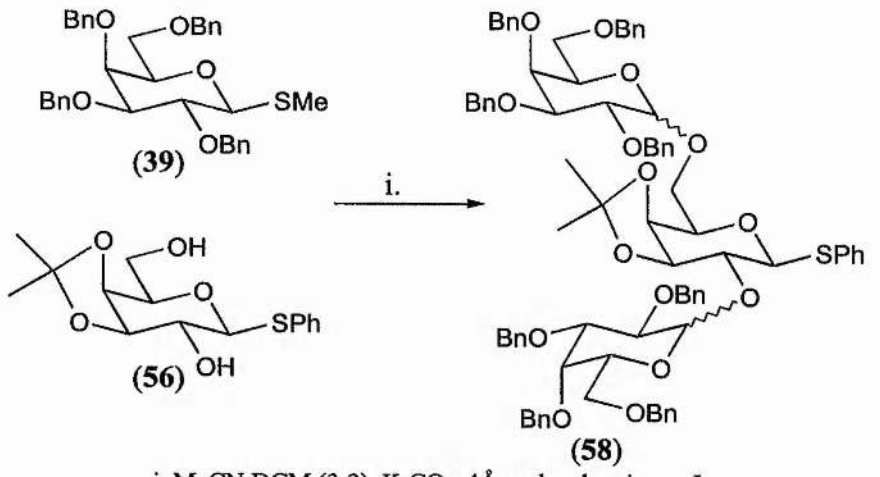


(**35**) (**56**)

i. DMF, 2,2-dimethoxy propane, *p*-toluenesulfonic acid (52%)

Figure 90 Synthesis of (56) from (35).

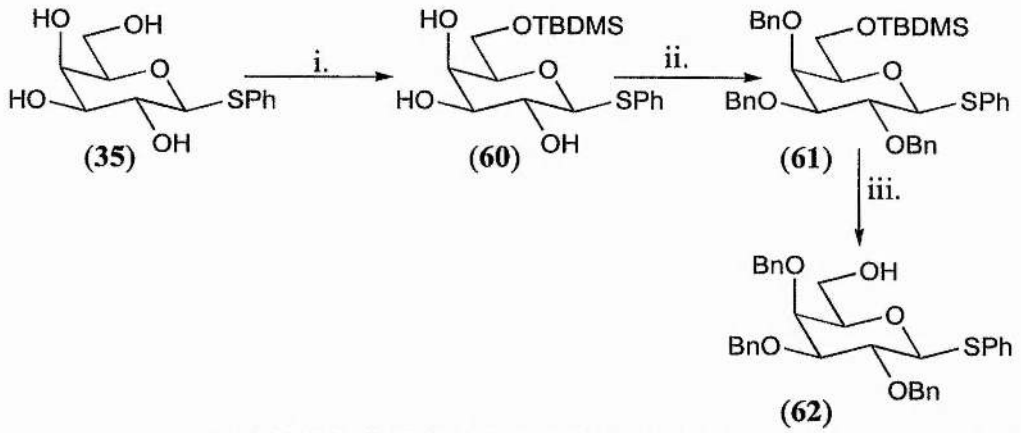
The diol acceptor allowed for formation of 4 different disaccharides (**59**)[1→6 α or β and 1→2 α or β] and 4 trisaccharides (**58**)[1→6 α, 1→2 α or β and 1→6 β, 1 →2 α or β]. Thus generation of a mixture of up to 8 products in addition to the starting materials could be expected upon reaction. Reaction of (**56**) with methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) (2.2 equivalents) activated with iodine (2.2 equivalents) at 0°C (Figure 91) did indeed give a complex mixture of products as determined by t.l.c.. When the reaction was repeated at –10°C (iodine in 3.4 equivalents) a complex mixture was still obtained. Unfortunately accurate identification of the products obtained was not possible. The formation of a large number of products in addition to hydrolysis of the donor and acceptor indicates that glycosylation does indeed take place. Although not optimised, these results were encouraging.



i. MeCN:DCM (3:2), K_2CO_3, 4Å molecular sieves, I_2.

Figure 91 Attempted iodine promoted one-pot trisaccharide synthesis.

To simplify matters a monohydroxyl acceptor was designed to allow only one site of reaction and therefore formation of only two isomers. Phenyl 6-*O*-*t*-butyldimethylsilyl-1-thio-β-D-galactopyranoside (**60**) was synthesised from phenyl 1-thio-β-D-galactopyranoside (**35**). This compound was then benzylated without further purification and the TBDMS group then cleaved with fluoboric acid to give phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**62**) in 43% yield over three steps (Figure 92).

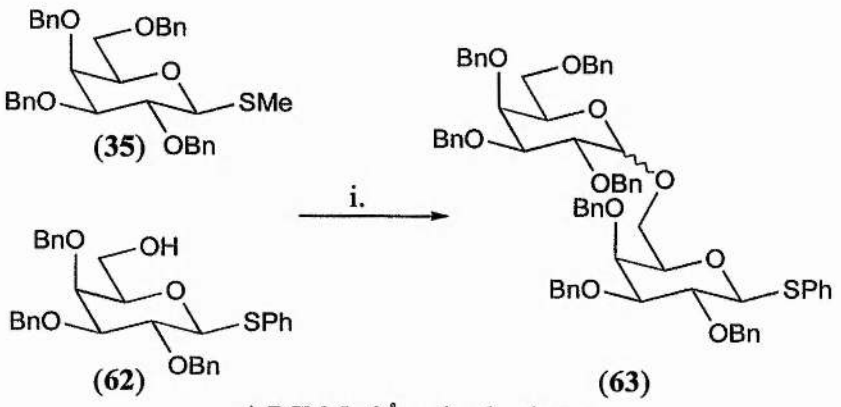


i. pyridine, 0°C, *t*-BDMSCl; ii. DMF, BnBr, NaH, 0°C; iii. MeOH, HBF_4 [43% from (**35**)]

Figure 92 Synthesis of (62) from (35).

Reaction of compound (**62**) with methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) (1.2 equivalents) activated with iodine (1 equivalent in two 0.5 equivalent portions) in DCM gave a mixture of products (Figure 93) (as

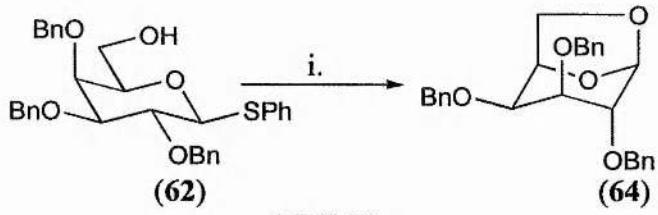
judged by t.l.c.) with one major component. Using these conditions with the addition of molecular sieves to prevent hemiacetal formation, did not reduce the mixture of products.



i. DCM, I_2, 3Å molecular sieves.

Figure 93 Attempted iodine promoted disaccharide synthesis.

Curiously addition of methanol to the reaction mixture did not appear to result in any noticeable glycosylation as would be expected if there were unreacted thioglycoside (either methyl or phenyl) remaining. When phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**62**) alone was reacted with iodine, a product identical (by t.l.c.) with 2,3,4-tri-*O*-benzyl-1,6-anhydro-β-D-galactopyranose (**64**) was observed. This indicated that phenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**62**) undergoes activation of the thiophenyl moiety which results in intramolecular cyclisation from the 6-hydroxyl group (Figure 94).

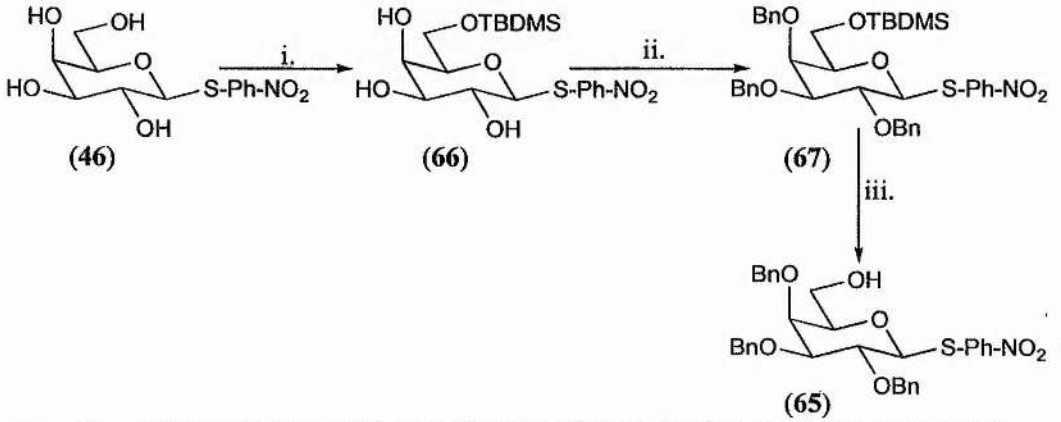


i. DCM, I_2.

Figure 94 Iodine promoted intramolecular reaction of (62) to give (64).

This result surprised us since we had already demonstrated that S-methyl thioglycosides activate faster than *S*-phenyl thioglycosides under the conditions tested already. It is conceivable that MeSI generated by activation glycoside (**39**)

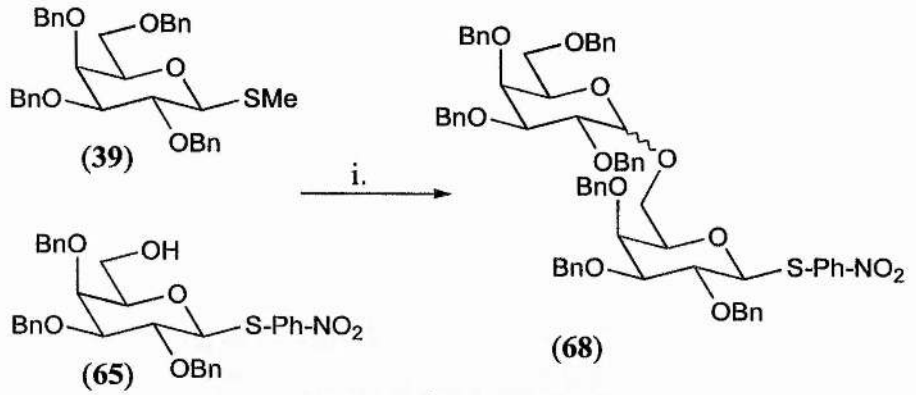
is better able to activate *S*-phenyl glycosides than is iodine. To overcome this problem the thiophenyl moiety was replaced with the less reactive *p*-nitrothiophenyl moiety. *p*-Nitrophenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactoside **(65)** is known to form the 1,6-anhydro derivative **(64)** on activation by DMTST but iodonium activating agents (IDCP and NIS/TfOH) in general do not effect this reaction.[241] Iodine should be a mild enough reagent to avoid this side reaction. Hence *p*-nitrophenyl-1-thio-β-D-galactoside **(46)** was silicon protected to give *p*-nitrophenyl 6-*O*-*t*-butyldimethylsilyl-1-thio-β-D-galactoside **(66)** in 52% yield. Compound **(66)** was then benzylated to give *p*-nitrophenyl 2,3,4-tri-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-1-thio-β-D-galactoside **(67)** in 42% yield. Cleavage of the TBDMS group with fluoboric acid was achieved in 48% yield to give *p*-nitrophenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactoside **(65)** (Figure 95).



i. pyridine, TBDMSCl, 0°C (52%); ii. DMF, BnBr, NaH, 0°C (42%); iii. MeOH, HBF_4 (48%).

Figure 95 Synthesis of (65) from (46).

Reaction of **(65)** with methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside **(39)** (1.1 equivalents) activated with iodine (2 equivalents) in the presence of molecular sieves gave two main products **(68)** (Figure 96) which were separated from other by-products by size exclusion chromatography (M.W. operating range 600-14000). In conjunction with the size-exclusion separation ^{1}H, ^{13}C and 2-D N.M.R. analysis indicated that disaccharides had probably been formed although the spectra could not be fully assigned.



i. DCM, I_2, 4Å molecular sieves.

Figure 96 Attempted iodine promoted disaccharide synthesis.

Unfortunately due to intense spectral overlap we have not been able to confirm the structure of the products of this reaction, mass spectroscopy data is awaited.

2.5.8 STEREOCONTROL OF THIOGLYCOSIDE REACTIONS

As can be seen from the results above, though the rate of reaction can be controlled by varying activating agents and solvents, stereocontrol is only modest. While acidic conditions are known to cause anomerisation of thioglycosides,[224] recently Boons[225] reported that treatment of thioglycosides with a catalytic amount of iodonium ions may result in anomerisation. Similar anomeric equilibrium mixtures were obtained irrespective of whether α or β-anomers were treated with IDCP. Additionally, Boons showed that anomerisation proceeded by intermolecular exchange of alkylthio groups by ^{13}C labelling studies.[225] Results indicated that anomerisation was important for the stereochemical outcome of glycosylation. The formation of both α and β iodides of glucose has been studied by Gervay to evaluate the factors effecting stereocontrol during formation.[170] The research showed that the initially formed β-iodides anomerise to the α-anomer faster than the reverse reaction when acetate (participating) protecting groups were used.[170] By contrast when benzyl (nonparticipating) groups were used the α-anomer was formed preferentially, though the β-anomer could be formed at –100°C.[170] The β-anomer readily anomerised to the α-anomer on warming.[170] In subsequent investigations on

glycosylation of α-iodides with anionic acceptors it appeared that some anomerisation occurred *in situ* to allow S_N2 attack on the more reactive β-iodide.[170] In relation to iodine activation of thioglycosides and glycosyl halides it is possible that reactions may involve formation of anomeric iodides (Figure 97).[170]

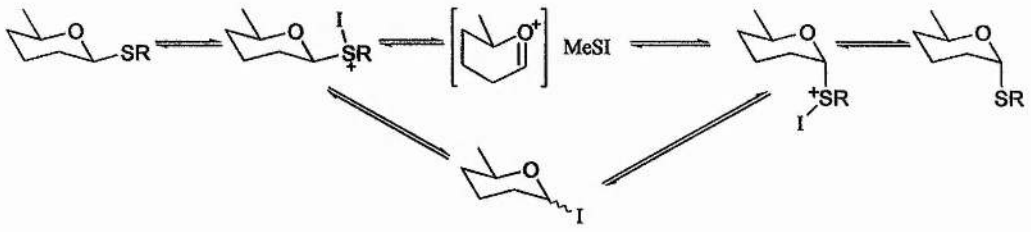
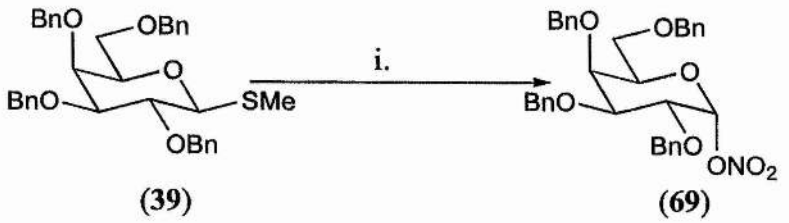


Figure 97 Iodine promoted anomerisation.

Hence in an effort to investigate whether iodine activated thioglycosides behaved in a similar fashion to those activated with iodonium ions, reactions with methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) were carried out. Reaction of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) with iodine (1.1 equivalents) without any acceptor were investigated. Epimerisation was observed (by t.l.c.) to occur rapidly (5-10 minutes) when acetonitrile was used as solvent.

Interestingly when methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) was reacted in acetonitrile with iodine (1.1 equivalents) and CAN (1.1 equivalents) the reaction was complete in 5 minutes. Upon analysis the product was found to be the α-anomeric nitrate (**69**) (IR 1654, 1648, 1282 cm^{-1}; 300MHz ^1H nmr; 6.1, d, 1H, $J = 4$ Hz, 1-H) (Figure 98), presumably formed from reaction with CAN.



(39) (69)

i. MeCN, CAN, I_2.

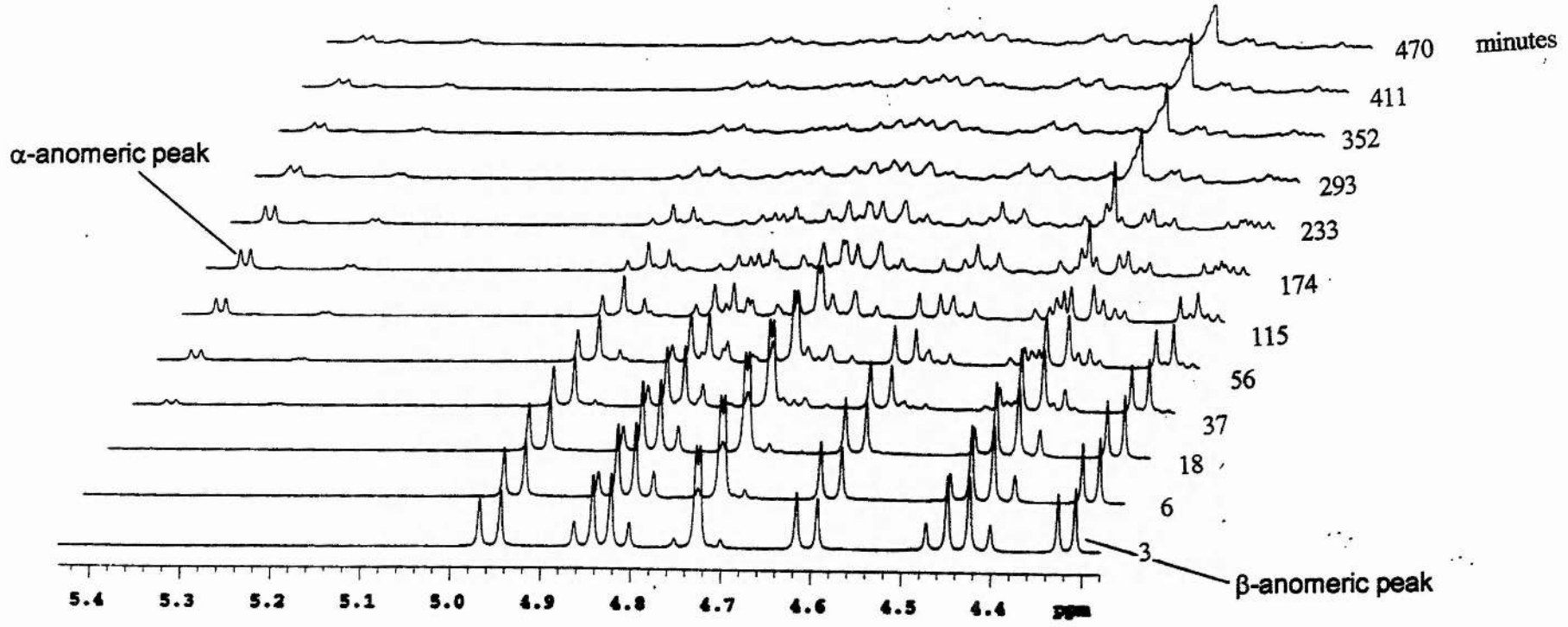
Figure 98 Iodine/CAN mediated formation of anomeric nitrate (69) from (39).

CAN promoted anomeric nitrate formation is known from CAN/NaN_3 azidonitration reactions. With reference to anomolous strereochemistry obtained with D-galactal, Lemieux[284] speculated that the nitrate might be formed as a result of nitrate attack on the oxocarbonium ion formed by formation of the 2-azido species. However he added that this was unlikely as otherwise 2-azido-2-deoxy glycosyl azide formation might be expected – a process that was not observed. Although the mechanism of I_2/CAN promoted formation of the nitrate **(69)** is unknown this finding implies that I_2/CAN promoted glycosylations may either proceed via the anomeric nitrate or be in competition with nitrate formation. Clearly such events could influence the efficiency and stereocontrol of I_2/CAN reactions.

2.5.9 NMR STUDIES ON EPIMERISATION

N.M.R. spectroscopy studies on iodine mediated reactions with carbohydrates have been carried out in the past. One such study followed the iodination of the 6-OH of 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose with an iodine-triphenylphosphine-imidazole reagent and observed the interaction of the phosphorous reagent with iodine, imidazole and the sugar with phosphorous N.M.R..[282] Work more relevant to stereocontrol was carried out by Lemieux[132,135,137,283] in the 1960s with ^{13}CN.M.R. on the effect of solvents on the anomeric effect. Directly related studies are those of Gervay who followed the formation of glycosyl iodides at various temperatures using N.M.R. tubes as reaction vessels.[170] As discussed earlier, Gervay was able to observe formation and anomerisation of α and β-iodides.[170] Hence in our hands direct real time

observation of thiogycoside epimerisation was possible by the use of time-lapse N.M.R. (Figure 99).



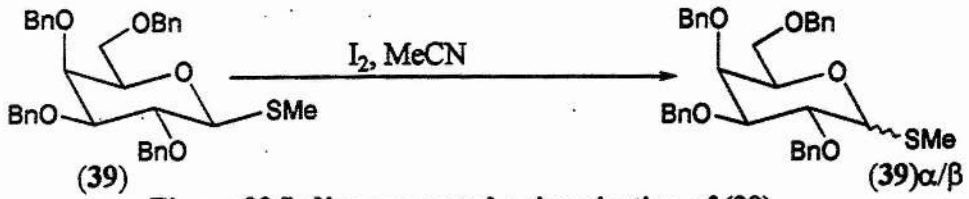


Figure 99 Iodine promoted epimerisation of (39)

N.M.R. observation of the analogous iodine promoted reaction in the presence of acceptor (methanol) showed that epimerisation and glycosylation occur simultaneously (Figure 100 and 101). This observation indicates that for methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**), epimerisation has an effect on the stereochemical outcome of iodine promoted glycosylations although no peaks corresponding to those of an anomeric iodide (H-1, δH 6.8 ppm)[170] were observed

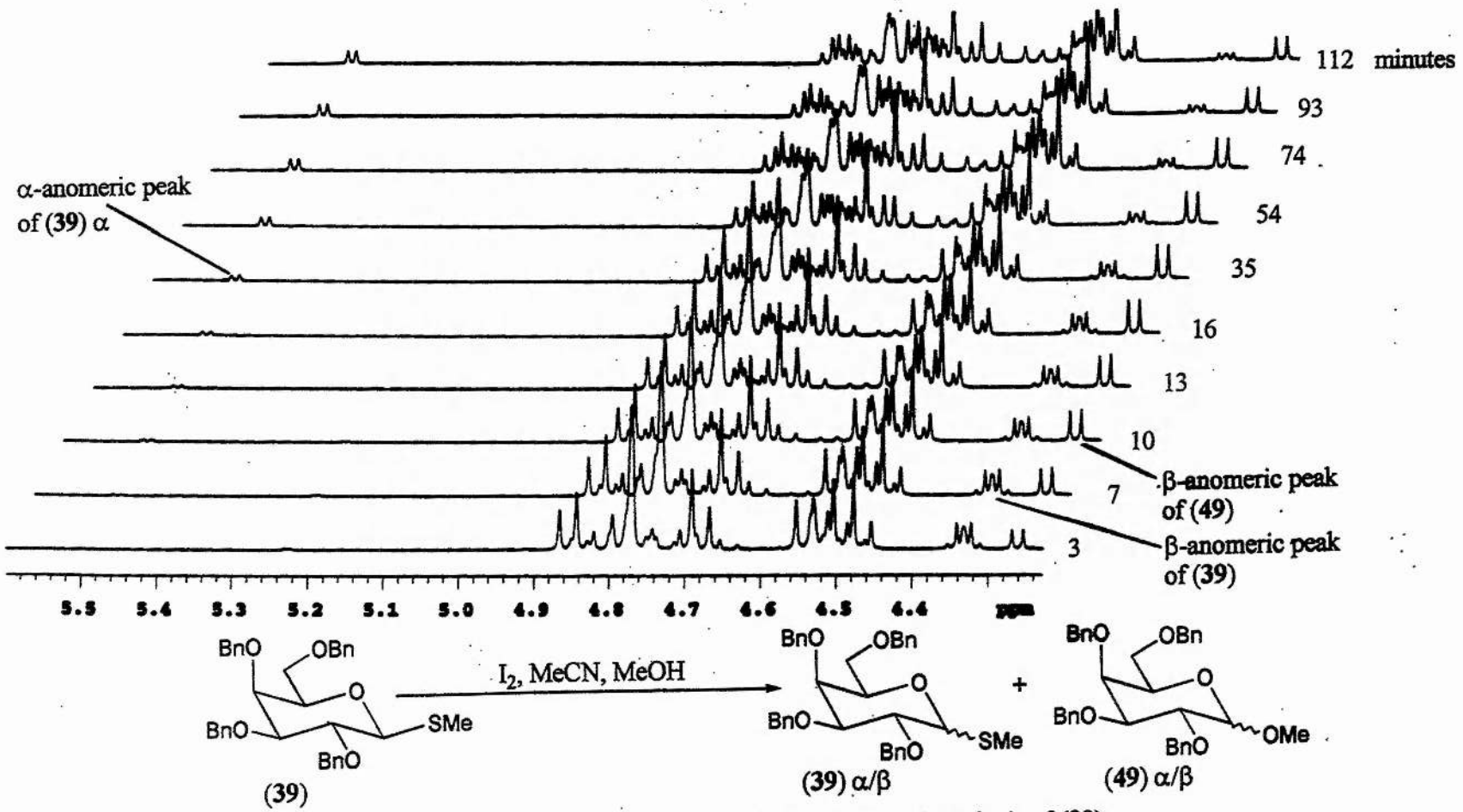


Figure 100 Iodine promoted epimerisation and methanolysis of (39)

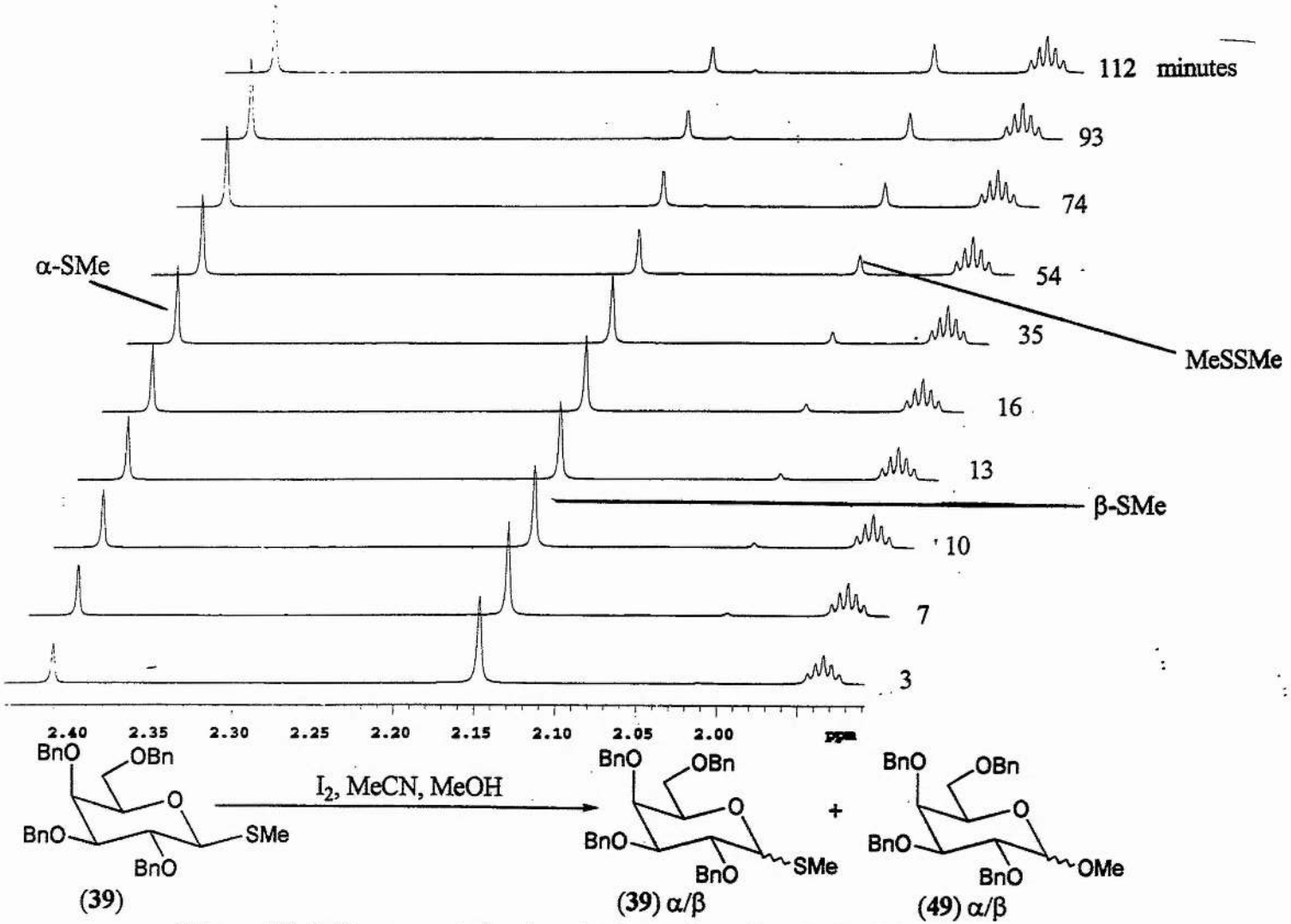


Figure 101 Iodine promoted epimerisation and methanolysis of (39)

2.5.10 SILICA SUPPORTED GLYCOSYLATIONS

Iodine activation for the synthesis of oligosaccharides has yet to be optimised as demonstrated in the reactions carried out in this project. There is a need for plentiful quantities of starting materials for optimisation and characterisation (which is difficult with small quantities of di or trisaccharides) during oligosaccharide synthesis. In an effort to overcome needless use of significant quantities of compound, development of a novel reaction system was initiated. As a result of using preparative t.l.c. for the attempted purification of some of the disaccharide products it was thought that if a reaction could be carried out on the surface of such a plate then the products could be purified *in situ*, dispensing with the need for workup. However, since preparative t.l.c. plates are quite expensive, conventional analytical t.l.c. plates were investigated first. For the visualisation of t.l.c. plates the use of iodine vapour is common. Since discolouration of the silica is less than that of the compounds on the plate it is clear that most compounds on the surface will interact with the iodine vapour. In the light of our investigations into the use of iodine as a thioglycoside activator, it was therefore interesting to find out whether iodine vapour would be sufficient to activate thioglycosides on a silica t.l.c. plate. Initially four t.l.c. plates were co-spotted with solutions of donor methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) and acceptor *p*-nitrophenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactoside (**65**) in DCM, and these were placed in a vessel containing solid iodine (Figure 102) whose vapour visibly discoloured the plates and the compounds spotted on them. At 10 minute intervals one plate was removed, the starting materials and products spotted by capillary as authentic markers, and eluted (10:1 toluene:ethyl acetate) before visualisation. After forty minutes the donor (**39**) had completely reacted to give a mixture of products including the hemiacetal of the donor (**39**) and a small quantity of disaccharides (using the products from the previous solution phase synthesis as markers).

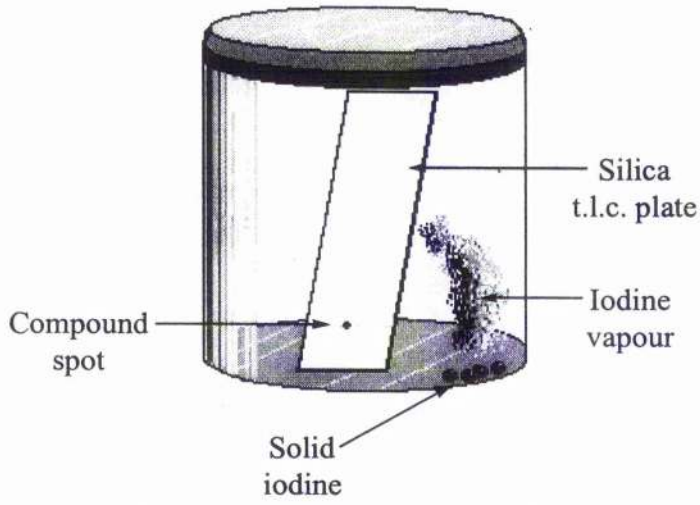


Figure 101 Iodine vapour activation of thioglycosides on silica t.l.c. plates.

Additionally, since both IBr and ICl fume noticeably in moist air it would be expected that they too would be capable of activating thioglycosides on silica. To this end *p*-nitrophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactoside (**47**) was spotted on a t.l.c. plate and methanol spotted from capillary on top of the previous spot. The plate was then placed in a jar with a trace of ICl liquid in it (the jar was tipped slightly to prevent the plate contacting the ICl liquid). The vapour from the ICl discoloured the plate slightly. After 5 minutes elution and visualisation showed that the thioglycoside had formed the hemiacetal rather than the methyl glycoside (**49**). The most obvious problem with the silica reactions was the formation of hemiacetal from the donor. In order to overcome this various alterations were made. Reactions were carried out under vacuum in an effort to remove any atmospheric moisture, unfortunately this did not improve matters. Predrying the t.l.c. plates over night in an oven at 100-110°C did not improve the yield of products either. To verify that the reaction was solid phase rather than taking place in the small quantity of solvent present, the solvent was removed from a plate under vacuum prior to activation. As expected this did not alter the progress of the reaction confirming that the reaction took place on the silica surface. Without iodine no reaction was observed demonstrating that silica alone was not enough to activate the thioglycoside donor. When the reaction was attempted with measured quantities of donor (**39**) and acceptor (**65**) (0.01 M each, premixed in DCE) with 6 fold excess of iodine (0.01 M in DCE) no

reaction was observed. These reactions demonstrate that although far from optimal, it is possible to activate thioglycosides with iodine or ICl vapour on silica.

Silica assisted activation of thioglycosides has been previously reported as a result of studies on activation of thioglycosides.[219] Addition of silica clearly increased the rate and stereoselectivity of the reactions investigated (Figure 103).[219]

Activator[#]	Solvent	Time	%	α:β
PhIO-Tf_2O	CH_3CN	1 min	81	1:12
PhIO-Tf_2O	$(CH_2Cl)_2$	1 h	42	0:1
PhIO-Tf_2O*[a]	$(CH_2Cl)_2$	instant	77	0:1
PhIO-Tf_2O*[b]	$(CH_2Cl)_2$	instant	94	0:1

Varying ratios of Donor/acceptor; * silica present in reaction; a. reaction added to silica; b. silica added to reaction

Figure 103 Effect of silica on rate, yield and stereoseectivity of glycosylation reactions.

In addition it was also shown that increasing the amount of silica added to the reaction mixture increased the rate markedly (Figure 104).

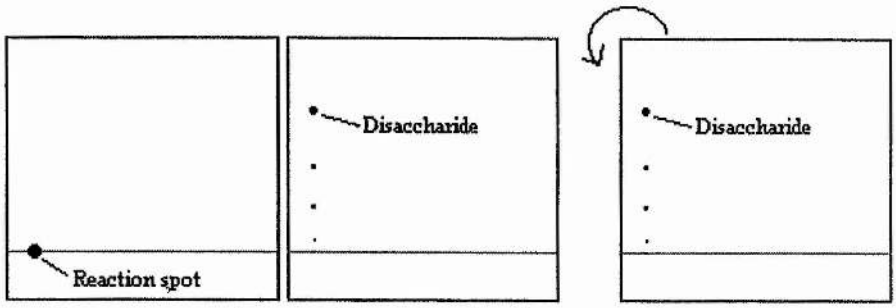
Additive[a]	Solvent	Silica Gel (mg)	Time	%	α:β
$LiNO_3$	ether	20	3.5 h	53	1:0
$LiNO_3$	ether	50	1.5 h	51	1:0
$LiNO_3$	ether	200	1 h	60	1:0
$LiNO_3$	ether	2000	45 min	61	1:0.12

a. 1.5 x NBS, 0.5 x additive, donor/acceptor; 1:0.8

Figure 104 Effect of the amount of silica on the rate of reaction.

Silica gel-mediated anomeric deacetylation has also been observed; per-acetylated 2-amino-2-deoxy-glycopyranose derivatives in methanol may be deprotected with high regioselectivity and almost without byproducts.[285] Microwave promoted reactions[286] and inadvertent reactions with moisture on t.l.c. plates[287] have also been observed previously on silica and solid phase reactions have also been carried out on alumina.[288] However none of these findings has

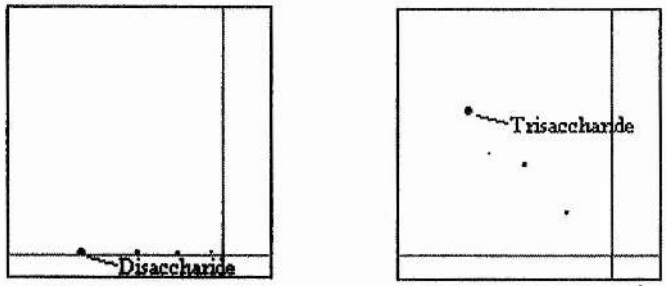
been used as a support for solid phase chemical-enzymatic synthesis of glycopeptides and oligosaccharides.[289] These syntheses were carried out quickly and in good yield, however they differ from the t.l.c. reactions in their requirement for amine linked spacer groups to be present on a modified silica surface.[289] If the t.l.c. system could be used in an analogous synthesis whereby the reagents were deposited or absorbed onto the silica surface rather than covalently linked this would obviously be more convenient. Reaction progress could be therefore be monitored by simple t.l.c. without need for cleavage of the products or the use of sophisticated n.m.r.[290] or mass spectroscopy techniques. Due the small quantities required for visualisation with t.l.c., it is conceivable that reactions on t.l.c. plates may be used to optimise reaction conditions without need for significant quantities of reagent. A potential use for this technology would be two-step synthesis of a trisaccharide using 2D t.l.c.. The initial disaccharide formation would be carried out in the first dimension. Subsequent elution of this reaction would give a pure product spot located by U.V. fluorescence. This spot could then be treated with a further acceptor and the reaction activated by iodine or if necessary IBr or ICl vapour. Elution of this would thus give a purified trisaccharide (Figure 105).



Spot donor and acceptor, react with iodine vapour.

Elute plate in 1st dimension.

Rotate plate 90°.



Spot next acceptor on top of disaccharide and react with iodine vapour.

Elute plate in 2nd dimension and scrape off "pure" product.

Figure 105 2-D iodine promoted disaccharide synthesis on preparative t.l.c. plates.

2.6 CONCLUSION

It has also been shown that iodine can efficiently activate "armed" thioglycosides of varying reactivity. Utilising different activating procedures (I_2, DDQ, IBr, ICl and various solvents) rate of reaction can be altered. In addition more potent activating systems can be used to activate unreactive thioglycoside donors. Thus it has been demonstrated that the reactivity of thioglycosides can be controlled using different activators and solvents. Attempts to take advantage of the different reactivities of various thioglycosides for the synthesis of oligosaccharides have as yet not led to clean reactions. Reasons for these complex product mixtures include poor stereocontrol in the reactions; lack of stereocontrol was observed even in the reaction of thioglycosides with methanol. N.M.R. Studies on iodine activation demonstrated that anomerisation takes place both in the presence and absence of an acceptor. In the presence of an acceptor, anomerisation occurs simultaneously with glycosylation.

A novel development that arose from these studies is the discovery that iodine vapour may activate thioglycosides deposited on the surface of silica t.l.c plates. This procedure has not been optimised but results indicate that formation of disaccharides on the surface of a t.l.c. plate is possible. The largest problem is the formation of hemiacetal by-products. This is presumably due to moisture on the t.l.c. plates and studies are under way to resolve this problem.

2.7 FUTURE WORK

The use of iodine activating systems for use with thioglycosides has not been fully optimised and while control of stereochemistry has not yet been considered varying solvents and temperature may enable this to be achieved. Further studies will hopefully result in new iodine promoted methods for the efficient synthesis of oligosaccharides from convenient thioglycoside building blocks.

In relation to the iodine promoted reactions studied there is the possibility that optimisation could be achieved on t.l.c. plates. If this were possible procedures could be optimised quickly and efficiently with minimum wastage of starting materials and of course such an approach need not be confined to carbohydrate chemistry. With technology currently available it is possible to identify products purely by t.l.c., referencing to known products in a computer database.[291] The computer programme reported uses data obtained from a variety of visualisation and detection techniques and reagents, such as U.V. fluorescence, iodinated solutions and chlorine vapour and can generate a list of matching compounds from only one of these properties.[291] Complimenting this technology is the ability to directly identify t.l.c. fractions by *in situ* F.T. Raman spectroscopy in the presence of iodine staining or fluorescor reagent.[292] Bearing both of these capabilities in mind, it is not difficult to envisage carrying out entire multi-step reaction sequences on a t.l.c. surface and identifying the products merely by eluting the plate and analysing each spot *in situ*, allowing bench chemists to develop syntheses without the need for large quantities of reagents, valuable starting materials or laborious purifications. Furthermore, depending on the conditions of reaction it may not even be necessary to elute the plate, allowing many reactions to be carried out on a single plate. This would have important implications for miniaturisation of combinatorial chemistry as well as being more environmentally friendly. In addition silica bound immunosensors are being investigated by several groups.[293,294] If one of the concepts is successful, sensor molecules could be covalently immobilised on silica and linked via nanostructured gold electrodes allowing electrochemical detection of the binding of ferrocene labelled molecules.[293] In light of such investigations it is conceivable that a t.l.c. plate could be impregnated with a sensor molecule that

could, for example change colour upon formation of the required product. Such a sensor could of course be used only for known products or specific functional groups. However for a molecule with known and limited sites of reaction this approach would be a cheaper method of following reaction progress than spectrometry. Hence any reactions that can be done on a solid support, whether directly bonded, linked or adsorbed, may potentially be reduced to miniature reactions and observed, studied, optimised and assessed before the need to scale up arises. Indeed, miniaturisation can been reduced to single silica covered silver particles (10 nm) upon which reactions (iodine diffusion through silica to react and give a single AgI particle on the silica surface) can be observed using transmission electron microscopy in real time.[295] Of course such technology is currently far from affordable or convenient due to the large costs of purchasing and housing the necessary equipment. However t.l.c. plates are commercially available and thus provide an affordable and user friendly starting point for development of miniaturisation in any synthetic laboratory.

EXPERIMENTAL

Elemental microanalyses were performed in the departmental microanalytical laboratory.

NMR spectra were recorded on a Varian Gemini 200 (200 MHz; FT ^{1}H-NMR and 50.31 MHz; ^{13}C-NMR) spectrometer, a Varian 300 (300 MHz, FT ^{1}H-NMR and,75.4 Hz ^{13}C-NMR) spectrometer and a Varian 500 (500 MHz, FT ^{1}H-NMR and 125 MHz, ^{13}C-NMR) spectrometer. ^{1}H-NMR spectra are described in parts per million downfield shift from TMS and are reported consecutively as position (δ_H or δ_C), relative integral, multiplicity (s.-singlet, d.-doublet, t.-triplet, q.-quartet, quin.-quintet, d.d.-doublet of doublets, sep.-septet, m.-multiplet, and br.-broad), coupling constant (Hz) and assignment (numbering according to the IUPAC nomenclature for the compound). ^{1}H-NMR were referenced internally to ^{2}HOH (4.68 p.p.m.) or $C^{2}HCl_3$ (7.27 p.p.m.). ^{13}C-NMR were referenced to $C^{2}H_3O^{2}H$ (49.9 p.p.m.) or $C^{2}HCl_3$ (77.5 p.p.m.).

I.R. spectra were recorded on a Perkin-Elmer 1710 FT IR spectrometer. The samples were prepared as Nujol mulls or thin films between sodium chloride discs. The frequencies (υ) as absorption maxima are given in wavenumbers (cm^{-1}) relative to a polystyrene standard. Mass spectra and accurate mass measurements were recorded on a VG 70-250 SE, a Kratos MS-50. Melting points were taken on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured at 23 °C on an Optical Activity AA-100 polarimeter using 5 cm or 10 cm path length cells. Thin layer chromatography plates were visualised with the aid of U.V. light (254 nm), and unless otherwise stated were subsequently dipped in 5% sulfuric acid/ethanol and heated until the compound charred. Thin layer chromatography was carried out using either 0.25 mm Macherey-Nagel DC Fertigplatten SIL G-25 UV_{254} plates or Merck 25-DC Platten Kieselgel 60 F_{254}. Column chromatography was carried out using Fluka Silicagel 60. Size exclusion

chromatography was carried out using BioRad BioBeads SX-1 mesh 200-400, with a M.W. operating range of 600-14000.

The solvents used were either distilled or of Analar quality, Light petroleum refers to that portion boiling between 40 and 60 °C. Solvents were dried according to literature procedures.[296] Ethanol and methanol were dried using magnesium turnings. Isopropanol and triethylamine and were distilled over CaH_2. THF and diethyl ether were dried over sodium/ benzophenone and distilled under nitrogen.

Benzyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**48**) was generously provided by Dr. M. Aloui. 2,3,4-Tri-*O*-benzyl-1,6-anhydro-β-D-galactopyranose (**64**) and some methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) were generously provided by Dr. K. P. R. Kartha.

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (10)

2-Acetamido-2-deoxy-D-glucopyranose (**9**) (0.09 mol, 20 g) was dissolved in acetyl chloride (0.56 mol, 40 cm^3) saturated with $HCl_{(g)}$ ($HCl_{(g)}$ was bubbled through the acetyl chloride for 15 minutes prior to reaction) and was allowed to react for 16 hours. The reaction was followed by t.l.c. (toluene:ethyl acetate; 3:1). Another portion of acetyl chloride was added (10 cm^3) and the reaction was allowed to proceed for a further 24 hours before toluene was added and the excess acetyl chloride and acetic acid were removed by azeotropic distillation with toluene. The resulting off-white solid was subjected to column chromatography (dichloromethane:ethyl acetate, 20:1-10:1) to yield the title compound (**10**) (24.86 g, 75%), m.p.110-115°C (from DCM:diethyl ether) {lit.,[60] m.p. 127-128°C (diethyl ether); $[\alpha]_D^{24}$ +113.3 (*c* 0.3 in $CHCl_3$) (Lit.,[60] $[\alpha]_D^{24}$ +110° (*c* 1.0 in $CHCl_3$)}; δ_H(200 MHz; $CDCl_3$) 1.98 (3 H, s, CH_3CO), 2.04 (6 H, s, CH_3CO), 2.09 (3 H, s, CH_3CO), 4.20 (3 H, m, 5-H, 6-H, 6'-H), 4.52 (1 H, m, 2-H), 5.21 (2 H, m, 3-H, 4-H), 5.89 (1 H, d, $J_{NH,2}$ 7, NH), 6.18 (1 H, d, $J_{1,2}$ 3, 1-H).

Octyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (11)[297]

Dry mercury cyanide (0.04 mol, 10.24 g) and ground calcium sulfate (14.4 g) were added to dry octanol (0.056 mol, 9.6 cm^3) in toluene (32 cm^3) and the mixture was stirred for one hour under nitrogen and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**10**) was then added (0.022 mol, 8.0 g). The reaction mixture was stirred for 2 days under nitrogen before dichloromethane was added (200 cm^3) and solids were removed by filtration through Celite. The filtrate was then washed with 10% aqueous NaCl solution and extracted with dichloromethane. The organic layer was washed with saturated $NaHCO_3$ solution and back extracted with dichloromethane. The resulting organic layer was then washed twice with water, which was back extracted with dichloromethane. The organic layers were combined, dried over $MgSO_4$, and solvent was removed under reduced pressure to yield a white solid which was purified by column chromatography (1:1, ethyl acetate:toluene) to give the title compound (**11**) (8.27 g, 76%), m.p. 113-115°C; $[\alpha]_D^{23}$ -21.3 (c 0.3 in methanol); δ_H(200 MHz; $CDCl_3$) 0.83-1.59 (15 H, 3 x m, octyl), 1.95 (3 H, s, CH_3CO), 2.02 (3 H, s, CH_3CO), 2.03 (3 H, s, CH_3CO), 2.08 (3H, s, CH_3CO), 3.48 (2 H, m, octyl), 3.78 (2 H, m, 2-H, 5-H), 4.19 (2 H, m, 6-H, 6'-H), 4.68 (1 H, d, $J_{1,2}$ 9, 1-H), 5.07 (1 H, t, $J_{3,4}$ 9, 4-H), 5.31 (1 H, t, $J_{3,4}$ 9, 3-H), 5.44 (1 H, d, $J_{NH,2}$ 8, NH).

Octyl 2-acetamido-2-deoxy-β-D-glucopyranoside (12)[297]

To octyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**11**) (0.048 mol, 22.0 g) in dry methanol (50 cm^3) was added a small piece of sodium metal. The reaction was followed by t.l.c. (dichloromethane:methanol, 4:1) and when it was complete Dowex ion exchange resin was added and the mixture was stirred for 30 minutes. The resin was removed by filtration and the solvent was removed under reduced pressure to yield the title compound (**12**) (15.83 g, 100%); m.p. 176-178°C (from DCM:light petroleum); δ_H(200 MHz; CD_3OD) 0.84-1.57 (15 H, 3 x m, octyl),

1.97 (3 H, s, CH_3CO), 3.40 (3 H, m, 5-H, 6-H, 6'-H), 3.70 (1 H, m, 3-H), 3.92 (1 H, m, 4-H), 4.42 (1 H, d, $J_{1,2}$ 8.2, 1-H).

Octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (13)

To octyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**12**) (3.02 mmol, 1.0 g) in pyridine (20 cm^3) at 0°C were added 5 aliquots of benzoyl chloride (each of 0.5 equivalents, 1.51 mmol, 0.212 g, 0.175 cm^3). The reaction was followed by t.l.c. (ethyl acetate:toluene, 1:8) and when complete the solution was washed with 2M HCl, extracted with dichloromethane. The organic extract was dried over magnesium sulfate, the solvent was removed under reduced pressure and the product was purified by column chromatography (ethyl acetate:toluene, 1:8) to yield the title compound (**13**) (0.91 g, 55%), m.p. 141-143°C (Found: C, 66.47; H, 7.24; N, 2.49 $C_{30}H_{38}NO_8$ requires C, 66.65; H, 7.08; N, 2.59%); $[\alpha]_D^{23}$ +4.2° (*c* 1.0 in $CDCl_3$); δ_H(200 MHz; $CDCl_3$) 0.85-1.56 (15 H, 3 x m, octyl), 1.87 (3 H, s, C{O}CH_3), 3.30 (1 H, m, OH), 3.50 (1 H, m, octyl), 3.82 (3 H, m, octyl, 4-H, 5-H), 4.10 (1 H, m, 2-H), 4.69 (3 H, m, 1-H, 6-H, 6'-H), 5.40 (1 H, m, 3-H), 5.73 (1 H, d, $J_{NH,2}$ 9.3, NH), 7.55 (6 H, m, Ph), 8.05 (4 H, m, Ph); δ_C(75.4 MHz; $CDCl_3$) 14.0, 22.7, 23.3, 25.9, 29.3, 29.5, 31.8, 54.5 (octyl, C{O}CH_3), 63.8, 69.6, 69.9, 74.3, 76.3 (ring carbons), 101.2 (1-C), 128.6, 128.7, 129.4, 129.9, 130.0, 130.1, 133.5, 133.7 (Ph), 167.2, 167.8 (C{O}Ph), 170.5 (NHCO).

Octyl 2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (14)

To octyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**12**) (0.30 mmol, 0.1 g) in pyridine (5 cm^3) was added benzoyl chloride (5 equivalents, 0.175 cm^3, 0.21 g) and a few crystals of 4-dimethylaminopyridine. The reaction was followed by t.l.c. (ethyl acetate:toluene, 1:10) and when it was complete the reaction solution was washed with 2M HCl and extracted with dichloromethane. The organic extracts were dried with $MgSO_4$, the solvent was removed under reduced pressure and the product was purified by column chromatography (ethyl acetate:toluene, 1:10) to give the title

compound (**14**) (0.07 g, 56%); δ_H(200 MHz; $CDCl_3$) 0.86-1.58 (15 H, 3 x m, octyl), 2.03-2.11 (3 H, 3 x s, CH_3CO), 3.53 (1 H, m, octyl), 3.87 (2 H, m, 4-H, 5-H), 4.11 (1 H, m, 2-H), 4.53 (1 H, d, $J_{1,2}$ 8, 1-H), 5.70 (4 H, m, NH, 3-H, 6-H, 6'-H), 7.32 (5 H, m, Ph), 8.00 (10 H, m, Ph).

Octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-*xylo*-hexopyranosid-4-ulose (15)

Octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (**13**) (0.05 g, 0.093 mmol) and nicotinium dichromate (0.086 g, 0.185 mmol, 2 equivalents) was added to dry dichloromethane (5 cm^3). Freshly activated 4Å molecular seives (0.1 g) were then added to the reaction vessel which was then flushed with nitrogen. The mixture was stirred and acetic anhydride (0.01 mmol, 0.001 cm^3) and then pyridine (0.346 mmol, 0.028 cm^3) were added. The solution turned a deep brown/black colour almost instantly and upon t.l.c. (toluene:ethyl acetate ; 4:1) the reaction was observed to be almost complete in 10 minutes. The reaction was then diluted with ether and filtered through a silica pad, the filtrate was then washed with ether and filtered through a silica pad. The resulting solution was azeotropically distilled with toluene to remove the pyridine. The product was crystallized from ether:hexane to give a white solid (**15**) (0.0186 g, 37%); m.p. 99-102°C (from DCM:light petroleum), (Found: C, 66.88; H, 6.85; N, 2.64. $C_{30}H_{37}NO_8$ requires C, 66.77; H, 6.91; N, 2.59%); $[\alpha]_D^{23}$ +30.4 (c 0.58 in $CHCl_3$); δ_H(200 MHz; $CDCl_3$) 0.83-1.61 (15 H, 3 x m, octyl), 1.97 (3 H, s, CH_3CO), 3.56 (1 H, m, octyl), 3.86 (1 H, m, 5-H), 4.12 (1 H, m, 2-H), 4.69 (2 H, m, 6-H, 6'-H), 5.34 (1 H, d, $J_{1,2}$ 7, 1-H), 5.94 (1 H, d, $J_{NH,2}$ 8, NH), 6.13 (1 H, d, $J_{2,3}$ 10, 3-H), 7.52 (10 H, m, Ph), 8.06 (5 H, m, Ph).

Octyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-methoxyimino-β-D-*xylo*-hexopyranoside (16)

Octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-*xylo*-hexopyranoside-4-ulose (**15**) (0.37 mmol, 0.2 g) and methoxylamine hydrochloride (5.27 mmol, 0.44 g) were

dissolved in ethanol (8 cm^3) and pyridine (1.1 cm^3) under argon atmosphere. The reaction was followed by t.l.c. (toluene:ethyl acetate; 4:1) and when complete the solution was washed with 2 M HCl and extracted with dichloromethane, the organic extracts were then dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue thus obtained was purified by silica chromatography (toluene:ethyl acetate;5:1→7:3) to yield the title compound as an oil (**16**) (0.188 g, 89%), (Found: C, 65.30; H, 7.46; N, 4.69. $C_{31}H_{40}N_2O_8$ requires C, 65.47; H, 7.08; N, 4.92%); $[\alpha]_D^{23}$ -18.3 (*c* 0.21 in $CHCl_3$); δ_H(200 MHz; $CDCl_3$) 0.80-1.53 (15 H, 3 x m, octyl), 2.03 (3 H, s, CH_3CO), 3.40 (1 H, m, octyl), 3.89 (4 H, m, 5-H, OCH_3), 4.46 (1 H, m, 2-H), 4.67 (2 H, m, 6-H, 6'-H), 5.45 (1 H, m, 3-H), 5.63 (1 H, d, $J_{1,2}$ 4, 1-H), 5.92 (1 H, d, $J_{NH,2}$ 9, NH), 7.40 (10 H, m, Ph), 8.05 (5 H, m, Ph).

Octyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-methoxyamino-β-D-gluco-pyranoside (17) and Octyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-methoxyamino-β-D-galactopyranoside (18)

Octyl 2-acetamido-3,6-di-*O*-benzoyl-2,4-dideoxy-4-methoxyimino-β-D-*xylo*-hexo-pyranoside (**16**) (0.474 mmol, 0.269 g) and sodium cyanoborohydride (0.473 mmol, 0.030 g) were dissolved in acetic acid (15 cm^3) and stirred under argon. The reaction was allowed to proceed for 6 days with the addition of a further 6 equivalents of sodium cyanoborohydride over this period. The reaction was followed by t.l.c. (ethyl acetate:toluene, 2:3) and when complete the reaction mixture was diluted with ether, made basic with $NaHCO_3$ and extracted with ether. The organic extracts were dried over magnesium sulfate and the solvent was removed under reduced pressure to give a mixture of two compounds which were separated by column chromatography (toluene:ethyl acetate; 1:1) to give the two title compounds as oils (**17**): δ_H(200 MHz; $CDCl_3$) 0.81-1.62 (15 H, 3 x m, octyl), 2.04 (3 H, s, CH_3CO), 3.42 (1 H, m, octyl), 3.91 (1 H, m, 5-H), 3.89 (3 H, s, CH_3ONH), 4.45 (1 H, m, 2-H), 4.72 (4 H, m, 1-H, 6-H, 6'-H, CH_3ONH), 5.45 (1 H, dd, $J_{3,4}$ 7, $J_{2,3}$ 9, 3-H), 5.63 (1 H, d, $J_{3,4}$ 3, 4-H), 5.72 (1 H, d, $J_{NH,2}$ 8, NH), 7.50 (10 H, m, Ph), 8.05 (5 H, m, Ph);

(18): δ_H(200 MHz; $CDCl_3$) 0.83-1.64 (15 H, 3 x m, octyl), 1.88 (3 H, s, CH_3CO), 3.46 (4 H, m, octyl, C*H*$_3$ONH), 3.69 (1 H, d, $J_{3,4}$ 4, CH_3ONH), 3.96 (3 H, m, 5-H, 6-H, 6'-H), 4.60-4.65 (2 H, m, 2-H, 1-H), 4.65 (1 H, d, $J_{4,5}$ 2, 4-H), (5.41 (1 H, dd, $J_{2,3}$ 12, $J_{3,4}$ 4, 3-H, NH), 7.42 (10 H, m, Ph), 8.04 (5 H, m, Ph).

Octyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (19)[297]

Octyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**12**) (0.044 mol, 14.5 g), *p*-toluene sulfonic acid monohydrate (0.035 equivalents, 0.29 g) were dissolved in dry acetonitrile (250 cm^3) under a nitrogen atmosphere. To this was added dropwise benzaldehyde dimethyl acetal (1.6 equivalents, 10.6 cm^3). Within 10 minutes a white precipitate had formed, by t.l.c. (ethyl acetate:toluene, 1:2) starting material was visible so the reaction was left overnight. When complete triethylamine (0.028 equivalents, 0.17 cm^3) and water (300 cm^3) were added and the precipitate filtered off, this was washed with water to give a white solid (**19**) (17.95 g, 98%), decomposed above 170°C (from DCM:light petroleum); δ_H(200 MHz; $CDCl_3$) 0.88-1.59 (15 H, 3 x m, octyl), 2.05 (3 H, s, C{O}CH_3), 3.47 (5 H, m, octyl, 5-H, 4-H, 3-H, OH), 3.75 (2 H, m, octyl, 2-H), 4.27 (2 H, m, 6-H, 6'-H), 4.72 (1 H, d, $J_{1,2}$ 8.2, 1-H), 5.55 (1 H, s, C*H*Ph), 5.71 (1 H, d, $J_{NH,2}$ 5.7, NH), 7.34 (3 H, m, Ph), 7.48 (2 H, m, Ph).

Octyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (20)[297]

Octyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**19**) (7.19 mmol, 3.037 g) was dissolved in DMF (100 cm^3) under argon and cooled in an ice bath. To the solution was added benzyl bromide (1.1 equivalents, 0.91 cm^3) and then sodium hydride in portions [(3.75 mmol, 0.15 g) from a 60% w/w oil dispersion washed in hexane]. The reaction was followed by t.l.c. (toluene:ethyl acetate, 2:1) and when complete was carefully quenched with ice cold water. The white precipitate that formed was filtered off to give the title compound (**20**) (3.13 g,

85%), m.p. 214-216°C (from DCM:light petroleum); (Found: C, 70.08; H, 8.17; N, 2.70. $C_{30}H_{41}NO_6$ requires C, 70.42; H, 8.07; N, 2.74%); $[\alpha]_D^{23}$ -3.3 (*c* 2.5 in $CHCl_3$); δ_H(200 MHz; $CDCl_3$) 0.87-1.62 (15 H, 3 x m, octyl), 1.88 (3 H, s, C{O}CH_3), 3.23 (1 H, m, octyl), 3.66 (5, m, octyl, 6-H, 6'-H, 5-H, 3-H), 4.32 (2 H, m, 2-H, 4-H), 4.77 (2 H, dd, $^2J_{AX}$ 11.72, CH_2Ph), 4.99 (1 H, d, $J_{1,2}$ 8.3, 1-H), 5.56 (2 H, m, NH, C*H*Ph), 7.39 (10 H, m, Ph).

Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (21)[297]

Octyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**20**) (0.012 mol, 6.00 g), sodium cyanoborohydride (0.118 mol, 7.44 g), some powdered activated molecular sieves (4Å) and several crystals of methyl orange were stirred in THF (350 cm^3) under a nitrogen atmosphere. To this was added via canula diethyl ether saturated with HCl gas until the solution effervesced and was just turning from yellow to pink. The reaction was followed by t.l.c. (toluene:ethyl acetate, 2:1) and when complete (3 days) the reaction was diluted with DCM and filtered. The filtrate was washed with saturated sodium bicarbonate solution and the aqueous portion extracted with DCM. The organic portion was washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure to give an oil. The oil was then dissolved in DCM:methanol (1:1, 70 cm^3) and slowly added to a column of IR120(H^+):IRA400(Cl^-) (1:1, ≈150 cm^3) ion exchange resin. The solution was allowed to slowly drip through the column until all effervescence had ceased. The eluate was concentrated under reduced pressure to give the title compound (**21**) (3.46 g, 57%) m.p. 106-108°C (from DCM:diethyl ether); δ_H(200 MHz; $CDCl_3$) 0.84-1.57 (15 H, 3 x m, octyl), 1.90 (3 H, s, C{O}CH_3), 3.23 (1 H, m, octyl), 3.46 (1 H, m, octyl), 3.82 (1 H, m, 2-H), 4.05 (1 H, dd, $J_{1,3}$ 10.2, $J_{2,3}$ 8.3, 3-H), 4.58 (2 H, dd, $^2J_{AX}$ 12.0, CH_2Ph), 4.75 (2 H, dd, $^2J_{AX}$ 11.7, CH_2Ph), 4.86 (1 H, d, $J_{1,2}$ 8.3, 1-H), 5.60 (1 H, d, $J_{NH,2}$ 7.7, NH), 7.33 (10 H, m, Ph). Diagnostic signals are given and the rest were in accordance with the expected compound.

Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methanesulfonyl-β-D-gluco-pyranoside (22)

Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**21**) (3.89 mmol, 2.00 g) was dissolved in pyridine (0.115 mol, 12 cm^3) and cooled to 0°C under a nitrogen atmosphere. To this was added methane sulfonylchloride (3.88 mmol, 0.30 cm^3) in 0.10 cm^3 aliquots, a further 6 aliquots of methanesulfonyl chloride (0.65 mmol, 0.05 cm^3) were required to drive the reaction to completion after allowing to warm to room temperature (over night). The reaction was followed by t.l.c. (toluene:ethyl acetate; 2:1) and when complete the reaction was quenched with water, diluted with DCM, washed with HCl (2M) and then saturated sodium bicarbonate solution. The organic extracts were dried over magnesium sulfate and the solvent was removed under reduced pressure to yield a yellow gum which was purified by column chromatography (toluene:ethyl acetate, 4;1) to give the title compound (**22**) (2.11 g, 91%), m.p. 120-122°C (from ethyl acetate:hexane) (Found: C, 62.68; H, 7.69; N, 2.32 $C_{31}H_{45}NO_8S$ requires C, 62.92; H, 7.66; N, 2.37%); $[\alpha]_D^{23}$ +17.6 (*c* 1.53 in $CHCl_3$); δ_H(200 MHz; $CDCl_3$) 0.87-1.55 (15 H, 3 x m, octyl), 1.87 (3 H, s, C{O}CH_3), 2.86 (3 H, s, CH_3SO_2), 3.30 (2 H, m, octyl), 3.80 (4 H, m, 6-H, 6'-H, 5-H, 4-H), 4.39 (1 H, t, $J_{2,3}$ 9.4, 3-H), 4.46 (1 H, m, octyl), 4.61 (2H, m, CH_2Ph), 4.73 (2 H, dd, $^2J_{AX}$ 11.3, CH_2Ph), 4.94 (1 H, d, $J_{1,2}$ 8.0, 1-H), 7.32 (10 H, m, Ph), 7.77 (1 H, d, $J_{NH,2}$ 7.3, NH).

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

Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methanesulfonyl-β-D-gluco-pyranoside (**22**) (3.57 mmol, 2.11 g) was dissolved in DMF (13 cm^3) and cesium acetate (17.8 mmol, 3.41 g) was added. This mixture was refluxed at 120°C for 3 days under a nitrogen atmosphere, a further portion of cesium acetate (2.60 mmol, 0.5 g) being added after 2 days. The reaction was followed by t.l.c. (toluene:ethyl acetate; 1:1 or DCM:methanol; 20:1) and when complete the DMF was removed

under reduced pressure. The residue obtained was dissolved in DCM and washed with saturated sodium bicarbonate solution and water, dried over magnesium sulfate and the solvent was removed under reduced pressure. Silica chromatography (toluene:ethyl acetate; 4:1) gave the title compound (**23**) (1.54 g, 78%), m.p. 80-82°C (from ethyl acetate:toluene) (Found: C, 68.80; H, 8.47; N, 2.54 $C_{32}H_{45}NO_7$ requires C, 69.16; H, 8.16; N, 2.52%); $[\alpha]_D^{23}$ +20.6 (*c* 1.85 in $CHCl_3$), δ_H(200 MHz; $CDCl_3$) 0.87-1.64 (15H, 3 x m, octyl), 1.89 (3 H, s, NC{O}CH_3), 2.04 (3 H, s, OC{O}CH_3), 3.26 (1 H, m, octyl), 3.50 (4 H, m, octyl, 5-H, 6-H, 6'-H), 3.82 (1 H, m, 2-H), 4.31 (2 H, m, C*H*Ph, 3-H), 4.45 (1 H, d, $^2J_{AX}$ 11.8, C*H*Ph), 4.57 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 4.71 (1 H, d, $^2J_{AX}$ 10.9, C*H*Ph), 4.99 (1 H, d, $J_{1,2}$ 8.3, 1-H), 5.48 (1 H, d, $J_{NH,2}$ 6.9, NH), 5.61 (1 H, d, $J_{3,4}$ 2.5, 4-H), 7.28 (10 H, m, Ph).

Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (24)

Octyl 2-acetamido-4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (**23**) (1.33×10^{-3} mol, 0.74 g) was dissolved in methanol (100 cm^3) and a small piece of sodium added to make the solution basic (≈pH 8). The reaction was left over night and followed by t.l.c. (toluene:ethyl acetate; 1:1). When complete the reaction was stirred with Amberlite IR 120 (H^+) ion exchange resin until the solution was pH 7, the resin was removed by filtration and the solvent was removed under reduced pressure to give the title compound (**24**) (0.64 g, 94%), m.p. 111-113°C (from methanol) (Found: C, 70.06; H, 8.46; N, 2.80 $C_{30}H_{43}NO_6$ requires C, 70.15; H, 8.44; N, 2.73%); $[\alpha]_D^{23}$ +0.21 (*c* 1.65 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 0.88-1.53 (15 H, 3 x m, octyl), 1.90 (3 H, m, C{O}CH_3), 3.40 (2 H, m, octyl), 3.75 (4 H, m, 6-H, 6'-H, 5-H, OH), 4,05 (1 H, s, 4-H), 4.23 (1 H, m, 2-H), 4.59 (4 H, m, CH_2Ph), 4.91 (1 H, m, 3-H), 5.68 (1 H, m, NH), 7.32 (10 H, m, Ph); δ_C(75.4 MHz; $CDCl_3$) 10.8, 19.4, 20.4, 22.7, 26.1, 26.3, 28.6, 51.4 (octyl, C{O}CH_3), 62.8, 66.1, 66.5, 68.5, 69.9, 70.5, 73.3 (ring carbons, CH_2Ph), 96.4 (1-C), 124.6, 124.9, 125.3, 125.4, 134.7, 134.9 (Ph), 167.6 (NHC{O}CH_3).

Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methanesulfonyl-β-D-galacto-pyranoside (25)

Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-galactopyranoside (**24**) (2.43 mmol, 1.248 g) was dissolved in pyridine (20 cm^3) and cooled to 0°C under a nitrogen atmosphere. To this solution was added methanesulfonyl chloride (4.78 mmol, 0.37 cm^3) and the reaction was followed by t.l.c. (toluene:ethyl acetate; 1:1). The reaction was quenched with water and diluted with DCM, and neutralised with 2M HCl. The organic extracts were washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 5:1) to give the title compound (**25**) (1.19 g, 82%), m.p. 116-118°C (from ethyl acetate:toluene); $[\alpha]_D^{23}$ +27.5 (*c* 0.47 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 0.78-1.56 (15 H, 3 x m, octyl) 1.84 (3 H, s, C{O}CH_3), 2.97 (3 H, s, CH_3SO_2), 2.34 (1 H, m, octyl), 3.37 (1 H, m, octyl), 3.63-3.75 (4 H, m, 2-H, 5-H, 6-H, 6'-H), 4.30 (1 H, m, 3-H), 4.35 (1 H, d, $^2J_{AX}$ 10.7, C*H*Ph), 4.43 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 4.59 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 4.75 (1 H, d, $^2J_{AX}$ 11.0, C*H*Ph), 4.88 (1 H, d, $J_{1,2}$ 7.9, 1-H), 5.28 (1 H, d, $J_{3,4}$ 2.7, 4-H), 5.47 (1 H, d, $J_{NH,2}$ 7.6, NH), 7.25 (10 H, m, Ph); δ_C(75.4 MHz; $CDCl_3$) 10.8, 19.4, 20.3, 22.6, 26.0, 26.2, 28.5, 35.8, 51.8 (octyl, NHC{O}CH_3, CH_3SO_2), 64.7, 66.8, 68.5, 69.4, 70.6, 71.6, 72.3 (ring carbons, CH_2Ph), 96.5 (1-C), 124.8, 125.1, 125.4, 125.4, 125.6, 134.1 (Ph), 167.6 (NHC{O}).

Octyl 2-acetamido-3,6-di-*O*-benzyl-4-azido-2-deoxy-β-D-glucopyranoside (26)

Octyl 2-acetamido-4-*O*-methanesulfonyl-3,6-di-*O*-benzyl-2-deoxy-β-D-galacto-pyranoside (**25**) (0.85 mmol, 0.5 g) was dissolved in DMF, sodium azide (8.46 mmol, 0.55 g) and 15-crown-5 (1.71 mmol, 0.34 cm^3) was added and the mixture refluxed at 120°C overnight under a nitrogen atmosphere. The reaction was followed by t.l.c. (toluene:ethyl acetate; 2:1) and when complete the solvent was removed under reduced pressure and the residue obtained dissolved in DCM. The organic solution was washed with saturated sodium bicarbonate solution and water,

dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 3:1) to give the title compound **(26)** (0.425 g, 93%), m.p. 100-102°C (Found: C, 67.02; H, 8.54; N, 9.66 $C_{30}H_{42}N_4O_5$ requires C, 66.89; H, 7.85; N, 10.4%) $[\alpha]_D^{23}$ +56.4 (*c* 0.96 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 0.77-1.55 (15 H, 3 x m, octyl), 1.80 (3 H, s, C{O}CH_3), 3.03 (1 H, m, octyl), 3.34 (2 H, m, 6-H, 6'-H), 3.53 (1 H, m, 2-H), 3.68 (3 H, m, octyl, 4-H, 5-H), 4.13 (1 H, t, $J_{2,3}$ 9.7, 3-H), 4.53 (3 H, d, dd, $^2J_{AX}$ 11.3, $^2J_{AX}$ 12.1, C*H*Ph, CH_2Ph), 4.77 (2 H, 2 x d, $^2J_{AX}$ 11.5, $J_{1,2}$ 8.2, C*H*Ph, 1-H), 5.55 (1 H, d, $J_{NH,2}$ 7.1, NH), 7.23 (10 H, m, Ph); δ_C(75.4 MHz; $CDCl_3$) 10.8, 19.4, 20.3, 22.7, 26.0, 26.2, 28.6, 55.4, 59.9 (octyl, C{O}*C*H_3, CH_3SO_2), 65.9, 66.7, 70.3, 70.7, 71.7, 75.9 (ring carbons, *C*H_2Ph), 96.2 (1-C), 124.4, 124.6, 124.8, 125.0, 125.3, 125.4, 134.8 (Ph), 167.6 (*C*{O}CH_3).

Octyl 2-acetamido-4-azido-3,6-di-$_O$-benzyl-2,4-dideoxy-β-D-galactopyranoside (27)

Octyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-methanesulfonyl-β-D-gluco-pyranoside **(22)** (0.06 mmol, 0.024 g) was dissolved in DMF (2 cm^3) and to this was added sodium azide (0.154 mmol, 0.01 g) and 15-crown-5 (0.24 mmol, 0.053 g, 0.047 cm^3) and the solution heated to 140°C under an argon atmosphere. The reaction was followed by t.l.c. (toluene:ethyl acetate; 1:1) and when complete (≈48 hours) the reaction was allowed to cool and the solvent was removed under reduced pressure. The residue obtained was dissolved in DCM and washed with saturated sodium bicarbonate solution extracting with DCM, the organic extracts were washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure to give the title compound **(27)** (0.013 g, 61%) m.p. 138-140°C; ν_{max}/cm^{-1} 2109s (azide); $[\alpha]_D^{23}$ +11.29 (c 0.39 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 0.84-1.59 (15 H, 3 x m, octyl), 1.91 (3 H, s, CH_3O), 3.20 (1 H, m, octyl), 3.40 (1 H, m, octyl), 3.71 (4 H, m, 2-H, 5-H, 6-H, 6'-H), 4.04 (1 H, d, $J_{4,5}$ 3.6, 4-H), 4.62 (5 H, m, 3-H, 2 x CH_2Ph), 5.00 (1 H, d, $J_{1,2}$ 8.2, 1-H), 5.68 (1 H, d, $J_{NH,2}$ 7.1, NH), 7.32 (10 H,

m, Ph); δ_C(75.4 MHz, $CDCl_3$) 13.9, 22.5, 23.5, 25.8, 29.2, 31.7, 55.8, 59.7 (octyl, C{O}CH_3), 68.5, 69.9, 71.4, 72.4, 73.6, 76.1 (ring carbons, CH_2Ph), 99.3 (1-C), 127.9, 128.3, 128.5, 128.6 (Ph), 170.9 (C{O}CH_3).

Octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-methylsulfonyl-β-D-gluco-pyranoside (28)

Octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (**13**) (1.86 mmol, 1.0g) was dissolved in pyridine (25 cm^3) and cooled to 0°C under argon. Methanesulfonyl chloride was added to this dropwise, and the reaction was stirred and followed by t.l.c. (methanol:dichloromethane, 1:20) until complete. The reaction was quenched with a few drops of water, the solution was diluted with dichloromethane and neutralised with 2M HCl. The aqueous solution was extracted with dichloromethane, dried over magnesium sulfate and the solvent was removed under reduced pressure to yield the title compound (**28**) (0.985 g, 87%), m.p. 129-131°C (from DCM:light petroleum); δ_H(200 MHz; $CDCl_3$) 0.83-1.65 (15 H, 3 x m, octyl), 1.87 (3 H, s, CH_3CO), 2.87 (3 H, s, CH_3SO_2), 3.49 (1 H, m, octyl), 3.90 (1 H, m, 5-H), 4.12 (1 H, m, 2-H), 4.69 (2 H, m, 6-H, 6'-H) 4.80 (1 H, d, $J_{1,2}$ 9, 1-H), 5.04 (1 H, d, $J_{2,3}$ 9, 3-H), 5.68 (2 H, m, NH, 4-H), 7.62 (10 H, m, Ph), 8.09 (5 H, m, Ph).

Octyl 2-acetamido-4-azido-3,6-di-*O*-benzoyl-2,4-dideoxy-β-D-galacto-pyranoside (29)

Octyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-methanesulfonyl-β-D-gluco-pyranoside (**28**) (0.33 mmol, 0.2 g) and sodium azide (0.49 mmol, 0.03 g) were dissolved in DMF (3.5 cm^3) and stirred at 110°C under nitrogen . The reaction was followed by t.l.c. (DCM:methanol; 20:1) and when complete the DMF was removed under reduced pressure. The resulting compound was dissolved in dichloromethane, washed with saturated $NaHCO_3$ solution and water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained was

purified by silica chromatography (toluene:ethyl acetate; 5:1) to yield the title compound (**29**) (0.09 g, 50%). m.p. 102-104°C (from diethyl ether:hexane); $[\alpha]_D^{23}$ -116.2 (*c* 1 in $CHCl_3$); ν_{max}/cm^{-1} 2110s (azide); δ_H(300 MHz; $CDCl_3$) 0.68-1.55 (15 H, 3 x m, octyl), 1.86 (3 H, s, CH_3CO), 3.49 (1 H, m, octyl), 3.85 (1 H, m, octyl), 4.13 (2 H, m, 2-H, 5-H), 4.22 (1 H, d, $J_{3,4}$ 3.0, 4-H), 4.55 (2 H, m, 6-H, 6'H), 4.83 (1 H, d, $J_{1,2}$ 8.5, 1-H), 5.78 (1 H, dd, $J_{3,4}$ 3.6, $J_{2,3}$ 11.2, H-3), 5.82 (2 H, d, $J_{NH,2}$ 8.5, NH), 7.40 (4 H, m, Ph), 7.55 (2 H, m, Ph), 8.03 (4 H, Ph), δ_C(75.4 MHz, $CDCl_3$) 13.9, 22.5, 23.2, 25.7, 29.1, 29.4, 31.7, 52.6 (octyl, C{O}*C*H3) 60.5, 63.1, 69.7, 70.6, 72.3 (ring carbons) 100.6 (1-C), 128.5, 128.7, 129.5, 129.7, 130.1, 133.4, 133.8 (Ph), 166.1, 166.2 (CO), 170.6 (NHCO).

Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (34)

Penta-*O*-acetyl-D-galactopyranose (**33**) (2.56 mmol, 1 g) and zinc iodide (0.018 mol, 5.8 g) were dissolved in dry 1,2-dichloroethane (50 cm^3) under a nitrogen atmosphere and stirred for several minutes. Phenylthiotrimethylsilane (9.98 mmol, 1.82 g, 1.89 cm^3) was added dropwise and the reaction was heated to 50°C under a nitrogen atmosphere. The reaction was followed by t.l.c (toluene:ethyl acetate; 2:1) and when complete (≈3 hours) it was allowed to cool, diluted with DCM and filtered through Celite. This solution was washed with sodium thiosulfate solution (2M) and the aqueous portion was extracted with DCM, the organic extracts were washed with saturated sodium bicarbonate solution and water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The oil obtained was purified by silica chromatography (neat toluene then neat ethyl acetate) to give the title compound as a glassy solid (**34**) (1.02 g, 90%); δ_H(300 MHz; $CDCl_3$) 1.96 (3 H, s, C{O}CH_3), 2.03 (3 H, s, C{O}CH_3), 2.08 (3 H, s, C{O}CH_3), 2.10 (3 H, s, C{O}CH_3), 3.92 (1 H, t, $J_{5,6}$ 6.6, 5-H), 4.12 (2 H, m, 6-H, 6'-H), 4.70 (1 H, d, *J* 9.9, 1-H), 5.04 (1 H, dd, $J_{3,4}$ 3.4, $J_{2,3}$ 9.9, 3-H), 5.23 (1 H, t, $J_{1,2}$ 9.9, 2-H), 5.41 (1 H, d, $J_{3,4}$ 3.3, 4-H), 7.28 (3 H, m, Ph), 7.50 (2 H, m, Ph); δ_C(75.4 MHz; $CDCl_3$) 20.5, 20.7

(C{O}CH_3), 61.6, 67.2, 71.9, 74.4 (ring carbons), 86.6 (1-C), 128.1, 128.9, 132.6 (Ph), 170.4 (C{O} x 4).

Methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (37)

Penta-*O*-acetyl-D-galactopyranose (**33**) (0.013 mol, 5 g) and zinc iodide (0.064 mol, 20.5 g) were dissolved in dry 1,2-dichloroethane (50 cm^3) under a nitrogen atmosphere and stirred for several minutes. Methylthiotrimethylsilane (0.032 mol, 3.85 g, 4.54 cm^3) was added dropwise and the reaction was stirred under a nitrogen atmosphere. The reaction was followed by t.l.c (toluene:ethyl acetate; 2:1) and when complete (1 hour) it was allowed to cool, diluted with DCM and filtered through Celite. This solution was washed with sodium thiosulfate solution (2M) and the aqueous portion was extracted with DCM. The organic extracts were washed with sodium bicarbonate solution (2M) and water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained crystallised under refrigeration, the crystals were filtered off and washed with copious amounts of hexane to give the title compound as a white crystalline solid (**37**) (4.52 g, 90%), m.p. 103-105°C (from DCM:hexane) (lit., [296] m.p. 110-111°C); δ_H(300 MHz; $CDCl_3$) 1.97-2.17 (15 H, m, C{O}CH_3, SCH_3), 3.94 (1 H, dt, $J_{4,5}$ 1.0, $J_{5,6}$ 6.6, 5-H), 4.11 (2 H, m, 6-H, 6'-H), 4.37 (1 H, d, $J_{1,2}$ 9.6, 1-H), 5.03 (1 H, dd, $J_{3,4}$ 3.3, $J_{2,3}$ 10.2, 3-H), 5.24 (1 H, t, $J_{1,2}$ 10.0, 2-H), 5.42 (1 H, dd, $J_{3,4}$ 1.1, $J_{4,5}$3.6, 4-H).

Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (36)

Phenyl 1-thio-β-D-galactopyranoside (**35**) (2.25 mmol, 0.612 g) was dissolved in dry THF (20 cm^3) and crushed KOH (0.016 mol, 0.909 g), 18-crown-6 (0.36 mmol, 0.095 g) and benzyl bromide (9.89 mmol, 1.19 cm^3) were added. The reaction (under a nitrogen atmosphere) was followed by t.l.c. (toluene:ethyl acetate; 10:1) and when complete (overnight) the reaction was quenched with methanol, diluted with DCM, washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained was purified by silica

chromatography (toluene:ethyl acetate; 20:1) to give the title compound (**36**) (5.71 g, 98%) m.p. 75-77°C (from DCM:light petroleum) (lit., [193] 86-88°C); $[\alpha]_D^{23}$ +3.13 (*c*1.15 in $CHCl_3$) (lit., [193] $[\alpha]_D^{23}$ +5, *c* 1 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 3.65 (4 H, m, 3-H, 5-H, 6-H, 6'-H), 3.97 (2 H, m, 2-H, 4-H), 4.47 (2 H, dd, $^2J_{AX}$ 11.5, CH_2Ph), 4.64 (2 H, m, 1-H, C*H*Ph), 4.79 (4 H, m, CH_2Ph), 5.00 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 7.32 (23 H, m, Ph), 7.60 (2 H, m, Ph).

Methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-methylthio-1-thio-β-D-galactopyranoside (40) and methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (37)

Penta-*O*-acetyl-D-galactopyranose (**33**) (1.0 mmol, 0.39 g) and zinc iodide (7.2 mmol, 2.29 g) were dissolved in dry 1,2-dichloroethane (20 cm^3) under a nitrogen atmosphere and stirred for several minutes. Methylthiotrimethylsilane (9.98 mmol, 1.82 g, 1.89 cm^3) was added dropwise and the reaction heated to 50°C under a nitrogen atmosphere. The reaction was followed by t.l.c (toluene:ethyl acetate; 2:1) and when complete (≈4.5 hours) it was allowed to cool and filtered through Celite. This solution was washed with sodium thiosulfate solution (2M) and the aqueous portion was extracted with DCM. The organic extracts were washed with saturated sodium bicarbonate solution and water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The oil obtained was purified by silica chromatography (toluene:ethyl acetate; 15:1) to give the title compounds, methyl 2,3,4-tri-*O*-acetyl-6-deoxy-6-methylthio-1-thio-β-D-galactopyranoside (**40**) (0.17 g, 44%); δ_H(300 MHz; $CDCl_3$) 1.94-2.17 (18 H, 5 x s, C{O}CH_3, SCH_3), 2.50 (1 H, dd, $^2J_{AX}$ 6.7, $^2J_{AX}$ 14.0, 6 or 6'-H), 2.70 (1 H, dd, $^2J_{AX}$ 6.7, $^2J_{AX}$ 14.0, 6 or 6'-H), 3.78 (1 H, t, $J_{5,6}$ 6.6, 5-H), 4.37 (1 H, d, $J_{1,2}$ 9.9, 1-H), 5.03 (1 H, dd, $J_{3,4}$ 3.3, $J_{2,3}$ 9.9, 3-H), 5.21 (1 H, t, $J_{1,2}$ 9.9, 2-H), 5.48 (1 H, d, $J_{3,4}$ 3.3, 4-H); δ_C(75.4 MHz; $CDCl_3$, assigned with the aid of a DEPT spectrum), 11.4, 16.4 (SCH_3), 20.4, 20.5, 20.6 (C{O}CH_3), 33.9 (6-C), 66.5, 68.4, 71.9, 76.9 (ring carbons), 83.4 (1-C), 169.7, 170.0, 170.2 (*C{O}*CH_3); and methyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (0.17 g, 43%) consistent with (**37**).

Methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (39)

Methyl 1-thio-β-D-galactopyranoside (**38**) (0.011 mol, 0.241 g) was dissolved in dry THF (40 cm^3) and crushed KOH (0.083 mol, 4.678 g), 18-crown-6 (1.85 mmol, 0.49 g) and benzyl bromide (0.05 mol, 6.065 cm^3) were added. The reaction (under a nitrogen atmosphere) was followed by t.l.c. (toluene:ethyl acetate; 10:1) and when complete [overnight, after addition of a further aliquot of benzyl bromide (1cm^3)] the reaction was quenched with methanol, diluted with DCM, washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 20:1) to give the title compound (**39**) (3.57 g, 55%) m.p. 52-54°C (from ethanol); δ_H(500 MHz; $CDCl_3$ assigned with the aid of 2-D N.M.R.) 2.19 (3 H, s, SCH_3), 3.58 (4 H, m, 3-H, 5-H, 6-H, 6'-H), 3.84 (1 H, t, $J_{1,2}$ 9.4, 2-H), 3.96 (1 H, d, $J_{3,4}$ 2.6, 4-H), 4.32 (1 H, d, $J_{1,2}$ 9.6, 1-H), 4.43 (2 H, dd, $^2J_{AX}$ 11.7, CH_2Ph), 4.60 (1 H, d, $^2J_{AX}$ 11.6, C*H*Ph), 4.72 (2 H, s, CH_2Ph), 4.83 (2 H, dd, $^2J_{AX}$ 10.2, CH_2Ph), 4.95 (1 H, d, $^2J_{AX}$ 11.7, C*H*Ph), 7.31 (20 H, m, Ph).

***p*-Methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (42)**

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**41**) (0.486 mmol, 0.2 g) and tetrabutylammonium hydrogen sulfate (0.49 mmol, 0.165 g) were dissolved in DCM (1 cm^3) and sodium carbonate solution (1M) (1 cm^3), to this was added *p*-methoxythiophenol (1.45 mmol, 0.179 cm^3), and the reaction was stirred vigorously. The reaction was followed by t.l.c. (toluene:ethyl acetate; 3:2) and when complete the mixture was diluted with DCM and washed with NaOH solution (1M), brine and water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 5:1) to give the title compound (**42**) (0.14 g, 61%); δ_H(300 MHz; $CDCl_3$) 1.88-2.26 (12 H, 4 x s, C{O}CH_3), 3.72 (3 H, s, OCH_3), 6.59 (1 H, t, $J_{5,6}$ 6.6, 5-H), 4.05 (2 H, m, 6-H, 6'-H), 4.49 (1 H, d, $J_{1,2}$ 9.9, 1-H), 4.95 (1 H, dd, $J_{3,4}$ 3.3, $J_{2,3}$ 9.9, 3-H), 5.09 (1 H, t, $J_{1,2}$ 9.9, 2-H), 5.30 (1 H, dd, $J_{3,4}$ 1.1, $J_{4,5}$ 3.3, 4-H), 6.77 (2 H, m,

PhOCH_3), 7.39 (2 H, m, *Ph*OCH_3); δ_C(75.4 MHz; $CDCl_3$) 20.3, 20.4, 20.6 (C{O}CH_3)., 55.1 (OCH_3), 61.4, 67.1, 67.2,. 71.9, 74.1 (ring carbons), 86.7 (1-C), 114.2, 135.8, 160.2 (Ph), 169.3, 170.0, 170.1, 170.3 (*C{O}*CH_3).

_p_-Methoxyphenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (44)

p-Methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**42**) was deacetylated in methanol with sodium methoxide (generated *in situ*) to give *p*-methoxyphenyl 1-thio-β-D-galactopyranoside (**43**) which was used directly in the next reaction. Compound (**43**) (0.27 mmol, 0.079 g) was dissolved in THF (5 cm^3), crushed KOH (1.92 mmol, 0.108 g), 18-crown-6 (0.06 mmol, 0.016 g) and benzyl bromide (1.19 mmol, 0.142 cm^3) were added under a nitrogen atmosphere. The reaction was followed by t.l.c. (toluene:ethyl acetate; 10:1) and when complete it was quenched with methanol, diluted with DCM, washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure to give the title compound (**44**) (0.166 g, 94%) m.p. 102-104°C (from DCM:ethanol); δ_H(300 MHz; $CDCl_3$) 3.67 (4 H, m, 3-H, 5-H, 6-H, 6'-H), 3.75 (3 H, s, OCH_3), 3.89 (1 H, t, $J_{1,2}$ 9.4, 2-H), 3.99 (1 H, d, $J_{3,4}$ 2.2, 4-H), 4.47 (2 H, dd, $^2J_{AX}$ 11.5, CH_2Ph), 4.55 (1 H, d, $J_{1,2}$ 9.6, 1-H), 4.62 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 4.74 (2 H, m, CH_2Ph), 4.80 (2 H, dd, $^2J_{AX}$ 10.2, CH_2Ph), 4.98 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 6.75 (2 H, m, *Ph*OCH_3), 7.32 (18 H, m, Ph), 7.44 (2 H, m, Ph), 7.54 (2 H, m, *Ph*OCH_3); δ_C(75.4 MHz; $CDCl_3$) 55.1 (OCH_3), 68.7, 72.6, 73.5, 74.3, 75.5, 77.1, 77.2 (ring carbons, CH_2Ph), 84.2 (1-C), 88.3 (*Ph*OMe), 114.3 (*Ph*OCH_3), 124.0 (*Ph*OCH_3), 127.4, 127.6, 127.7, 127.8, 127.9, 128.2, 128.3, 128.4 (Ph), 134.7 (*Ph*OCH_3), 138.0, 138.3, 138.5, 138.9 (Ph), 159.5 (*Ph*OCH_3).

_p_-Nitrophenyl 2,3,4,6-tetra-_O_-acetyl-1-thio-β-D-galactopyranoside (45)

2,3,4,6-Tetra-_O_-acetyl-α-D-galactopyranosyl bromide (**41**) (2.43 mmol, 1.0 g) and tetrabutylammonium hydrogen sulfate (2.42 mmol, 0.823 g) were dissolved in DCM (5 cm^3) and sodium carbonate solution (1M)(5 cm^3). _p_-Nitrothiophenol (7.28 mmol, 1.13 cm^3) was added and the reaction was stirred vigorously. The reaction was followed by t.l.c. (toluene:ethyl acetate; 10:1) and when complete (≈2 hours) the mixture was diluted with DCM and washed with NaOH solution (1M). The organic extracts were washed with brine then water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 5:1) to give the title compound (**45**) (0.74 g, 63%) (Found: C, 49.82; H, 4.81; N, 2.81 $C_{20}H_{23}NO_{11}$ requires C, 49.48; H, 4.77; N, 2.88%) $[\alpha]_D^{23}$ -8.7 (_c_ 0.94 in CH_3Cl) (ref., [268] $[\alpha]_D^{23}$ -8.3, _c_ 1 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 1.97-2.15 (12 H, 4 x s, C{O}CH_3), 4.03 (1 H, t, $J_{5,6}$ 6.4, 5-H), 4.18 (2 H, m, 6-H, 6'-H), 4.86 (1 H, d, $J_{1,2}$ 9.9, 1-H), 5.09 (1 H, dd, $J_{3,4}$ 3.3, $J_{2,3}$ 9.9, 3-H), 5.29 (1 H, t, $J_{1,2}$ 10.0, 2-H), 5.46 (1 H, d, $J_{3,4}$ 3.3, 4-H), 7.61 (2 H, m, $PhNO_2$), 8.14 (2 H, m, $PhNO_2$); δ_C(75.4 MHz; $CDCl_3$) 20.4, 20.5, 20.6 (C{O}CH_3), 61.7, 66.7, 67.0, 71.7, 74.8 (ring carbons), 84.8 (1-C), 123.9, 130.5, 142.4, 146.9 (Ph), 169.5, 170.0 (_C{O}_CH_3).

_p_-Nitrophenyl 2,3,4,6-tetra-_O_-benzyl-1-thio-β-D-galactopyranoside (47)

_p_-Nitrophenyl 1-thio-β-D-galactopyranoside (**46**) (0.33 mmol, 0.103 g), KOH (2.32 mmol, 0.13 g) and (0.07 mmol, 0.018 g) were dissolved in THF (5 cm^3). To this solution was added benzyl bromide (1.40 mmol, 0.17 cm^3) under a nitrogen atmosphere and the reaction was stirred. The reaction was followed by t.l.c. (toluene:ethyl acetate; 10:1) and when complete (≈9 hours) the reaction was quenched with methanol (≈30 minutes), diluted with DCM, washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 20:1) to give the title compound (**47**) (0.178 g, 80%); δ_H(300 MHz; $CDCl_3$) 3.64 (4 H, m,

3-H, 5-H, 6-H, 6'-H), 4.02 (2 H, m, 2-H, 4-H), 4.47 (2 H, dd, $^2J_{AX}$ 11.6, CH_2Ph), 4.58 (1 H, d, $^2J_{AX}$ 11.0, C*H*Ph), 4.72 (5 H, m, 1-H, CH_2Ph), 4.97 (1 H, d, $^2J_{AX}$ 11.0, C*H*Ph), 7.30 (20 H, m, Ph), 7.59 (2 H, m, $PhNO_2$), 7.87 (2 H, m, $PhNO_2$)

Methyl 2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranoside (49)

The standard procedure for iodine activation of a thioglycoside was as follows: Thioglycoside (0.125 mmol) was dissolved in DCM (1 cm^3) and methanol (0.0123 mol, 0.5 cm^3). To this solution was added iodine (2 mol equivalents, 0.248 mmol, 0.063 g) and the reaction followed by t.l.c. (toluene:ethyl acetate; 10:1). When complete the reaction was quenched with Amberlite IRA 400 (OH^-) ion exchange resin until all visible colour was gone from the solution, the resin was filtered off and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 50:1) to give the title compound as a mixture of anomers. The ratio of α to β anomers was determined from the OCH_3 group intensity by ^{13}CNMR; δ_C(75.4 MHz; $CDCl_3$) 55.2 (α-OCH_3), 56.9 (β-OCH_3), 98.8 (α-1-C), 105.0 (β-1-C).

The standard procedure for iodine/DDQ activation of a thioglycoside was as follows: Thioglycoside (0.125 mmol) was dissolved in DCM or acetonitrile (1 cm^3) and methanol (0.0123 mol, 0.5 cm^3). To this solution was added iodine (2 mol equivalents, 0.248 mmol, 0.063 g) and DDQ (1 mol equivalent, 0.125 mmol, 0.135 g) and the reaction followed by t.l.c. (toluene:ethyl acetate; 10:1). When complete the reaction was quenched with sodium thiosulfate (2M) extracting with DCM, the organic extracts were washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 50:1) to give the title compound as a mixture of anomers, the ratio of α to β anomers was determined from the OCH_3 group intensity by ^{13}CNMR.

The standard procedure for iodine monohalide activation of a thioglycoside was as follows:

Thioglycoside (0.125 mmol) was dissolved in DCM or acetonitrile (1 cm^3) and methanol (0.0123 mol, 0.5 cm^3). To this solution was added IBr or ICl (1M in DCM)(2 mol equivalents, 0.25 mmol, 0.25 cm^3) and the reaction followed by t.l.c. (toluene:ethyl acetate; 10:1). When complete the reaction was quenched with sodium thiosulfate (2M) extracting with DCM. The organic extracts were washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure to give the title compound as a mixture of anomers, the ratio of α to β anomers was determined from the OCH_3 group intensity by ^{13}CNMR.

_p_-Aminophenyl 2,3,4,6-tetra-_O_-benzyl-1-thio-β-D-galactopyranoside (50)

_p_-Nitrophenyl 2,3,4,6-tetra-_O_-benzyl-1-thio-β-D-galactopyranoside (**47**) (0.74 mmol, 0.5 g) and tin (II) chloride (3.69 mmol, 0.7 g) were suspended in ethanol (25 cm^3) and refluxed under a nitrogen atmosphere. The reaction was followed by t.l.c. (toluene:ethyl acetate; 10:1) and when complete [after addition of a further portion of tin (II) chloride (0.14 g] the reaction was allowed to cool, filtered through Celite and washed with saturated sodium bicarbonate solution extracting with DCM. The organic extracts were washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure to give the title compound which was not purified further (**50**); δ_H(300 MHz; $CDCl_3$) 3.51 (4 H, m, 3-H, 5-H, 6-H, 6'-H), 3.76 (1 H, t, $J_{1,2}$ 9.5, 2-H), 3.87 (1 H, d, $J_{3,4}$ 2.6, 4-H), 4.35 (3 H, dd, $^2J_{AX}$ 11.6, CH_2Ph), 4.40 (1 H, d, $J_{1,2}$ 9.7, 1 H), 4.51 (1 H, d, $^2J_{AX}$ 11.8, C*H*Ph), 4.63 (2 H, dd, $^2J_{AX}$ 11.8, CH_2Ph), 4.71 (2 H, dd, $^2J_{AX}$ 10.1, CH_2Ph), 4.87 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 6.41 (2 H, m, *Ph*NH_2), 7.27 (22 H, m, Ph, *Ph*NH_2); δ_C(75.4 MHz; $CDCl_3$) 68.7, 72.6, 73.5, 74.2, 75.6 (ring carbons, CH_2Ph)), 84.3 (1-C), 88.8 (PhNH_2), 115.3, 120.9, 127.4, 127.6, 127.7, 127.9, 128.0, 128.2, 128.4, 128.5, 135.2, 138.0, 138.4, 138,.5, 138.9, 146.5 (Ph).

_p_-Acetamidophenyl 2,3,4,6-tetra-_O_-benzyl-1-thio-β-D-galactopyranoside (51)

_p_-Aminophenyl 2,3,4,6-tetra-_O_-benzyl-1-thio-β-D-galactopyranoside (**50**) was dissolved in pyridine (10 cm^3) and acetic anhydride (3.18 mmol, 0.3 cm^3) under a nitrogen atmosphere. The reaction was followed by t.l.c. (toluene:ethyl acetate; 10:1) and when complete (overnight) the solution was diluted with DCM and neutralised with HCl (2M) extracting with DCM. The organic extracts were washed with saturated sodium bicarbonate solution, dried over magnesium sulfate and the solvent was removed under reduce pressure to give the title compound (**51**) (0.179 g, 35% from _p_-nitrophenyl 2,3,4,6-tetra-_O_-benzyl-1-thio-β-D-galactopyranoside); ν_{max}/cm^{-1} 1592, 1685 (NHCO); δ_H(300 MHz; $CDCl_3$) 2.07 (3 H, s, NHC{O}CH_3), 4.52 (4 H, m, 3-H, 5-H, 6-H, 6'-H), 3.80 (1 H, t, $J_{1,2}$ 9.3, 2-H), 3.89 (1 H, d, $J_{3,4}$ 2.5, 4-H), 4.35 (2 H, dd, $^2J_{AX}$ 11.8, CH_2Ph), 4.49 (1 H, d, $J_{1,2}$ 9.89, 1-H), 4.52 (1 H, d $^2J_{AX}$ 11.5, C*H*Ph), 4.68 (4 H, m, CH_2Ph), 4.87 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 7.28 (24 H, m, Ph, *Ph*NHAc); δ_C(75.4 MHz; $CDCl_3$) 24.5 (C{O}CH_3), 68.6, 72.6, 73.5, 74.4, 75.6 (ring carbons), 84.1 (1-C), 88.1 (PhNHAc), 119.9, 127.6, 128.0, 128.3, 128.4, 128.5, 133.1, 137.4, 138.3, 138.8 (Ph, *Ph*NHAc), 168.3 (NHC{O}).

Phenyl 2,3,4,6-tetra-_O_-pivaloyl-1-thio-β-D-galactopyranoside (52)

Phenyl-1-thio-β-D-galactopyranoside (**35**) (2 mmol, 0.57 g) was dissolved in pyridine (20 cm^3) and pivaloyl chloride (8.8 mmol, 1.135 cm^3) added under a nitrogen atmosphere. The reaction was followed by t.l.c. (toluene:ethyl acetate; 1:1) and when complete (overnight) methanol was added to quench the reaction. The solution was neutralised with HCl (2M) and extracted with DCM. The organic extracts were washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 5:1) to give the title compound (**52**) (0.215 g, 20%) m.p. 107-110°C (from light petroleum:diethyl ether); (Found: C, 63.46; H, 8.15; N $C_{32}H_{48}O_9S$ requires C, 63.13; H, 7.95); δ_H(300 MHz; $CDCl_3$) 1.15 (9 H, s, $(CH_3)_3$C{O}), 1.16 (9 H, s, $(CH_3)_3$C{O}), 1.19 (9 H, s, $(CH_3)_3$C{O}), 1.21 (9 H, s,

$(CH_3)_3C\{O\}$), 3.79 (1 H, t, $J_{5,6}$ 6.1, 5-H), 3.88 (1 H, t, $J_{1,2}$ 9.7, 2-H), 4.00 (1 H, d, $J_{3,4}$ 3.0, 4-H), 4.27 (2 H, m, 6-H, 6'-H), 4.63 (1 H, d, $J_{1,2}$ 9.9, 1-H), 4.88 (1 H, dd, $J_{3,4}$ 3.3, $J_{2,3}$ 9.5, 3-H), 7.21 (3 H, m, Ph), 7.53 (2 H, m, Ph); δ_C(75.4 MHz; $CDCl_3$) 26.9 ($^tBuC\{O\}$), 63.2, 67.6, 75.6, 76.1 (ring carbons), 89.4 (1-C), 127.8, 128.9, 132.0 (Ph), 178.2, 178.5 ($^tBuC\{O\}$).

Methyl 2,3,4,6-tetra-*O*-pivaloyl-α/β-D-galactopyranoside (54)

The standard procedure for activation of phenyl 2,3,4,6-tetra-*O*-pivaloyl-1-thio-β-D-galactopyranoside (**53**) was as follows:

Phenyl 2,3,4,6-tetra-*O*-pivaloyl-1-thio-β-D-galactopyranoside (**53**) (0.125 mmol, 0.065 g) was dissolved in acetonitrile (1 cm^3) and methanol (0.0123 mol, 0.5 cm^3). To this solution was added iodine (1.1 mol equivalents, 0.138 mmol, 0.035g) and either no other reagent or DDQ (1.2 mol eqivalents, 0.154 mmol, 0.035g) or CAN (1.1 mol equivalents, 0.136 mmol, 0.075 g). The reaction was followed by t.l.c. (toluene:ethyl acetate; 1:1) and when complete the reaction was quenched with Amberlite IRA 400 (OH^-) ion exchange resin until all visible colour was gone from the solution, the resin was filtered off and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 3:1) to give the title compound as a mixture of anomers, the ratio of α to β anomers was determined from the OCH_3 group intensity by ^1H NMR; δ_H(300 MHz; $CDCl_3$) 3.43 (α-OCH_3), 3.55 (β-OCH_3).

Phenyl 3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (56)

Phenyl 1-thio-β-D-galactopyranoside (**35**) (0.545 mmol, 0.156 g) was dissolved in DMF (1 cm^3) and 2,2-dimethoxy propane (0.041 mol, 5 cm^3) and p-toluenesulfonic acid (0.031 mmol, 0.006 g) were added under a nitrogen atmosphere. The reaction was followed by t.l.c. (toluene:ethyl acetate; 1:1) and when complete the solution was made slightly basic (≈pH 7.5-8) with triethylamine and the solvent was removed under reduced pressure. The residue obtained was dissolved in DCM and TFA (50%

aqueous, 0.0025 mmol, 0.05 cm^3) was added and the reaction was followed by t.l.c. (toluene:ethyl acetate; 2:1) until complete (10 minutes). The reaction was made pH8 with triethylamine. The solution was diluted with DCM and washed with HCl (2M). The organic extracts washed with saturated sodium bicarbonate solution dried over magnesium sulfate and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (hexane:ethyl acetate; 5:1→ 1:1) to give the title compound (**56**) (0.057 g, 52%) δ_H(300 MHz; $CDCl_3$) 1.33 (3 H, s, CH_3), 1.41 (3 H, s, CH_3), 3.56 (1 H, m, 2-H), 3.82 (2 H, m, 4-H, 6-H), 3.98 (1 H, m, 6-H), 4.10 (1 H, t, $J_{5,6}$ 6.3, 5-H), 4.18 (1 H, dd, $J_{3,4}$ 2.2, $J_{2,3}$ 5.5, 3-H), 4.46 (1 H, d, $J_{1,2}$ 10.4, 1 H), 7.29 (3 H, m, Ph), 7.54 (2 H, m, Ph); δ_C(75.4 MHz; $CDCl_3$) 26.2, 27.9 (CH_3), 62.6, 71.4, 73.8, 79.1, 87.8 (ring carbons), 110.5 (1-C), 128.2, 129.1 (Ph), 131.8 (CMe_2), 132.6 (Ph).

Attempted synthesis of phenyl 2,6-di-*O*-(2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopyranosyl)-3,4-isopropylidene-1-thio-β-D-galactopyranoside (58)

Phenyl 3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (**56**) (0.0032 mmol, 0.01 g) and methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) (0.0088 mmol, 0.05 g) were dissolved in MeCN:DCM (0.4 cm^3, 3:2). To this was added 4Å molecular sieves (0.05 g) and potassium carbonate (0.014 g) and the reaction cooled to 0°C. To this mixture was added iodine (0.0098 mmol, 0.025 g) and the reaction monitored by t.l.c. (1/2 run toluene:ethyl acetate;1:1 then with hexane:ethyl acetate;3:1). After 10 minutes the reaction was diluted with DCM and quenched with sodium thiosulfate (2M). The solution was extracted with DCM and the organic extracts washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure.

This reaction was also carried out at -10°C and stopped after 20 minutes.

Attempted synthesis of phenyl 2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (60)

Methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) (0.0044 mmol, 0.025 g) and phenyl 2,3,4-tri-*O*-benzyl-1-thio-β–D-galactopyranoside (**63**) (0.0037 mmol, 0.02 g) were dissolved in DCM (1 cm^3) and to this solution iodine (0.002 mmol, 0.005 g) was added. The reaction was followed by t.l.c. (toluene:ethyl acetate; 10:1) and after 1 hour and 8 minutes a further portion of iodine was added (0.005 g). The reaction was quenched with sodium thiosulfate after 3 hours and 20 minutes. The solution was extracted with DCM and the organic extracts washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure.

This reaction was also attempted with the conditions given above but with the addition of 3 Å molecular sieves.

Phenyl 6-*O*-*t*-butyldimethylsilyl-1-thio-β–D-galactopyranoside (61)

Phenyl 1-thio-β-D-galactopyranoside (**35**) (7.35 mmol, 2.0 g) was dissolved in ice cold pyridine (10 cm^3) and *t*-butyldimethylsilyl chloride (8.36 mmol, 1.26 g) added. The reaction was followed by t.l.c. (DCM:methanol; 9.2:0.8) and when complete [≈2 hours, after addition of a further portion of TBDMSCl (0.4 g)] the reaction was quenched with methanol and the solvent was removed under reduced pressure by azeotropic distillation with toluene. The residue obtained was purified on a short silica pad (toluene then toluene:ethyl acetate1:1 then ethyl acetate) to give the title compound (**61**) which was used directly in the next step.

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-1-thio-β–D-galacto-pyranoside (62)

Phenyl 6-*O*-*t*-butyldimethylsilyl-1-thio-β–D-galactopyranoside (**61**) was dissolved in DMF (10 cm^3) and cooled to 0°C and benzyl bromide (0.024 mol, 2.88 cm^3) and then sodium hydride (60% in mineral oil, washed with hexane) were added portionwise (each portion ≈8.33 mmol, 0.2 g). The reaction was followed by t.l.c. (DCM:methanol; 9.2:0.2) and portions of sodium hydride added every 15-20 minutes until complete (≈4 hours). When complete the reaction was quenched with methanol and the solvent was removed under reduced pressure. The residue obtained was dissolved in DCM and washed with sodium hydroxide solution (1M). The organic extracts were washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure to give the title compound (**62**); δ_H(300 MHz; $CDCl_3$) 0.06 (6 H, s, Me_2Si), 0.91 (9 H, s, Me_3CSi), 3.47 (1 H, t, $J_{5,6}$ 6.6, 5-H), 3.62 (1 H, dd, $J_{3,4}$ 2.8, $J_{2,3}$ 9.3, 3-H), 3.75 (2 H, m, 6-H, 6'-H), 3.97 (1 H, t, $J_{1,2}$ 9.5, 2-H), 4.70 (6 H, m, 1-H, CH_2Ph), 5.00 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 7.35 (15 H, m, Ph); δ_C(75.4 MHz; $CDCl_3$) 18.1, 25.8 (Me_2Si), Me_3CSi)), 61.5. 72.7, 73.5, 74.4, 75.5, 77.3, 78.9 (ring carbons, *C*H_2Ph), 84.2 (1-C), 87.7, 126.9, 127.4, 127.6, 127.7, 128.2, 128.3, 128.4, 128.8, 131.4 (Ph).

Phenyl 2,3,4-tri-*O*-benzyl-1-thio-β–D-galactopyranoside (63)

Phenyl 2,3,4-tri-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-1-thio-β–D-galactopyranoside (**62**) was dissolved in methanol (100 cm^3) and fluoboric acid (48% w/w in water) (9.18 mmol, 1.2 cm^3) added to this solution, the reaction was followed by t.l.c. (toluene:ethyl:acetate; 10:1) and when complete the reaction was made basic with sodium hydrogen carbonate (solid) and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 10:1 then 5:1) to give the title compound (**63**) (1.75 g, 43% from phenyl 1-

thio-β-D-galactopyranoside) m.p. 80-82°C (from diethyl ether hexane); ν_{max}/cm^{-1} 3482s (OH); δ_H(300 MHz; $CDCl_3$) 2.57 (1 H, br s, OH), 3.53 (1 H, t, $J_{5,6}$ 6.0, 5-H), 3.70 (2 H, m, 6-H, 6'-H), 3.94 (2 H, m, 3-H, 4-H), 4.10 (1 H, t, $J_{1,2}$ 9.3, 2-H), 4.85 (6 H, m, 1-H, CH_2Ph), 5.01 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 7.40 (18 H, m, Ph), 7.70 (2 H, m, Ph); δ_C(75.4 MHz; $CDCl_3$) 61.8, 72.6, 73.2, 74.0, 75.3, 77.1, 78.7 (ring carbons, *C*H_2Ph), 83.9 (1-C), 87.3, 127.0, 127.4, 127.6, 127.9, 128.1, 128.2, 128.3, 128.7, 131.2, 133.8 (Ph).

_p_-Nitrophenyl 6-_O_-_t_-butyldimethylsilyl-1-thio-β-D-galactopyranoside (66)

_p_-Nitrophenyl 1-thio-β-D-galactopyranoside (**46**) (4.438 mmol, 1.41 g) was dissolved in pyridine (15 cm^3) and the solution cooled to 0°C. To this was added _t_-butyldimethylsilyl chloride (7.39 mmol, 1.115 g) under a nitrogen atmosphere and the reaction was followed by t.l.c. (ethyl acetate). When complete the reaction was quenched with methanol and the solvent was removed under reduced pressure. The residue obtained was purified on a short silica pad (toluene then toluene:ethyl acetate; 1:1 then ethyl acetate) to give the title compound (**66**) (1.01 g, 52%) which was used directly in the next step.

_p_-Nitrophenyl 2,3,4-tri-_O_-benzyl-6-_O_-_t_-butyldimethylsilyl-1-thio-β-D-galacto pyranoside (67)

_p_-Nitrophenyl 6-_O_-_t_-butyldimethylsilyl-1-thio-β-D-galactopyranoside (**66**) (2.35 mmol, 1.013 g) was dissolved in DMF (10 cm^3) and benzyl bromide was added (7.76 mmol, 0.923 cm^3). The solution was cooled to 0°C under a nitrogen atmosphere and sodium hydride (60% in mineral oil, washed with hexane) was added in portions (each portion ≈4.16 mmol, 0.1 g). The reaction was followed by t.l.c. (toluene:ethyl acetate; 10:1) and portions of sodium hydride were added every 15-20 minutes until

the reaction had stopped (≈4 hours). When the reaction had stopped it was quenched with methanol and the solvent was removed under reduced pressure. The residue obtained was dissolved in DCM and washed with sodium hydroxide solution (1M) extracting with DCM. The organic extracts were washed with saturated sodium bicarbonate solution and water, dried over magnesium sulfate and the solvent was removed under reduced pressure to give the title compound (**67**) which was not purified further (0.698 g, 42%) and was used directly in the next step.

p-Nitrophenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (65)

p-Nitrophenyl 2,3,4-tri-*O*-benzyl-6-*O*-*t*-butyldimethylsilyl-1-thio-β-D-galacto-pyranoside (**67**) (0.99 mmol, 0.70 g) was dissolved in methanol (5 cm^3) and fluoboric acid (48% w/w in water) (1.91 mmol, 0.25 cm^3) added. The reaction was followed by t.l.c. (toluene:ethyl acetate; 10:1) and when complete (after addition of a further 0.1 cm^3 fluoboric acid after 1.5 hours) the reaction was made basic with solid sodium hydrogen carbonate and the solvent was removed under reduced pressure. The residue obtained was purified by silica chromatography (toluene:ethyl acetate; 10:1→15:1→12.5:1→10:1) to give the title compound (**65**) (0.28 g, 48%) m.p. 143-144°C (from DCM:hexane); $[\alpha]_D^{23}$ -3.5 (c 0.85 in $CHCl_3$); δ_H(300 MHz; $CDCl_3$) 3.62(3 H, m, 3-H, 6-H, 6'-H), 3.87 (1 H, dd, $J_{5,6}$ 6.7, 11.1, 5-H), 3.95 (1 H, d, $J_{3,4}$ 2.5, 4-H), 4.06 (1 H, t, $J_{1,2}$ 9.3, 2-H), 4.65 (1 H, d, $^2J_{AX}$ 11.0, C*H*Ph), 4.72 (1 H, d, $^2J_{AX}$ 10.4, C*H*Ph), 4.77 (4 H, m, CH_2Ph), 5.02 (1 H, d, $^2J_{AX}$ 11.2, C*H*Ph), 7.28 (15 H, m, Ph), 7.61 (2 H, d, $J_{A,B}$ 8.2, $PhNO_2$), 7.96 (2 H, d, J 8.2, $PhNO_2$); δ_C(75.4 MHz; $CDCl_3$) 62.0, 72.8, 73.1, 74.4, 75.7, 76.6, 79.0 (ring carbons, CH_2Ph), 84.0 (1-C), 85.6 (*Ph*NO_2), 123.8, 127.6, 127.9, 128.2, 128.3, 128.4, 128.6, 129.1 (Ph), 137.8, 137.9, 138.0 (Ph), 144.3, 146.0 ($PhNO_2$).

Attempted synthesis of *p*-nitrophenyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (68)

Methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) (0.11 mmol, 0.063 g) and *p*-nitrophenyl 2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside (**65**) (0.1 mmol, 0.059 g) were dissolved in DCM and 4Å molecular sieves (0.1 g) and iodine (0.22 mmol, 0.051 g) were added. The reaction was followed by t.l.c. (toluene:ethyl acetate; 10:1) and after 6.5 hours the reaction was quenched with sodium thiosulfate solution. The mixture was extracted with DCM, the organic extracts was washed with water, dried over magnesium sulfate and the solvent was removed under reduced pressure. The mixture of products obtained was partially purified by size exclusion chromatography (50 g BioBeads SX-1 200-400 mesh, toluene); δ_H(500 MHz; $CDCl_3$) 7.78 (2 H, m, $PhNO_2$), 8.10 (2 H, m, $PhNO_2$) ; δ_C(75.4 MHz; $CDCl_3$) 98.4, 104.0 (2 x 1-C)

Methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α/β-D-galactopyranoside (39)

The standard procedure for epimerisation of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) was as follows:

Methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside was dissolved in CH_3CN and iodine (1.1 equivalents) with either no other reagent or DDQ (1.1 equivalents), CAN (1.1 equivalents) or CAS (1.1 equivalents) added. The course of epimerisation was followed by t.l.c. (toluene:ethyl acetate; 10:1) and when complete the reactions were either quenched with Amberlite IRA 400 (OH^-) ion exchange resin until all visible colour was gone from the solution, the resin filtered off and the solvent was removed under reduced pressure (for iodine alone) or the solvent was removed under reduced pressure and the residue obtained purified by silica chromatography (toluene:ethyl acetate; 50:1) (for reactions with DDQ, CAN or CAS).

^{1}H N.M.R. studies on reactions of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α/β-D-galactopyranoside (39)

The general procedure for ^{1}H N.M.R. studies on epimerisation of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α/β-D-galactopyranoside (**39**) was as follows:
Methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside was dissolved in either CD_3CN (0.7 cm^3) or D_3CCN (0.7 cm^3) and iodine (1.1 equivalents) added. The course of epimerisation was followed by 500 MHz ^{1}H N.M.R..

The general procedure for ^{1}H N.M.R. studies on epimerisation and methanolysis of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-α/β-D-galactopyranoside (**39**) was as follows:
Methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside was dissolved in either CD_3CN (0.7 cm^3) or D_3CCN (0.7 cm^3) and iodine (1.1 equivalents) and MeOH (7 equivalents) added. The course of the reaction was followed by 500 MHz ^{1}H N.M.R..

2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl nitrate (70)

Methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (0.125 mmol, 0.0712 g) was dissolved in either acetonitrile (1 cm^3) and iodine (1.1 mol equivalents, 0.138 mmol, 0.035 g) and CAN (1.1 mol equivalents, 0.137 mmol, 0.075 g) were added. The course of reaction was followed by t.l.c. (toluene:ethyl acetate; 10:1) and when complete (≈5 minutes) the solvent was removed under reduced pressure and the residue obtained was purified by silica chromatography (toluene:ethyl acetate; 50:1) to give the title compound (**70**) (0.017 g, 24%); ν_{max}/cm^{-1} 1282s, 1648s, 1654s (ONO_2); δ_H(300 MHz; $CDCl_3$) 3.46 (2 H, m, 6-H, 6'-H), 3.78 (1 H, dd, $J_{3,4}$ 2.6, $J_{2,3}$ 10.8, 3-H), 3.98 (2 H, m, 4-H, 5-H), 4.20 (H, dd, $J_{2,3}$ 4.2, $J_{1,2}$ 10.3, 2-H), 4.31 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 4.38 (1 H, d, $^2J_{AX}$ 12.4, C*H*Ph), 4.48 (1 H, d, $^2J_{AX}$ 11.2, C*H*Ph), 4.60 (1 H, d, $^2J_{AX}$ 11.8, C*H*Ph), 4.67 (1 H, d, $^2J_{AX}$ 11.5, C*H*Ph), 4.76 (1 H, d, $^2J_{AX}$ 11.8, C*H*Ph), 4.77 (1 H, d, $^2J_{AX}$ 12.1, C*H*Ph), 4.86 (1 H, d, $^2J_{AX}$ 11.2, C*H*Ph), 6.09 (1 H, d, $J_{1,2}$ 4.1, 1-H), 7.23 (20 H, m, Ph).

Studies on silica t.l.c. plate supported reactions.

Thin layer chromatography plates (silica t.l.c. plates cut to approximately 7 cm x 1.5 cm) were visualised with the aid of U.V. light (254 nm), and unless otherwise stated were subsequently dipped in 5% sulfuric acid/ethanol and heated until the compound charred. Thin layer chromatography was carried out using either 0.25 mm Macherey-Nagel DC Fertigplatten SIL G-25 UV_{254} plates or Merck 25-DC Platten Kieselgel 60 $F_{254.}$ Column chromatography was carried out using Fluka Silicagel 60.

The solvents used were either distilled or of Analar quality, solvents were dried according to literature procedures.[296]

Attempted synthesis of *p*-nitrophenyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (68)

Solutions (DCM) of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) and *p*-nitrophenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**65**) were co-spotted on four t.l.c. plates and these placed in a vessel containing solid iodine, whose vapour visibly discoloured the plates and the compounds spotted on them. At 10 minute intervals one plate was removed, spotted with the starting materials and products for reference and eluted (10:1 toluene:ethyl acetate) before visualisation. After forty minutes the donor had completely reacted to give a mixture of products including the hemiacetal of the donor and a small quantity of disaccharides (using the products from the previous solution phase synthesis of (**68**) as markers).

General procedure for silica t.l.c. plate supported reactions in the attempted synthesis of *p*-nitrophenyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (68)

Compounds (dissolved in DCM) were spotted by capillary on top of each other on the surface of the plate which was placed in a vessel containing solid iodine. The plate and the compounds spotted on it was visibly discoloured by the iodine vapour liberated from the solid form. After 20 minutes the plate was removed from the

vessel, the starting materials were spotted by capillary for reference and the plate was eluted (10:1; toluene:ethyl acetate).

The reaction was repeated using various combinations of the following conditions: under vacuum, with removal of solvent prior to reaction, with plates pre-dried overnight in an oven at 100°C. No improvement on the example given was found.

Attempted synthesis of *p*-nitrophenyl-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-galactopyranosyl)-2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (68)

Solutions of methyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**39**) (0.0015 cm^3, 0.01M in DCE) and *p*-nitrophenyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**65**) (0.001 cm^3, 0.01M in DCE) were mixed and spotted on a t.l.c. plate that had been dried overnight at ≈110°C. To the reaction spot was spotted iodine (0.009 cm^3, 0.01M in DCE) and the plate placed in a vessel with a nitrogen atmosphere. After 20 minutes the plate was removed, spotted with the starting materials and products for reference and eluted (10:1 toluene:ethyl acetate) before visualisation. No reaction was observed.

Methyl 2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside (49)

A solution (DCM) of *p*-nitrophenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside (**47**) was spotted on a t.l.c. plate and methanol spotted on this. The plate was then placed in a jar with ICl liquid in it. The vapour from the ICl discoloured the plate slightly, after 5 minutes the plate was removed, spotted with starting material for reference and eluted (toluene:ethyl acetate; 10:1). Visualisation showed partial reaction of the donor and hemiacetal formation.

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